Acta Paediatrica Hungarica

VOLUME 31, NUMBER 1, 1991

Akadémiai Kiadó, Budapest

ACTA PAEDIATR HUNG HU ISSN 0231-441X

ACTA PAEDIATRICA

HUNGARICA

A Quarterly of the Hungarian Academy of Sciences

Editor

M. MILTÉNYI

Acta Paediatrica publishes original reports of studies in paediatrics and borderline fields.

Manuscripts and editorial correspondence should be addressed to

ACTA PAEDIATRICA HUNGARICA

Bókay J. u. 53.
H-1083 Budapest, Hungary

Subscription information

Orders should be addressed to

KULTURA Foreign Trading Company
P.O. Box 149,
H-1389 Budapest

or its representatives

Acta Paediatrica Hungarica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ
Publishing House of the Hungarian Academy of Sciences
Prielle Kornélia u. 19–35.
H-1117 Budapest

Acta Paediatrica Hungarica is abstracted/indexed in Biological Abstract, Chemical Abstracts, Excerpta Medica, Index Medicus

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Acta Paediatrica Hungarica

VOLUME 31

AKADÉMIAI KIADÓ, BUDAPEST
1991

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MYELODYSPLASIA IN CHILDHOOD

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Received 3 Oct 1989

Two simple statements can be given which reflect our helplessness with respect to myelodysplasia:

- MDS are rare in childhood, but features and course of disease resemble those in adults.
- JCMML should be considered as a pediatric subtype of MDS, but with a worse prognosis than CMML in adult patients.

MDS = Myelodysplastic syndrome
JCMML = Juvenile chronic myelomonocytic leukaemia

INTRODUCTION

Myelodysplastic syndrome (MDS) is a term rarely used in pediatric literature. Although the various disease entities have been defined by the FAB cooperative group /3/, synonyma like preleukemic syndrome, subacute or smoldering leukemia, refractory anemia, childhood monosomy 7 syndrome, juvenile chronic myelocytic leukemia (JCML), chronic myelomonocytic leukemia (CMML) are still used for MDS classification.

Other reasons why the pediatrician is not so familiar with this group of diseases are its low incidence, lacking awareness of the problem and the absence of clear diagnostic criteria. MDS is said to encompass approximately 1-2 % of acute leukemia cases /9/. On the other hand JCML accounts for 0,5-2 % of all leukemias /3/. It is therefore not surprising that even in the most recent literature few data are available and only small series of patients are described /4, 7, 9, 17, 25, 26/.

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There is still a lot of controversy regarding the distinction of myeloproliferative from myelodysplastic diseases and in particular regarding the use of the terms JCML and MDS /1, 8, 16, 22/. Pediatricians distinguish Ph+ chronic myelocytic leukemia (CML) (the so called adult type) from the juvenile type of CML /21/. The latter disease is characterized by the lack of the Ph chromosome, by its manifestation in children under 5 years of age, by the presence of thrombocytopenia, often elevated hemoglobin F(HbF), the poor response to treatment and by a short survival time (no more than one or two years).

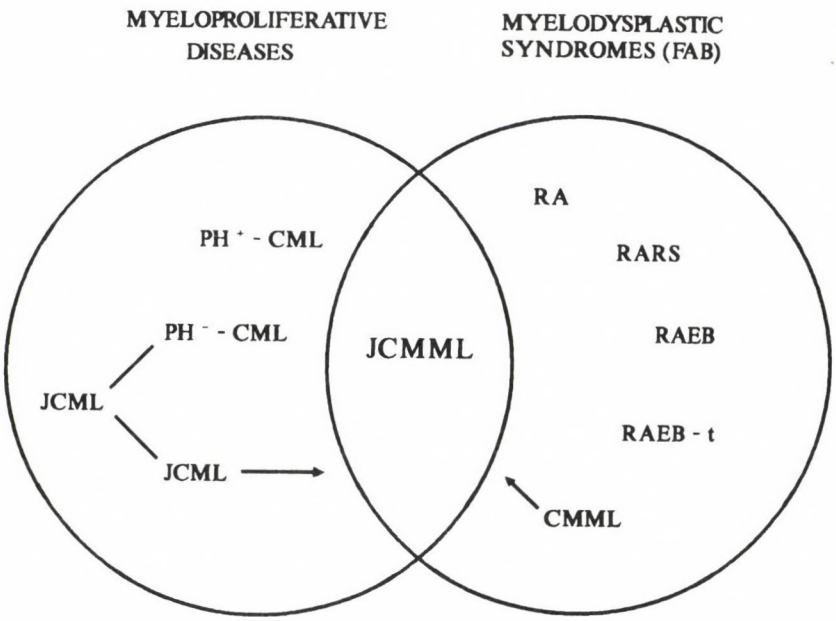

Fig. 1. FAB - French-American-British co-operative Group;
PH±-CML - Philadelphia pos./neg. chronic myeloic leukemia;
CMML - chronic myelomonocytic leukemia;
JCML - juvenile CML;
JCMML - juvenile CMML;
RA - refractory anemia;
RARS - refractory sideroblastic anemia;
RAEB - RA with excess of blasts;
RAEB-t - RAEB in transformation;

Applying the criteria proposed by the FAB group to define the MDS subtype CMML, which usually occurs in elderly patients, the clinical and hematological findings do not resemble those of JCML. Therefore it seems acceptable to consider JCML a special form of CMML and to introduce the term juvenile chronic myelomonocytic leukemia (JCMML) (Fig. 1).

PATIENTS AND METHODS

Clinical and hematological findings in JCMML

In 1984, Castro-Malaspina et al /8/ published a survey of 38 patients treated at the Hospital St.Louis in Paris and the Memorial Sloan Kettering Cancer Center in New York. The age distribution showed that the majority of these children were under 4 years at diagnosis, the predominating age being the first and second year of life. The male sex clearly prevailed.

The main symptoms and clinical findings were failure to thrive, fever, recurrent infections and bleeding tendency with anemia. Frequently children showed variable papular and ekzematoid skin rashes especially of the face. In addition to generalized lymphadenopathy most of the children presented with hepatosplenomegaly. The small series collected during the last 8 years in our own institution demonstrated very similar features (Table I).

Hematologically a leukocytosis of usually not more than 100.000/ul was characteristic with the presence of immature granulocytes in the peripheral blood. In all these cases a monocytosis of over 1500/ul was present. Often few blasts and red cell precursors in the peripheral blood were encountered, accompanied by a low hemoglobin, elevated HbF concentration and thrombocytopenia. Usually an increase of gamma-globulins and a higher lysozyme titer are detected in the serum also /8/.

Clinical and hematological findings in MDS

As it was mentioned, pediatric experience in MDS is very limited. In the recently published article of Creutzig et al. /9/ data on 21 children were collected from several institutions in Germany and Italy. 16 were diagnosed as RAEB in transformation, 4 as RAEB and 1 as adult type CMML. During the last 8 years we saw 5 cases in Austria. 1 child had RA, 3 had RAEB and 1 patient RAEB -t. There was also a clear prevalence of males, but the disease manifestation occured in a higher age group than JCMML (Table II). The patients' characteristics resembled those in adults. Long history, not necessarily an organ involvement and blood pictures of great variability with or without the presence of blasts. HbF and leucocyte alkaline phosphatase score were not always pathologic.

Bone marrow findings and chromosomal abnormalities in JCMML and MDS

Bone marrow cellularity and number of blasts varied markedly but signs of dysplasia were always present in at least one of the three cell lineages /7, 9, 25/.

TABLE I

Symptoms and clinical findings at diagnosis in JCMML

Symptoms and Clin. Findings	Castro-Malaspina et al (Cancer 54, 675, 1984) (N=38)	Own Pts. (N=6)
o Sex	26 ♂, 12 ♀	5 ♂, 1 ♀
o Age	0-6 yrs (most < 4 yrs)	3 MTS - 3 3/12 yrs
o Malaise and Failure to thrive	23 (60 %)	6/6
o Rec. infections	22 (58 %)	5/6
o Bleeding	19 (50 %)	4/6
o Cutaneous manifestations	16 (42 %)	5/6
o Lymphadeno-pathy	8 (21 %)	6/6
o Hepatospleno-megaly	34 (89 %)	6/6

Comparing bone marrow smears of JCMML and MDS we could not find any striking differences concerning cellularity, number of blasts and megakaryocytes or cytochemistry. The dysplasia was more pronounced in MDS and atypical monocytoid cells were more common in JCMML. In all our cases the immunological phenotyping showed the prevalence of a myelomonocytic population in JCMML, as evidenced by the presence of the epitop CDw14 in 25 - 70 % of cells.

Neither stem cell cultures nor chromosome analyses could clearly discriminate between both disease groups. As the result of a clonal defect the colony-forming capacity of all of the marrow hemopoietic precursor cells is quite low or absent in the majority of MDS patients in adulthood, this is also true for the diseases in childhood /13/. Besides an impaired growth of normal hematopoietic progenitors an excessive proliferation of monocyte macrophage colonies in the absence of exogenous colony-stimulating activity were detected in some cases of JCMML /1, 11/, an observation that was not shared by our small number of patients.

TABLE II

Patients characteristics in MDS in childhood

	Creutzig et al. (1987)	Own Pts.
No of Pts.	21	5
Sex	12 ♂ 9 ♀	4 ♂ 1 ♀
Age (Median)	2 - 17 yrs (11 2/12 yrs)	4 8/12 - 14 11/12 yrs
RA	-	1
RAEB	4	3
RAEB-t	16	1
CMML	1	-
Duration of History (Weeks)	1 - 32 (10)[1]	3 - 24 (8)[1]
Hepatomegaly	12/21 (57 %)	2/5
Splenomegaly	4/21 (19 %)	3/5
Lymphadenopathy	5/21 (24 %)	3/5
Bleeding	8/21 (38 %)	1/5
WBC (x 10^9/l)	0,8-27,0 (5,5)[1]	1,5-22.5 (11,8)[1]
Hemoglobin (g/dl)	3,8-12,9 (7,8)[1]	8,7-11,7 (8,9)[1]
Platelet Count (x 10^9/l)	6 -141 (37)[1]	42-50 (78)[1]
Blasts (%)	1 - 26	0- 7
Fetal hemoglobin ↑	5/ 9 (56 %)	1/3
Lap* Score ↓	3/ 9 (33 %)	0/2

* leucocyte alkaline phosphatase

1 median

Out of 6 patients with JCMML one patient each showed a monosomy 7 and a trisomy 8, one further patient developed a 7p+ anomaly in blast crisis. Two out of 5 patients with MDS had also a monosomy 7 and one patient a 11 q - deletion.

Surveying the data in the literature, there is a surprising similarity of chromosomal abnormalities detected in MDS and JCMML /9, 12, 14, 15, 19, 27/. In both disease groups monosomy 7 and trisomy 8 are most frequently encountered. So far it seems unlikely that other possible changes are typical for one or the other group (Table III).

TABLE III

Chromosome anomalies in childhood MDS

MDS
o No Anomalies (Approx. 40 %)
o Monosomy 7
o Trisomy 8
o Other changes (5q-, 11q, 18q-)
JCMML
o No anomalies (~ 80 %)
o Monosomy 7
o Trisomy 8
o Other anomalies (chromosomes 1, 3, 5, 8, 15, 17, Y)

DISCUSSION

Prognosis and treatment

The prognosis of JCMML is poor. 26 out of 38 patients in the series of Castro-Malaspina et al. /8/ died within 2 years, irrespectively of the treatment (25 of them had received chemotherapy). Only 12 patients survived more than 2 years. The development of acute leukemia was the cause of death in 11 patients. We had a similar experience in our small group of

patients: 4 out of 6 died between 5 and 45 months after diagnosis due to progressive disease and/or therapy related septicemia (median 15 months). All had received several treatment schedules, 3 children were also splenectomized. In 3 patients, alfa-Interferon (alfa-INF)- treatment was unsuccessful except for a transient response of 4 months in one case /20/. Two children are living with stable disease 5 and 12 months, respectively, after diagnosis.

The survival statistics clearly show the bad prognosis of MDS in childhood. Only 5 out of 21 patients in the cohort of Creutzig et al /9/ were alive between 16 to 69 months after diagnosis (3 of them in complete remission) and the median survival was 20 months (1-69+ months). The benefit of intensive chemotherapy remains doubtful in these series. 2 out of 5 of our own patients developed a leukemic transformation 5 and 7 months, respectively, after diagnosis. 2 children died after a survival time of 2 and 22 months, respectively 3 patients are in stable disease without treatment 1, 1 and 23 months after diagnosis.

So far it is not clear what kind of treatment can be recommended in JCMML and MDS /23/. Clinical observation without chemotherapy but with support of blood products seems to be the treatment of choice. The median time until progression in the entire group of Creutzig et al /9/ was 12 months (range 1-21 months). A low dose chemotherapy with 6-mercaptopurine or cytosine arabinosid did not show convincing results /5, 7, 9/.

Intensive chemotherapy may be of certain value for inducing remission (generally of short duration) in progressive disease /6, 9, 24/. 11 out of 21 patients in the series of Creutzig et al. /9/ were treated with intensive chemotherapy, 6 of them achieved complete remission. Only casuistic experiences are reported with alfa-INF up till now /10, 20/. Only one of our 3 patients showed a transient response at a dose of 2×10^6 U/day s.c. Allogenic matched bone marrow transplantation is certainly the treatment of choice for eliminating the underlying stem cell defect.

Approximately 30 patients including children with MDS have been transplanted since 1979, two third of which are still in remission /2/.

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Acta Paediatrica Hungarica 31 (1), pp 13-21 (1991)

FAMILIAL OCCURRENCE OF BILATERAL RENAL AGENESIS

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Received 13 Oct 1989

The 58 cases of bilateral renal agenesis (Potter syndrome) registered in the Genetic Counselling unit of our institute in the last 12 years are reviewed. The only familial recurrent case which has been prenatally diagnosed is described in detail. A urinary bladder anomaly like that of the subsequent third child has not been previously reported.
The authors analyze the possible inheritance patterns. They suggest the malformation is a genetically heterogeneous entity. They emphasize that nowadays the birth of a newborn with bilateral renal agenesis can be prevented in all cases.

INTRODUCTION

The 135 cases of Potter - the first author who described the bilateral renal agenesis (BRA) and the consequent deformities - do not include any familial recurrences /14/. Although such cases have already been published, they can be considered even nowadays as rarities /1,2,4,6,7,9,10,12,13,15-21/. The incidence rate of BRA is estimated between 0.1 - 0.3/1000 births, and the recurrence rate is referred as 1 - 5 % after the first affected newborn or fetus /4,16,21/. Unilateral renal agenesis can be detected by ultrasound in 5 % of parents and siblings of the affected infants /16/.

Since the establishment of our Genetic Counselling unit twelve years ago, we have come across only one familial case of BRA. After the birth of a newborn with BRA and the induced abortion of a similar mid-trimester fetus, a child suffering from lower urinary tract malformation was born.

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MATERIALS AND METHODS

After the delivery of a newborn or fetus with BRA or an induced abortion because of prenatally diagnosed Potter syndrome we registered 58 women (i.e. couples) at our Genetic Counselling between 1st January, 1977 and 31st December, 1988. Autopsy records of newborns or stillbirths were collected from other institutes as well. During their next pregnancy they were regularly in contact with our Genetic Counselling unit. The state of health of infants born in other institutes are documented in all cases.

After the definite diagnosis of the malformation we informed the couple of its fatal outcome. They asked for the interruption of the mid-trimester pregnancies in all cases. We performed the induction by extraovular injection of Rivanol (0.1 %) and continuous infusion of oxytocin /8/. Autopsy of the fetuses was performed in our fetal pathology laboratory.

Our cases are presented in Table I. We give a detailed report on the cases observed in the same family.

TABLE I

BRA cases registered in the Genetic Counselling of the University of Medicine, Debrecen, between 1977 and 1988

	Cause of the first attendance	
	A previous child/fetus with BRA	Prenatally diagnosed BRA in the current pregnancy
Outcome of the following pregnancies	35	23
No more pregnancy undertaken or registered	7	18
Healthy newborn	26	4
Aborted in the 1st trimester	-	1
Infant suffering from congenital heart anomaly	1	-
Recurrent BRA	1	-

CASE REPORT

The parents are healthy. The mother was 23, the father 34 years of age at the time of the first delivery. They are not known to be related and have negative history concerning the usage of unusual medicines or drugs. It is noteworthy that the mother of the gravida had been operated on because of renal stones, but she was not aware of any urinary tract malformation.

The first pregnancy of the mother resulted in the birth of a premature male infant by breech delivery at 32 weeks' gestation, following an attempt of tocolysis. His Apgar score at one minute was 4. Gasping, bradycardia, deep cyanosis could be observed, and in spite of resuscitation death occurred at 15 minutes of age. During her pregnancy the mother visited neither an ultrasound laboratory, nor a genetic counselling unit. The 45 cm male newborn with a mild degree of Potter's face weighed 1980 gms. Besides hypognathia, the narrow skull was elongated mento-occipitally, the ears were low-set. The left fingers and nails were hypoplastic.

The autopsy findings revealed the total absence of both kidneys and ureters. The urinary bladder was 1 cm in diameter without any urine in it. Other remarkable findings are the hypoplastic lungs, absence of the left umbilical artery and a small additional spleen near to the hilus lienis.

Before deciding about the next pregnancy the parents visited our Genetic Counselling. After studying the autopsy record we estimated the recurrence risk under 5 % based on the data of the literature, and suggested to undertake the pregnancy with prenatal diagnostic measurements.

A few months later the woman reported again, pregnant and in the 15th week of gestation. The ultrasonography showed oligohydramnios, and compressed position of the limbs. Subsequent examinations performed at the 17th and the 19th weeks showed similar conditions, urinary bladder and kidneys could not be observed. The maternal serum AFP level was normal.

Because of the fatal outcome the parents were advised the pregnancy be interrupted, which they accepted. The male fetus was not alive, weighed 255 gms, had Potter's face and limbs strictly pressed to the body. The autopsy findings were: total absence of both kidneys and ureters, as well as the urinary bladder. The renal arteries also could not be found. The size of adrenal glands was normal, but they were discoid in shape. The testes were undescended. The lungs were mildly hypoplastic. The number and the running of the umbilical vessels were normal.

A year later - being aware of the higher risk of conceiving an unhealthy infant - the pregnant mother reported for prenatal diagnostic measurements. This time we could not detect any kidney malformations throughout the ultrasonographic series (at the 9th, 18th, 28th weeks).

At the 39th week of gestation a boy, weighing 4000 gms was delivered. After having recurrent pyelonephritis, cystography and intravenous pyelography were performed at the age 1 year, and several diverticuli of the urinary bladder and a consequent hydroureter - caused by the dislocation because of the largest diverticulu - were detected. Using continuous

catheter application, the congestion of the upper urinary tract was eliminated. After this, diverticulectomy and ureterneoimplantation was carried out. (It was performed at the Department of Pediatric Surgery of Borsod-Abauj-Zemplén County Hospital).

At the age of two and a half years in 1989 the child is normal, but sometimes needs urodesinficients because of pyuria. No abnormality was detected during the control pyelography, except a mild congestion on one side. The urinary bladder was of normal size, but irregular shape because of surgical manipulations.

Following strict genetic care the woman gave birth to another male newborn some months ago, who is - according to the first examinations - healthy.

DISCUSSION

Our Eastern Hungary Regional Genetic Counselling Unit is visited by couples from distant counties too, so an exact incidence rate cannot be calculated. Based on the data of the prenatal screening program going on in three Eastern-Hungarian counties (Hajdú-Bihar, Szabolcs-Szatmár, Szolnok) we found the incidence rate of BRA to be 0.32/1000 births.

Table II lists the 22 cases found in a review of the literature, where two or more members of the family were noted to have BRA. Familial occurrence means mostly siblings, except two cases. Another four relatives suffer from other kinds of upper urinary tract malformation. In the family No. 16 two unilateral and two bilateral renal agenesis occurred. Until now, none has reported on the birth of a child with lower urinary tract malformation following two siblings with BRA.

These reports show a considerable variability of the effect of genetic factors on the phenotype in the familial cases. The case observed by Mauer et al. has a crucial importance: one of two monoamniotic co-twins had bilateral, and the other unilateral renal agenesis. A sibling with soliter dysplastic kidney of a newborn with BRA has also been observed /3/.

Concerning the inheritance pattern several possibilities therefore present themselves. The autosomal recessive trait seems to be likely, but the predominance of affected males suggests that some sex limiting factors may be involved /6/.

The cases observed in cousins /9,13/ do not support this explanation: the low probability of a marriage of heterozygous persons is decreased by the necessity of a third heterozygous person without consanguinity. However, in the case of consanguineous parents it is the most probable possibility /18/.

Several family cases suggest an X-linked recessive inheritance /13/. The great number of siblings of different sex, however, makes it unlikely that this possibility could play an important role, except if we suppose that BRA is a genetically heterogeneous entity.

Taking into account all the cases presented in Table II, in particular the case of those families which have members with a urinary tract malformation of less severe degree, the autosomal dominant trait with variable expressivity presents itself, too. Roodhoft et al. found unilateral renal agenesis in three cases, double ureter in two, and multicystic kidney in one case among the 71 parents of 41 infants with BRA, who were evaluated by ultrasonography for renal malformations. It is difficult to fit our case to these findings because of the lack of reports on similar cases. However, presuming a gene defect influencing the formation of the ureteric buds one can give the explanation.

The multifactorial etiology with sex limiting factors seems to be the most probable one. After the birth of one affected child we estimate the recurrence risk under 3-5 %.

The increased risk of pregnants with an affected child or fetus makes it necessary to submit them to careful ultrasound examination that can assure the detection of fetuses suffering from a life-incompatible urinary tract malformation with oligohydramnios, and the interruption of the mid-trimester pregnancy. Given the routine screening of all pregnants the oligohydramnios sequence can be detected in almost every case (i.e. in 100 %).

Diagnostic value of the increased level of maternal serum AFP, which was reported in some cases with BRA /5/, is questionable. Therefore, BRA can be detected only by a screening program including ultrasonography, too.

TABLE II

No.	Authors	BRA male	BRA female	Siblings with other urinary tract anomaly	Healthy siblings	Other relatives with urinary tract anomaly
1.	Madisson	2	-		2	
2.	Carter et al.	2	-		1	
3.	"	1	1		1	
4.	"	1	1		2	
5.	"	1	1		2	
6.	"	-	2			
7.	Roodhoft et al.	1	1			mother (multi-cystic kidney)
8.	Schmidt et al.	-	2		2	
9.	Baron	2	-		1	
10.	Rizza and Downing	-	2		1	
11.	Whitehouse and Mountrose	1	1		1	
12.	Hack et al	2	-		1	

13. Buchta et al	1	1			mother (unilateral agenesis)
14. "	2	-			
15. "	4	-			
16. Kohn and Borns	-	1	1 male (unilateral agenesis)	3	father (unilateral agenesis) father's cousin (BRA)
17. Kaffe et al.	1	1			
18. Pashayan et al.	2	-			mother's cousin (BRA)
19. Schinzel et al.	2	-			
20. Sangal et al.	2	-			mother (horseshoe kidney)
21. Morse et al.	1	2		1	
22. Wilson and Baird	2	-			
23. present report	2	-	1 male (diverticulum of the urinary bladder)	1	
Total	32	16	2	17	BRA 2 other 4

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Acta Paediatrica Hungarica 31 (1), pp 23-30 (1991)

BURKITT'S LYMPHOMA WITH 8;14 AND 2;8 TRANSLOCATIONS SIMULTANEOUSLY

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Received 10 March 1989

Burkitt's lymphoma of a ten-year-old boy with specific 8;14 and variant 2;8 translocations is reported. The post mortem diagnosis of Burkitt's lymphoma was based on histological picture and the cytogenetic findings of the tumor biopsy. The child died four days after clinical admission. Because of the rapid progression of the disease immunological and serological investigations could not be performed. Therefore several questions remained unclarified. It is supposed that in the patient's B-lymphocytes multiple transformation events occurred leading to the development of the polyclonal lymphoma, similarly to that described in trasplant-associated lymphoproliferations.

INTRODUCTION

A clinical entity described by Burkitt in 1958 /10/ in East-African children occurs at high frequency in equatorial Africa and New Guinea but has a much lower incidence in the rest of the world. Since 1965, however, there have been several sporadic cases reported from all over the world /2, 3, 17/. Burkitt's lymphoma can be characterized by a specific reciprocal translocation between chromosome 8 and 14. This translocation can be found in 80 % of patients with this disease. In the remaining 20 % two variant translocations: t(2;8) and t(8;22) can be observed /9/.

Hereby we report a case of non-endemic Burkitt's lymphoma in the cell lines of which the typical translocation t(8;14) and variant translocation t(2;8) simultaneously appeared.

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CASE REPORT

The patient, a ten-year-old boy, did not suffer from any serious disease before. His mother has been treated for Hodgkin lymphoma for a year. In the history of the patient abdominal pain and meteorism were mentioned. On clinical admission the extremely thin, dysmorphic patient had enlarged abdomen and serious dyspnoe. In the right part of the abdomen a hard painless tumor of infant's head size filling the pelvis could be presumed. Ultrasonography revealed a high density tumor mass of 12 x 14 cm size. Haemostatus was abnormal. No abnormal cells in peripheral smear and bone marrow were seen. On the third day after his admission laparotomy was performed. A tumor with lobulated surface, in close connection with the surrounding tissues became visible. Metastatic infiltrations of the mesenterium, omentum maius, peritoneum and that of abdominal wall were observed. Biopsy was made without any effort to remove the tumor mass. The child died in two days.

Post mortem histologic diagnosis was lymphoblastic lymphoma of Burkitt's type (Figure 1) with the starry sky pattern. Because of the rapid progress of the disease immunological and serological investigations could not be performed.

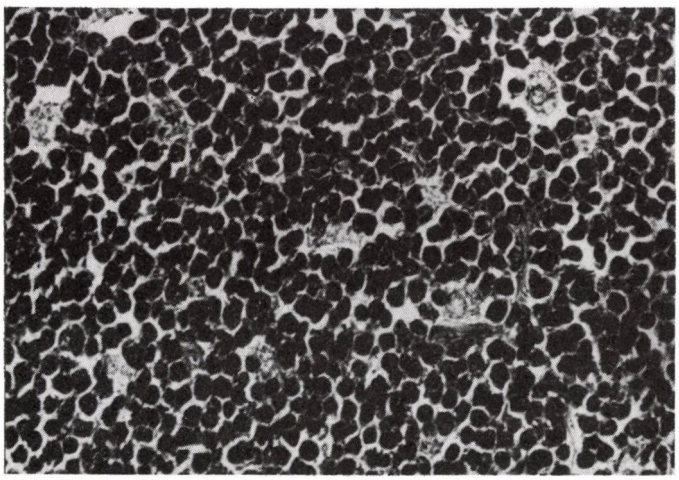


Fig. 1. Burkitt's lymphoma with the starry sky pattern (PAS, 320X)

CYTOGENETIC STUDIES

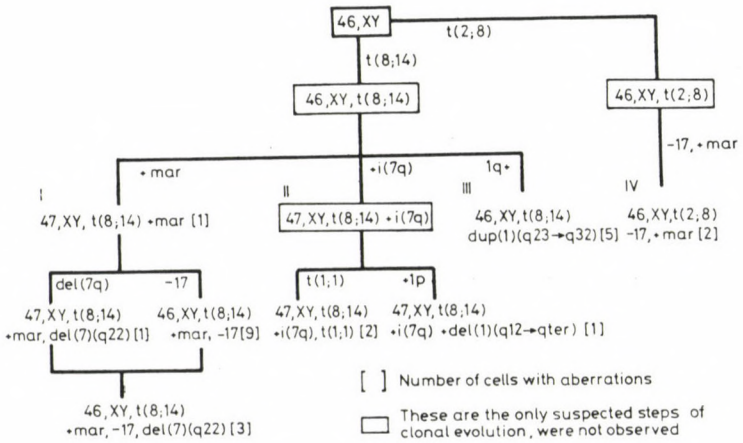
Chromosome analyses were carried out from the tumor tissue. It was cut into small pieces and cells obtained were then suspended in the culture medium (TC 199). Chromosome analyses were performed by direct method. After using modified Giemsa banding technique /28/, karyotypes were described according to the International System for Human Cytogenetic Nomenclature

/18/. We karyotyped as many cells as we could. 24 metaphases were photographed and arranged into karyograms. Each of them was abnormal. Cytogenetic picture proved to be rather complicated.

The modal chromosome number was 46. Four various cell lines were found, in which the steps of clonal evolution could be followed (Table I).

TABLE I

Clonal evolution in cells of Burkitt's lymphoma



In the first cell line of the analysed cells the specific t(8;14) (q24;q32) together with an unidentified marker chromosome was found. In twelve of these cells loss of chromosome 17 occurred. Besides, in three metaphases it was combined with a deletion of the long arm of chromosome 7.

In the second cell line and extranumeral isochromosome of the long arm of chromosome 7 associated with t(8;14). In addition to this, an extra 1p (breakpoint q12), while in two others a tandem translocation of chromosomes 1:t(1;1)(q32;q32) appeared.

Besides t(8;14) the third line was characterized by duplication of one part of the long arm of chromosome 1 (dup 1q)(q23;q32).

The fourth cell line had a t(2;8)(p13;q23) the variant translocation of Burkitt's lymphoma consisted of only two cells. Both of them had monosomy 17 and a marker chromosome.

It is interesting that the marker chromosome appeared in the metaphase with the loss of chromosome 17 only. This implies that chromosome 17 may take part in forming the marker chromosome.

DISCUSSION

It was Manolov and Manolova /21/ who discovered that Burkitt's lymphoma can be characterized by the specific 14q+ marker chromosome Zech et al. /32/ identified the exact translocation between chromosomes 8 and 14. Manolova and Manolov /22/ demonstrated this translocation to be reciprocal. This specific translocation /t(8;14)/ has been described in many cases of both endemic and non-endemic Burkitt's lymphomas and in acute leukemias of Burkitt's type as well /6, 26, 31/. Recognition of the variant translocations: the t(2;8) and the t(8;22) in sporadic cases seemed to help to clarify the etiology /1, 4, 5, 23/. In two years, however, Bernheim et al. /7/ managed to show variant translocations in endemic cases, too, so he concluded that there was no direct connection between the type of translocation and Epstein-Barr Virus (EBV) - association. It was Sandberg and Wake /27/ who suggested that the anomaly of the long arm of chromosome 8 is more specific for Burkitt's lymphoma than the 14q+ marker. Gene mapping studies of these tanslocations have cleared the underlying molecular events. While at breakpoint 8q24 the c-myc protooncogene, at the breakpoints of chromosomes 2, 14 and 22, one of the immunoglobulin genes are located: at 14q32 the heavy chain gene (μ), at 2p12 the light chain (κ) and at 22q11 the λ light chain gene have been assigned /13, 15, 20, 30/. By the rearrangement in the t(8;14) the c-myc is translocated near the μ heavy chain gene. In the other two translocations the immunoglobulin genes are translocated next to the c-myc oncogene /9, 14/. All the three situations result in the deregulation of c-myc oncogene.

The challenging fact in our case is that the two different translocations, the t(8;14) and (2;8) appeared simultaneously in the same sample of the tumor. While the (8;14) was observed in 22 out of 24 cells, the t(2;8) appeared only in two remaining cells. This may indicate a greater proliferative advantage of the cell line with t(8;14) compared to cells with the variant translocation t(2;8). The question arises that, if

the disease had not been of so rapid progression, this variant translocation perhaps would not have been visible in a later stage of the disease.

In the additional chromosome changes 1, 7, 17 and marker chromosomes were involved. According to the Fifth Workshop /16/ 14 out of 21 with standard or variant 8q24 translocations had additional chromosome abnormalities. The most common additional change was duplication of part of 1q, always including 1q21-32 which was seen in four patients. One patient had +7 Knuutila et al. /19/ reported chromosome abnormalities in 16 patients with Burkitt's lymphoma or L3 acute lymphocytic leukemia. Trisomy 7 was the most frequent numerical aberration. In addition to one patient had t(1;7) (q21;q32) a gain of a 7q- chromosome and a t(7;?)(7q22;?). They observed structural aberrations of chromosome 1 in five patients. Besides, they wrote -17, 17p+ and appearance of marker chromosomes in patients with Burkitt's lymphoma. Duplications in 1q have been detected in a wide variety of human neoplasms /24, 25/, whereas trisomy 7 is often detected in patients with the t(8;14) /8/.

Cleary et al. /11, 12/ established that most of the patients in transplant-associated lymphoproliferations developing lymphomas have monoclonal tumors, but the disease is multiclonal in biopsy material from different anatomic sites showing different proliferating clones of tumor cells. It is suggested that in these patients multiple independent transformation events occur as a consequence of uncontrolled EBV infection /29/.

In our case it is particularly interesting that cells with various karyotype were obtained from the same part of tumor mass. It is possible that an unknown infection disturbed the immune system's defensive mechanism resulting in an insufficient elimination of the latently EBV-infected B-lymphocytes.

Both primary and secondary immunodeficiencies may induce specific karyotypic abnormalities by the reactivation of a latent EBV-infection /29/. Our data suggest that in the patient's B-lymphocytes multiple transformation events occurred leading to the development of the polyclonal lymphoma.

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Acta Paediatrica Hungarica 31 (1), pp 31-45 (1991)

POSTNATAL DEVELOPMENT OF UREA- AND AMMONIA-EXCRETION IN URINE OF VERY-LOW-BIRTH-WEIGHT INFANTS SMALL FOR GESTATIONAL AGE

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Received 12 August 1989

In 12 very-low-birth-weight (VLBW) infants with intrauterine growth retardation and in 14 VLBW-infants appropriate for gestational age (AGA) fed a human milk (HM) formula (HM enriched with 6 g freeze dried HM per 100 ml) the renal excretion of urea and ammonia was studied on the 10th, 21st and 42nd days of life.

The lowest excretion of urea was found in both groups on the 10th day of life. Up to the 42nd day of life the excretion raised significantly more in the AGA- than in the small for gestational age (SGA)-infants. In contrast to the urea excretion the excretion of ammonia was highest on the 10th day of life in both groups, but the excretion was significantly higher in the SGA-infants if compared to the AGA-infants. In the AGA-infants excretion of ammonia decreased with postnatal age whereas in the SGA-infants the high excretion remained up to the 42nd day of life.

The data suggest that in VLBW-infants the urea synthesizing capacity is decreased and develops within the first weeks of postnatal life. The postnatal development is delayed in SGA-infants when compared to AGA-infants. The differences are more pronounced with increasing degree of intrauterine growth retardation.

INTRODUCTION

Since the first description of a transient hyperammonemia of preterm infants by Ballard et al /1/ many reports have been published concerning this metabolic disturbance during postnatal life. Up to now the transient hyperammonemia is a condition of unknown etiology. Nevertheless, an imbalance between ammonia production and deposition via urea synthesis could be found in all these infants /2, 16, 20/.

Own previous studies have shown that very-low-birth-weight (VLBW) infants, appropriate for gestational age (AGA) are not

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able to respond to a protein load with increasing urea concentrations in serum and that the renal excretion of ammonia is very high at the end of the first week of life /3, 4/. The signs of an immaturity of the urea synthesizing system disappear during the first weeks of life /2, 3, 4, 20/. From these results it can be assumed that the urea synthesizing system of VLBW-infants is immature and develops during postnatal life.

In VLBW-infants small for gestational age (SGA) the postnatal development of liver function have been found as delayed if compared to AGA-infants in dependence on the degree of intrauterine growth retardation (IUGR) /5, 6/.

In all investigations concerning the transient hyperammonemia the SGA-infants were unmentioned /1, 2, 16, 20/. Thus, it was the aim of this study to investigate the influence of the IUGR on the urea and ammonia excretion in urine during the first 6 weeks of postnatal life.

PATIENTS AND METHODS

26 VLBW-infants were enrolled in the prospective study. 12 were classified as SGA- and 14 as AGA-infants according to Lubchenco et al /24/.

All infants were without detectable clinical problems so that enteral feeding could be started within the first day of postnatal life. Supplementary i.v. infusions were necessary at least up to the 4th day of life. For enteral feeding a human milk (HM) formula (fresh preterm HM enriched by 6 g freeze-dried HM per 100 ml) was given using a nasogastric bolus injection technique (Table II).

Clinical data are presented in Table I.

On the 10th, 21st and 42nd days of life 24-hour urine samples were collected. On the same days blood samples were drawn 60 minutes postprandially and aliquots of the HM-formula were separated. All samples were frozen (- 20°C) and stored until analysis.

In the urine total nitrogen (Kjeldahl-method), alpha-amino-nitrogen (ninhydrin-reaction), urea (urease-reaction), ammonium (according to Berthelot) /29/, and pH-values were measured.

In the serum urea (urease-reaction), alpha-amino-nitrogen (ninhydrin-reaction), were estimated. On the 42nd day of life plasma amino acids were assayed on a LKB 4151 Alpha-plus analyzer /7/. The acid-base status was estimated in capillary blood by the Astrup-method.

Nutrient intakes were calculated on the basis of daily

TABLE I

Data (M ± SD, range) of the studied small for gestational age (SGA) and appropriate for gestational age (AGA) very low birth weight infants

	SGA-infants	AGA-infants
Gestational age (weeks)	32.1 (30 - 37)	30.6 (29 - 32)
Weight at birth (g)	1251 (1020 - 1495)	1286 (980 - 1480)
n	12	14

measurement of feeding volume and the composition of the HM-formula as described previously /9/.

To evaluate the degree of IUGR the difference between weight at birth and the 10th percentile according to Lubchenco /24/ were calculated and expressed as g/kg /5/.

For statistical analysis Student's t-test and linear regression analysis (ABSTAT-System) were performed.

RESULTS

The protein, as well as the caloric intakes did not differ either between AGA- and SGA-infants or between the study days (Table II).

In both study groups the urea excretion in the urine was lowest on the 10th day of life but were raising significantly more in AGA- than in SGA-infants. Thus, on the 21st and the 42nd days of life the excretion was significantly lower in SGA- than in AGA-infants (Fig. 1).

TABLE II

Mean (M ± SD) daily feeding volumes and the calculated protein as well as caloric intakes in the studied small for gestational age (SGA) and appropriate for gestational age (AGA) very-low-birth-weight infants on the three study days

	Feeding volume (ml/kg)		Protein intake (g/kg)		Caloric intake (kcal/kg)	
Day	SGA	AGA	SGA	AGA	SGA	AGA
10	178 ± 29	181 ± 32	2.93 ± 0.35	2.94 ± 0.43	129 ± 19	121 ± 16
21	201 ± 32	186 ± 30	3.09 ± 0.41	2.98 ± 0.38	131 ± 19	119 ± 21
42	195 ± 33	194 ± 41	3.12 ± 0.41	3.13 ± 0.43	124 ± 15	128 ± 18

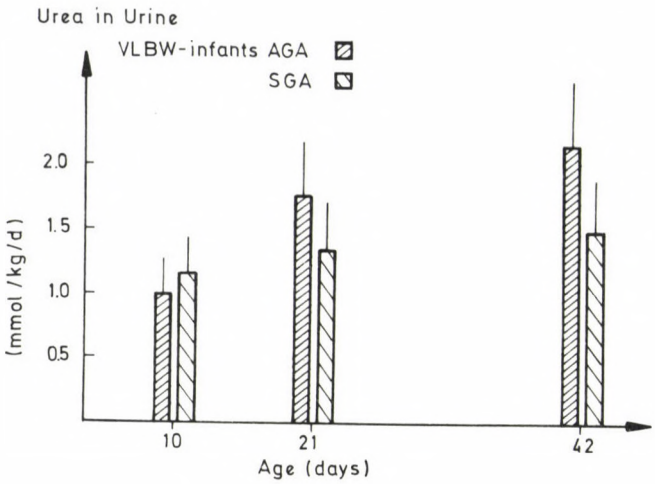

Fig. 1. Daily (M ± SD) excretion of urea in urine of very-low-birth-weight infants small for gestational age (SGA) (n = 12) or appropriate for gestational age (AGA) (n = 14) on the three study days.

In AGA-infants the renal excretion of ammonia decreased significantly from the 10th to the 42nd day of life ($r = -0.871$; $p < 0.001$). In SGA-infants the excretion of ammonia was significantly higher than in AGA-infants on all study days and the differences became more pronounced with postnatal age (Fig. 2).

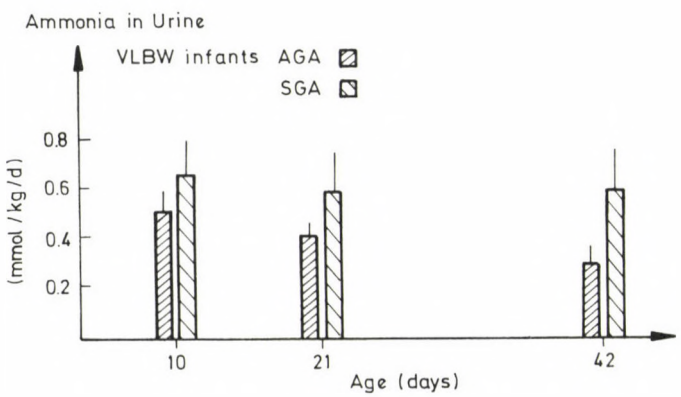

Fig. 2. Daily (M ± SD) excretion of ammonia in the same infants as in Fig. 1.

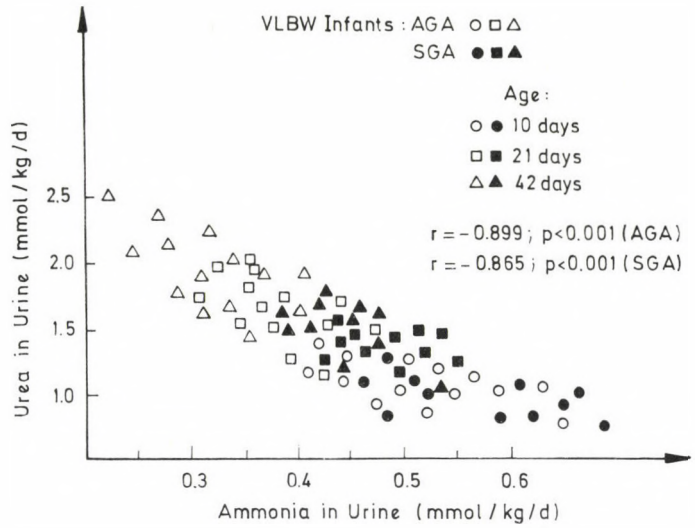

Fig. 3. Relationship between the excretion of urea and of ammonia in all studied infants.

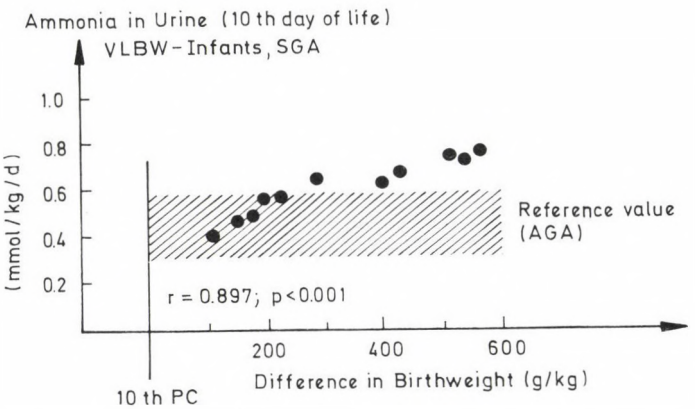

Fig. 4. Relationship between the excretion of ammonia and the degree of intrauterine growth retardation, expressed as difference between weight at birth and the 10th percentile according to Lubchenco in g/kg birthweight on the 10th day of life.

Also a significant correlation between the serum urea concentration and the urea concentration in urine could be found in SGA- as well as AGA-infants (SGA: $r = 0.796$, $p < 0.001$; AGA: $r = 0.874$, $p < 0.001$).

Despite similar nutrient intakes (Table II) the renal excretion of total nitrogen and alpha-amino-nitrogen was significantly higher in SGA-than in AGA-infants (Table III).

There were no differences of parameters of the acid base balance in blood as well as of the pH values in urine between the study groups (Table IV).

Serum concentrations of urea tended to be higher in SGA than in AGA-infants but significant differences could be found only on the 42nd day of life. In contrast, the concentrations of alpha-amino-nitrogen were significantly higher in SGA-infants if compared to AGA-infants on all study days (Table V).

The plasma concentrations of amino acids connected with the ornithine urea cycle were significantly higher in SGA- than in AGA-infants on the 42nd day of life (Fig.5).

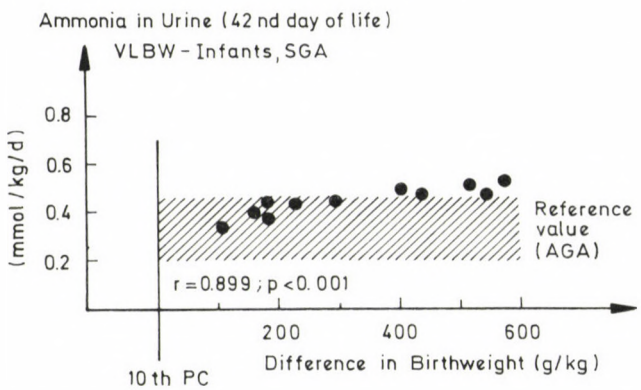

Fig. 5. Mean (M $\pm$ SD) plasma concentrations of amino acids involved in the ornithine-urea cycle in the same infants shown in Figure 1 on the 42nd day of life (GLX = plasma glutamine and glutamate concentrations are combined and reported as one-half the total).

On the 10th day of life the renal excretion of ammonia was significantly correlated with the severity of intrauterine growth retardation, expressed as difference of weight at birth

TABLE III

Mean (M ± SD) daily excretion of total and alpha-amino-nitrogen in the urine of the studied small for gestational age (SGA) and appropriate for gestational age (AGA) very-low-birth-weight infants on the three study days

	Total nitrogen (mmol/kg)			Alpha-amino-nitrogen (mmol/kg)		
Day	SGA	AGA	p	SGA	AGA	p
10	8.1 ± 0.6	7.7 ± 0.8	n.s.	0.96 ± 0.12	0.43 ± 0.09	< 0.01
21	7.9 ± 0.9	6.8 ± 0.8	< 0.05	0.82 ± 0.14	0.38 ± 0.08	< 0.01
42	7.2 ± 0.8	6.1 ± 0.5	< 0.01	0.71 ± 0.13	0.29 ± 0.09	< 0.01

TABLE IV

Mean (M ± SD) parameters of acid base balance in the blood and urine of the studied small for gestational age (SGA) and appropriate for gestational age (AGA) very-low-birth-weight infants on the three study days

Day	Capillary blood pH values		Base excess (mmol/l)		Urine pH values	
	SGA	AGA	SGA	AGA	SGA	AGA
10	7.36 ± 0.05	7.39 ± 0.06	-2.3 ± 1.5	-2.5 ± 1.3	6.42 ± 0.6	6.15 ± 0.5
21	7.38 ± 0.07	7.41 ± 0.07	-2.1 ± 1.6	-1.5 ± 1.4	6.39 ± 0.3	6.28 ± 0.4
42	7.38 ± 0.06	7.42 ± 0.09	-0.9 ± 1.6	0.4 ± 1.4	6.31 ± 0.4	6.34 ± 0.6

TABLE V

Mean (M ± SD) serum concentrations of urea and alpha-amino-nitrogen in the studied small for gestational age (SGA) and appropriate for gestational age (AGA) infants of very-low-birth-weight infants on the three study days

Day	Urea (mmol/l)			Alpha-amino-nitrogen (mmol/l)		
	SGA	AGA	p	SGA	AGA	p
10	2.23 ± 0.7	2.54 ± 0.6	n.s.	2.89 ± 0.39	2.16 ± 0.31	< 0.01
21	2.14 ± 0.6	2.68 ± 0.7	n.s.	2.74 ± 0.36	2.09 ± 0.24	< 0.01
42	2.19 ± 0.7	2.79 ± 0.6	< 0.05	2.63 ± 0.31	2.11 ± 0.25	< 0.01

to the 10th percentile of Lubchenco /24/ (Fig. 4). The urea excretion was also significantly correlated with the degree of IUGR ($r = -0.796$, $p < 0.001$). The excretion of urea as well as of ammonia was outside the reference values of the AGA-infants when the difference between weight at birth and the 10th percentile was more than 200 g/kg.

On the 42nd day of life there was still a dependence of the degree of IUGR on the excretion of ammonia ($r = 0.899$, $p < 0.001$) as well as on the excretion of urea ($r = -0.609$, $p < 0.05$).

The weight gain was not significantly different in both study groups (SGA: 17.6 $\pm$ 3.1 g/kg/day; AGA: 19.3 $\pm$ 2.9 g/kg/day, respectively (Fig.3).

DISCUSSION

The renal excretion of urea increases in AGA-infants of VLBW more rapidly than in SGA-infants from the 10th day up to the 42nd day of life. In the same time the excretion of ammonia decreases significantly in the AGA-infants whereas in SGA-infants the excretion of ammonia remains on the level of the 10th day of life during the observation period.

The renal excretion of urea depends on multiple factors: serum urea concentration, glomerular filtration rate, renal tubular urearecycling, utilization of ingested protein for growth and the capacity of the urea synthesizing system of the liver.

The weight gain was adequate for the given protein and energy intakes and reached intrauterine values /19/. The elevated serum concentrations and the higher renal excretion of alpha-amino-nitrogen in SGA- than in AGA-infants indicate that protein synthesis was not limited by the supply of amino acids in SGA-infants. Thus, the protein synthesis rate can be assumed to be nearly optimal in both groups and the higher excretion of ammonia and the lower excretion of urea in the SGA-infants are not simply explained by different protein synthesis rates

between SGA- and AGA-infants. During the first days of life the functional glomerular-tubular inbalance of the kidney function may influence the urea excretion in urine /8/. In the present study there was a similar correlation between urea concentration in serum and urine in both study groups so that different functional capacities between SGA- and AGA-infants can be excluded as reason for the observed lower urea excretion in the SGA-infants.

No significant differences could be found in the acid-base-status as well as in the urinary pH-values (Table IV). Thus, differences of the excretion of ammonia in the urine cannot be explained as renal response to metabolic acidosis.

The elevated concentrations of amino acids involved in the ornithine-urea cycle in SGA-infants exclude a lack of substrates as reason for the lower urea synthesis rate in these infants if compared to AGA-infants.

Since the first descriptions of infants SGA it was realized that IUGR results in an inhomogeneous somatic, as well as functional reduction of different organs and especially to those of the liver /11, 17, 25/.

Metabolic studies in SGA-infants have concerned with glucose homeostasis /14, 15, 21/, metabolism of fatty acids and triglycerides /13, 15, 29/ as well as protein metabolism /5, 6, 12/. The results of these and experimental studies /22, 23, 26/ indicate that different hepatocellular functions are affected by IUGR /10/.

Thus, from the presented data it can be speculated that the urea synthesis of the liver is limited in SGA-infants if compared to AGA-infants of similar birthweight.

As seen in studies concerning the metabolism of bile acids or the nitrogen metabolism the differences between SGA- and AGA-infants become more pronounced with increasing severity of the IUGR /5/.

The underlying reasons for our findings in the SGA-infants cannot be explained by the present data, however, many factors could be involved. The metabolic situation found in the SGA-infants seems to be caused by the state of intrauterine malnutrition /12, 22/ which is more or less reversible if the

IUGR is of moderate degree /6/.

The present data suggest that metabolic investigations in VLBW-infants have to consider the metabolic differences between SGA- and AGA-infants especially when serum urea or urea as end product in 15-N-tracer studies /30/ are used for evaluation of protein or nitrogen metabolism.

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Acta Paediatrica Hungarica 31 (1), pp 47-52 (1991)

TRANSIENT HYPERINSULINISM IN ASPHYXIATED NEWBORN INFANTS

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Received 28 April 1989

Hypoglycemia in birth asphyxiated infants is attributed to glycogen depletion. We observed three term AGA (Appropriate for Gestational Age) infants with birth asphyxia, who developed hyperinsulinemic hypoglycemia postnatally. All had inappropriately high serum insulin concentrations for their blood glucose levels, and needed glucose infusion rates of > 8 mg/kg/min for several days to maintain normoglycemia. All infants recovered spontaneously.

INTRODUCTION

Hypoglycemia in birth asphyxiated infants is commonly due to reduced glycogen stores /1/. In addition to premature depletion of carbohydrate reserves the negative effect of asphyxia on glycogenolytic and gluconeogenic reactions may also contribute to a larger postnatal fall in blood glucose /2/. Recently we have observed three term AGA infants, who suffered birth asphyxia and developed severe symptomatic hypoglycemia in early postnatal life. All had inappropriately high serum insulin concentrations for their blood glucose levels, and needed glucose infusion rates more than 8 mg/kg/min for several days to maintain normoglycemia. All infants recovered spontaneously.

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CASE REPORTS

Case 1. A male infant was born after an uneventful pregnancy by normal delivery. Birthweight 2950 g, gestational age 39 weeks. The umbilical cord was twisted tightly around the neck at birth, the Apgar score was 6 at 1 minute and 7 at 5 minutes. The baby required mask and bag ventillation in the delivery room. At six hours of age he was transferred to the NICU because of cyanosis, grunting and generalized muscle hypotony. The chest X-ray revealed cardiomegaly. The blood glucose was 0.4 mmol/l on admission, and 10 percent glucose infusion was started. At 1 day of age he needed iv. glucose infusion at a rate of 16 mg/kg/min to control hypoglycemia. Hyperinsulinism was subsequently confirmed. The glucose infusion rate was gradually decreased, and after 5 days of treatment the infusion of glucose was stopped, normal blood glucose levels were maintained with oral feeding only.

Case 2. This girl was born at term by normal delivery and weighed 3370 gms. She was blue and gasping at birth and the umbilical cord was around the neck. The Apgar score was 5 at 1 minute and 7 at 5 minutes. At 4 hours of age blood glucose was 0.8 mmol/l and she had apnea and generalized convulsions. 10 percent glucose infusion was started. Hypoglycemia continued to be a major problem, and she needed iv. glucose at a rate of 13 mg/kg/min to prevent hypoglycemia for 5 days. Hyperinsulinism was confirmed, and she tolerated oral feeding only after 10 days of treatment.

Case 3. This boy was born at term, with a gestational age of 40 weeks and a birthweight 3800 gms. Labour was induced, because of maternal toxemia. The Apgar score was 3 at 1 minute, and 6 at 5 minutes. The baby required intubation and suction of the trachea after birth because of meconium aspiration. At 25 hours of age he developed cyanosis and convulsions and the blood glucose was 1.5 mmol/l. After one bolus dose of glucose, he was put on a 10 percent glucose infusion at a rate of 9 mg/kg/min. This infusion rate was gradually decreased. At 1 week of age he was off all treatment, with no symptoms or signs of hyperinsulinism.

RESULTS AND DISCUSSION

The clinical and laboratory data of the three newborn infants are summarized in the Table. Hypoglycemia developed between the 4th and 25th hours of postnatal age. Hyperinsulinism was subsequently confirmed in all three cases on the basis of the following criteria:

TABLE I

Clinical and laboratory data of 3 hyperinsulinemic newborn infants

N°	Age at onset of hypoglycemia (h)	Symptoms	Simultaneous concentrations		Maximum glucoe infusion rate to maintain normoglycemia (mg/kg/min)	I.v. glucose tolerance K_G	Age when 6h fast tolerated off treatment (days)
			glucose (mmol/l)	insulin (mU/l)			
1	10	cyanosis hypotony	0.7	17	16	4.1	21
2	4	apnea convulsions	0.8	45	13	3.8	10
3	25	cyanosis convulsions	1.5	101	9	3.7	7

a/ glucose infusion rate > 8 mg/kg/min to maintain normoglycemia

b/ iv. glucose tolerance test K_G value > 2

c/ inappropriately high serum insulin concentrations for their blood glucose levels.

The relationship between blood glucose and plasma insulin concentrations is shown in Figure 1.

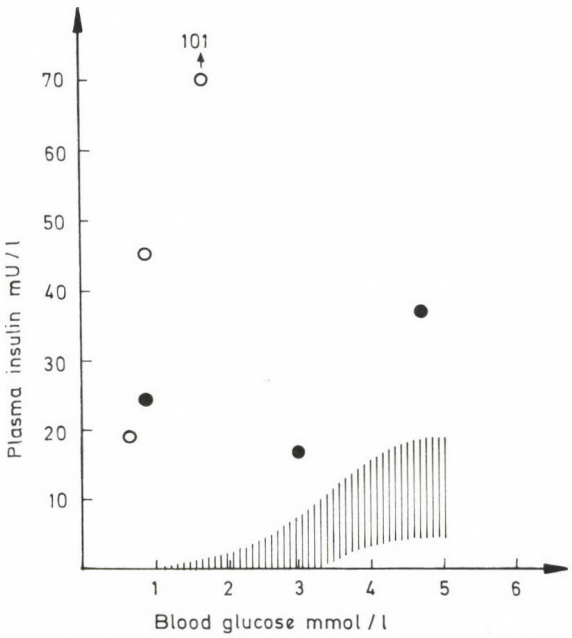

Fig. 1. Relationship between blood glucose and plasma insulin concentrations (The shaded area represents normal controls from our laboratory)
o before treatment
● during treatment
(Plasma insulin was measured by a radio-immunoassay method)

Hyperinsulinism was transient, it lasted for 7-21 days in our cases, and all infants recovered spontaneously. Hypoglycemia was controlled by iv. glucose infusions, none of the newborns required diazoxide treatment.

The association of birth asphyxia and hyperinsulinism in newborn infants was first recognized by Collins and Leonard /3/. The pathomechanism of hyperinsulinism is not known. Although a bolus dose of glucose can produce insulin release, only one of our patients received such a bolus at birth, it is however, unlikely to induce prolonged hyperinsulinism. Mobilization of liver glycogen and a large increase in blood glucose concentration in association with oxygen lack have been demonstrated in the human newborn /4/. An increased release of cathecolamines associated with intrauterine asphyxia produces a rise in blood glucose by inhibiting insulin release and stimulating glucagon release and hence glycogenolysis /5/. Increase of blood glucose, lactate and base deficit occur in shocked patients too, but this initial phase of hyperglycemia and hypoinsulinemia is followed within 2-3 hours by a hypersecretion of insulin, which may last for weeks /6/. This may occur in birth-asphyxiated infants, who are often hypotensive /i.e. shocked/. Their blood glucose concentration may be high initially, and be unrecognized until rebound hypoglycemia causes clinical symptoms.

In addition to glycogen depletion and low gluconeogenic rate, functional hyperinsulinism may be an additional mechanism responsible for hypoglycemia in birth-asphyxiated infants. Early recognition and vigorous treatment are required to prevent neurological damage.

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Acta Paediatrica Hungarica 31(1), pp 53-65 (1991)

NEONATAL EFFECTS OF METHYLDOPA THERAPY IN PREGNANCY HYPERTENSION[x]

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Received 23 Oct 1989

This study has been performed to assess the effect of methyldopa (MD) therapy in pregnancy hypertension on the neonatal adaptation. Infants born to mothers on MD for several weeks prior to delivery and presenting with excessive tremor and irritability were evaluated according to the dose of maternal MD. Pregnancy hypertension and high dose MD was associated with impaired placental perfusion, compromised function of fetoplacental unit and more frequent surgical delivery. Infants of mothers on high (1.25-2.0 g/day) or low (less than 1 g/day) MD had gestational age, head circumference, acid-base balance, Apgar score and blood pressure similar to those born to healthy control mothers. The birth weight of infants of the high MD group, however, were significantly lower than in the low-dose or control groups. MD therapy resulted in a dose-dependent increase in plasma levels of prolactin, thyrotropin and triiodthyronine indicating decreased dopaminergic inhibition of pituitary hormone release. Plasma thyroxine concentration, however, decreased significantly. Cerebrospinal fluid noradrenaline was found to be markedly depressed after maternal MD showing disturbed central nervous system monoamine metabolism. It is suggested that MD administration to mothers presenting with pregnancy hypertension interferes with cerebral monoamine metabolism of the neonate and induces alterations in some endocrine functions under dopaminergic control. The possible role of chronic fetal distress frequently associated with pregnancy hypertension should also be considered.

INTRODUCTION

Methyldopa (MD) has been widely used for the control of hypertension in pregnancy and it has been shown to improve

[x]Presented in part at the 1st Congress of the European Society for Developmental Pharmacology, Les Diablerets, Switzerland, 1988

Akadémiai Kiadó, Budapest

perinatal outcome without short-term or long-term major adverse effects on the infant and child /7,18,19,22,23/.

However, systolic blood pressure of full-term newborn infants of the treated mothers was lower during the first two days after delivery and boys born to mothers taking MD from 16-20 weeks of gestation had smaller head circumference at birth and when aged between 4 and 7 1/2 years /7,17,22/.

Boutroy et al recently observed episodes of arterial hypotension, bradycardia, disturbances of respiratory adaptation, decreased urine flow rate and functional ileus in newborn infants exposed to maternal MD /5/.

In a preliminary study we reported on 3 infants presenting with excessive tremor and irritability whose mothers were given MD for pregnancy hypertension in a dose of 1.5-2.0 g/day for 8-10 weeks before delivery. These neurological symptoms were associated with markedly depressed noradrenaline levels in the cerebrospinal fluid without clinical and laboratory evidences of perinatal asphyxia, infection, electrolyte and metabolic disturbances. They were assumed, therefore, to represent the neonatal equivalent of iatrogenic parkinsonism probably due to the reduction of dopaminergic tone in the central nervous system /3/.

On the basis of these observations we extended our clinical investigations to explore in more details the influences of MD prescribed during pregnancy on neonatal adaptation, in particular on cerebrospinal fluid noradrenaline levels and on pituitary release of hormones under dopaminergic control (prolactin, thyrotropin).

PATIENTS AND METHODS

Three groups of newborn infants were enrolled in the study. Infants of group I and II were born to mothers on MD for pregnancy hypertension and presented with excessive tremor and irritability lasting for several days, therefore, as a part of routine clinical evaluation lumbar puncture was performed.

Pregnancy hypertension was defined as systolic and diastolic pressure repeatedly higher than 140 and 90 mmHg, respectively,

under strictly controlled conditions. Patients whose blood pressure could not be controlled with MD alone other drugs such as beta-adrenergic blockers, diuretics and hydralazine were given, but these patients were excluded from the study. Infants of the trial were allocated to group I or II according to the daily dose of maternal MD needed to achieve good blood pressure, i.e. maintain systolic and diastolic pressure below 140 and 90 mmHg, respectively.

Group I consisted of 10 newborn infants of mothers treated with methyldopa (Dopegyt, EGYT, Budapest) in a dose of up to 1 g/day (mean daily dose: 0.7 g, range: 0.5-1.0 g) for a period of 8.7 weeks (range: 6-12 weeks) prior to delivery.

Group II included 15 newborn infants whose mothers were given MD in a higher dose (mean: 1.83 g/day, range: 1.25-2.0 g/day) for a period of 8.9 weeks (range. 5-14 weeks).

Group III comprised 20 neonates born to healthy normotensive mothers without any drug therapy during their pregnancy. The indication for lumbar puncture in this group was suspected perinatal infection which was excluded later on.

All infants were carefully monitored and checked for perinatal asphyxia, infection, hypocalcemia, hypomagnesemia, hypoglycemia, and hypo/hypernatremia but no pathological alterations could be detected and the therapeutical trial with glucose, calcium, magnesium and pyridoxine proved to be unsuccessful.

Lumbar punctures were performed and blood samples were taken at 8-10 a.m. for hormone measurements (prolactin PRL, thyrotropin TSH, thyroxine T_4, triiodthyronine T_3) at the 12-76 hours of postnatal age (mean: 38.2 hours).

Patients with pregnancy hypertension were admitted to the department where placental functions and fetal well-being were regularly assessed as follows:

Serum beta$_1$-glycoprotein (SP_1), human placental lactogen (HPL) and urinary excretion of estriol were measured twice a week, fetal ultrasonography and non-stress test were performed weekly, fetal movements were counted twice a day and after the 38th week of gestation regular amnioscopy was carried out. Placental perfusion parameters were also measured before and about one week after MD therapy when adequate blood pressure control could be achieved using the method described by Lunnell et al /15/ and modified by Bódis et al /4/.

Pregnant women of the three groups did not differ significantly in age, parity and weight gain during their pregnancy. 1 mother in group I and 4 in group II had proteinuria of greater than 1 g/day.

In addition to routine laboratory procedures, neonatal cerebrospinal fluid noradrenaline was determined by spectrofluorimetry according to Hahn /12/, and radioimmunoassays were applied for TSH /16/, PRL /1/, and also for T_4 and T_3 determination using commercial kits. The inter - and intraassay coefficient of variation was less than 10 % for each hormone.

Results are expressed as means $\pm$ SDM, statistical evaluations were done by using Student's t-test and X^2-test.

Approval of the institutional ethical comittee and informed parental consent were obtained for the study.

RESULTS

Placental perfusion parameters, the results of biochemical tests assessing the condition of foetoplacental unit and the mode of delivery in the three groups are shown in Table I and II.

It can be seen that pregnancy hypertension resulted in impaired placental perfusion as indicated by the significantly longer vascular and intervillous phase, the significantly greater intervillous perfusion index and uteroplacental vascular resistance and by the significant fall of blood flow index. In response to MD therapy the control of blood pressure was associated with a significant improvement of time parameters of placental perfusion and a significant decrease of uteroplacental vascular resistance. However, intervillous perfusion index remained unaltered and there was only a moderate, insignificant increase in blood flow index. (Table I).

As shown in Table II biochemical tests of the function of foetoplacental unit yielded pathological results more freqently in the high-dose MD group than in those mothers treated with low-dose MD.

Clinical and laboratory data of newborn infants of mothers with or without MD treatment are summarized in Table III. There were no significant differences in gestational age, head circumference, Apgar score and acid-base parameters of the neonates born to treated or untreated mothers. High-dose MD, however, was found to be associated with a significantly lower birth weight which may be accounted for the more severe maternal hypertension and the subsequent impaired placental perfusion independent of MD administration. Similarly, fetal systolic blood pressure appeared to be uninfluenced by MD therapy.

Table IV demonstrates neonatal hormone parameters on the first day of life after maternal MD. In response to MD therapy a dose-dependent increase occurred in the plasma levels of PRL, TSH, and T_3 indicating decreased dopaminergic inhibition of pituitary hormone release. Interestingly, plasma T_4

TABLE I

Placental perfusion parameters in patients with pregnancy hypertension before and after Methyldopa administration (mean ± SDM)

		T_{max} sec	T_V sec	T_i sec	IPI %	BFI	UVR	Blood pressure mmHg systolic	diastolic
Control (n=20)		65.5 ±12.5	44.1 ±8.8	21.4 ±8.6	31.6 ±10.7	22.0 ±16.0	5.36 ±2.8	118.0 ±3.2	82.1 ±2.2
	before Methyldopa	207.0^{xx} ±44.0	76.8^{x} ±18.0	130.0^{xx} ±33.0	60.0^{x} ±15.2	6.0^{xx} ±4.8	26.3^{x} ±8.4	158.5^{xx} ±7.5	96.0^{xx} ±4.4
	after Methyldopa	132.6^{x} ±76.0	±40.5 18.0	92.2^{x} ±86.0	64.2^{x} ±12.8	9.2^{xx} ±5.4	14.3^{x} ±6.1	132.0^{x} ±5.6	88.0^{x} ±3.6

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

Asterisks indicate significant differences from the control value

T_{max} = time of maximum activity
T_V = time of vascular phase
T_i = time of intervillous phase
IPI = intervillous perfusion index
BFI = blood flow index
UVR = uteroplacental vascular resistance

TABLE II

Results of intensive monitoring of the function of fetoplacental unit and the mode of delivery in healthy and hypertensive pregnant women on Methyldopa therapy

		Methyldopa g/day 1.25-2.0	Methyldopa g/day <1.0	Control
Number of patients		15	10	20
Serum SP_1	pathological	7	4	3
	normal	8	6	17
Serum HPL	pathological	8^{xx}	2	2
	normal	7	8	18
Urinary estriol	pathological	8^{xx}	2	3
	normal	7	8	17
Non stress test	pathological	5^{xx}	1	1
	normal	10	9	19
Aminioscopy	pathological	5^{xx}	1	0
	normal	10	9	20
Delivery	vaginal	9	7	19
	forceps	1	1	0
	caesarean sect	5	2	1

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

Asterisks indicate significant differences from the control group

TABLE III

Clinical and laboratory data of the newborn infants born after maternal Methyldopa (mean ± SDM)

Methyldopa g/day	Gest. age weeks	Birth weight g	Head circ. cm	Acid-base balance			Apgar score		Blood pressure mmHg	
				pH	BE mEq/l	pCO_2 mmHg	1 min	5 min		
1.25 - 2.0 n = 15	38.1 ±1.6	2528^{xx} ±438	33.6 ±1.6	7.32 ±0.08	-4.8 ±3.0	38.8 ±7.9	9.0 ±0.2	9.5 ±0.3	78	±8.5
1.0 n = 10	38.6 ±1.7	2910 ±294	34.2 ±1.4	7.34 ±0.10	-5.2 ±2.8	36.4 ±6.8	9.2 ±0.5	9.9 ±0.2	81	±9.2
control n = 20	38.7 ±1.4	3055 ±254	34.1 ±1.0	7.29 ±0.09	-6.9 ±3.1	40.6 ±9.2	8.7 ±0.6	9.8 ±0.3	83	±10.1

$^{x}p < 0.05$ Asterisks indicate significant differences from the control values

$^{xx}p < 0.01$

TABLE IV

Neonatal hormone parameters on the 1st day of life after maternal Methyldopa (mean ± SDM)

Methyldopa g/day	PRL	TSH	T_4 nM/l	T_3 nM/l	CSF-NA ng/ml
1.25 - 2.0 n = 15	2410^{x} ±507	-16.0^{xx} ±5.2	84.1^{xx} ±35.1	2.76^{x} ±0.81	0.91^{xx} ±0.18
1.0 n = 10	2011 ±384	11.1 ±4.8	121.4 ±46.4	1.68 ±1.0	1.81 ±0.68
control n = 10	1884 ±404	9.6 ±3.1	149.8 ±38.1	1.29 ±0.12	2.35 ±0.94

$^{x}p < 0.05$ Asterisks indicate significant
$^{xx}p < 0.01$ differences from the control values

concentration decreased significantly. The reason for this decrease is not apparent, one can speculate, however, that the enhanced peripheral conversion of T_4 to T_3 induced by MD may play a role.

As in our preliminary study cerebrospinal fluid noradrenaline was found to be markedly depressed after maternal MD providing strong evidence that in this group of selected patients the central nervous system monoamine metabolism is disturbed.

DISCUSSION

The antihypertensive effect of MD has been claimed to be accounted for by its ability to deplete endogenous catecholamines and to induce synthesis of false neurotransmitters in the central nervous system. In support of this notion MD administration has been found to reduce hypothalamic noradrenaline, adrenaline and dopamine and to increase its metabolic product - methyl-noradrenaline which can be stored and released as false neurotransmitter in place of noradrenaline /26/.

Clinical and pharmacological studies of the placental transfer of MD to human fetus and neonates have revealed similar free and conjugated MD concentrations in maternal and umbilical cord plasma /13/. Moreover, its elimination from the newborn plasma was found to be markedly prolonged with a halflife of about 14 hours as opposed to the value of less than 2 hours in healthy adults /5/. Interestingly, the conjugated and total MD concentration appeared to be higher in the amniotic fluid than in the corresponding plasma providing evidence that:
1.) the drug is mainly eliminated by the fetal kidney and
2.) the fetus is exposed to an environment containing MD in a relatively high concentration /13/.

In agreement with these observations the results of the present study indicate that MD therapy for pregnancy-induced hypertension may have serious influences on neonatal adaptation by interfering with fetal-neonatal central monoamine metabolism and inducing alterations in some endocrine functions under dopaminergic control.

The most striking clinical findings in this study were that infants born after maternal MD exhibit excessive tremor and irritability unrelated to the well-known clinical and biochemical disturbances of neonatal adaptation and unresponsive to therapeutic measures commonly applied. We assume a causal relationship of MD administration to the observed neurological symptoms and depletion of central

catecholamines. In support of this assumption we found significantly depressed cerebrospinal fluid noradrenaline in infants of treated mothers and rapid improvement in clinical symptoms of hyperactivity and irritability after atropine therapy /3/.

This latter finding can be interpreted to indicate that the reduction in adrenergic tone in favour of cholinergic tone may contribute substantially to the development of these neurological symptoms.

In addition to this interpretation, an alternate mechanism for the observed changes in monoamine metabolism and clinical symptoms should also be considered. Chisholm et al reported strong correlation between newborn irritability and second trimester mean arterial pressure without specific antihypertensive drug therapy /6/.

It seems therefore, that newborn irritability is more likely the result of impaired placental perfusion and chronic fetal distress frequently associated with hypertension than that of antihypertensive drug therapy.

In an attempt to explore whether the observed clinical symptoms and biochemical alterations are the result of the maternal MD therapy or they are due to pregnancy hypertension independent of drug administration pregnant rats were given MD in a dose of 14 mg/kg/day from the 10th day of their pregnancy until delivery.

MD administration during pregnancy resulted in a significant decrease of noradrenaline and dopamine but not of serotonin in brain tissue homogenate on day 5, but such alterations could not be seen on day 21 (Bódis, J. unpublished observation).

In this regard it is of interest that exposure to moderate hypoxia induced significant reduction in central nervous system monoamine biosynthesis as reflected by the decreases in brain monoamine levels /14,20/ and by the delayed and long-lasting decrease in brain dopamine when hypocarbic hypoxia was applied /21/. Furthermore, we have recently demonstrated decreased cerebrospinal fluid noradrenaline in premature infants recovering from perinatal asphyxia /2/.

Data presented in this study on plasma levels of PRL and TSH provide indirect evidence that the pituitary release of these hormones is under dopaminergic control and MD administration to the mothers during late pregnancy relieves dopaminergic inhibition and results in elevated plasma PRL and TSH in the neonate. It is of concern, however, that when metoclopramide, a dopamine receptor antagonist, was given to mothers in term labor no significant influence on cord PRL and TSH could be observed /24,25/.

The clinical significance of elevated hormone levels in the immediate neonatal period is not completely understood, PRL, however, has been shown to play a role in respiratory adaptation /11/, in the control of neonatal tissue hydration /8/ and in the regulation of renal handling of water and electrolytes /9,10/.

The increase in plasma TSH levels appears to have less clinical significance. When neonatal TSH screening program is performed, however, the possible effects of maternal MD therapy should be carefully evaluated.

In conclusion, MD administration to mothers presenting with pregnancy hypertension seems to interfere with central monoamine metabolism and to induce alterations in neuroendocrine functions of the neonates. Further studies are to be conducted to explore whether the observed clinical symptoms and biochemical changes are the result of maternal MD therapy or they are at least in part due to the associated chronic fetal distress.

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Acta Paediatrica Hungarica 31 (1), pp 67-73 (1991)

EUTHYROID SICK SYNDROME IN TYPE I DIABETES MELLITUS IN CHILDREN AND ADOLESCENTS*

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We studied concentrations of thyroid hormones (T_3, T_4, FT_4, rT_3, TBG and TSH) in 62 type I diabetic children and adolescents. The patients were classified into group A (n = 27, good control, HbA_{1c}<10 %), group B (n = 19, poor control, HbA_{1c}>10 %) and group C (n = 16, diabetic ketoacidosis, pH <7.1 and HCO_3 <15 mmol/L. All patients were treated with two daily injections of purified monocomponent insulins. Thirty healthy subjects of the same age served as control group. Patients in group B and C had significantly lower T_3 and higher rT_3 levels ($p < 0.001$) compared to the matched controls (1.5 vs 2.2; 0.9 vs 2.2; 0.58 vs 0.3 and 0.6 vs 0.3 nmol/L). Serum TBG levels were significantly lower ($p < 0.01$) in the group A (19.5 $\pm$ 4.3 mg/L), group B (20.3 $\pm$ 3.3) and group C (18.0 $\pm$ 3.4) compared with control group (24.2 $\pm$ 3.1). There was significantly negative correlation between T_3 and HbA_{1c} in group B ($r = 0.546$; $p < 0.02$). The results of this study confirm that euthyroid sick syndrome does exist in type I diabetic children and adolescents with poor metabolic control and ketoacidosis. The inverse relationship between T_3 and HbA_{1c} percentage (low T_3 and high HbA_{1c}) points to the poor diabetic control.

INTRODUCTION

The biochemical changes in circulating thyroid hormones, described as euthyroid sick syndrome /5/ have been observed in uncontrolled type I diabetic children and adolescents /2,3,4/.

It is well recognised that deficiency of insulin, disordered glucose metabolism and ketoacidosis are accompanied by

*This paper was presented in part at the 19th International Congress of Pediatrics, Paris, July 1989

Akadémiai Kiadó, Budapest

alternation in the peripheral metabolism of thyroid hormones /1,3,7/. Pittman et al /9/ have suggested that T_3 production from peripheral T_4 monodeiodination is impaired in uncontrolled diabetic patients.

In the present study we investigated circulating thyroid hormone concentrations, thyrotropin (TSH) and thyroxine binding globulin (TBG) in various stages type I diabetes in children and adolescents.

PATIENTS AND METHODS

Sixty-two type I diabetic children and adolescents were studied. The patients were classified into three groups according to the results of diabetic control. The diabetic patients were treated by two daily injections of purified monocomponent insulins (Actrapid MC and Monotard MC, Novo Industri, Copenhagen).

Group A comprised 27 patients (14 females and 13 males; 12 prepubertals and 15 pubertals) aged 4.5 to 16 years (mean 11.5 years), body weight from 15 to 49 kg (mean weight 28.3 $\pm$ 6.2), body height from 108 to 158 cm (mean height 130.2 $\pm$ 12.0). The glycosylated haemoglobin (HbA_{1c}) amounted from 5.5 to 10 % (mean 8.3 %).

Group B comprised 19 patients (9 females and 10 males; 7 prepubertals and 12 pubertals) aged 7 to 16 years (mean 12.7 years), body weight from 20 to 50 kg (mean weight 30.2 $\pm$ 6.8 kg), body height from 122 to 155 cm (mean height 133.1 $\pm$ 11.5 cm). The HbA_{1c} amounted from 11.1 to 19.3 % (mean 14.7 %).

Group C consisted of 16 diabetic ketoacidosis patients (10 females and 6 males; 6 prepubertals and 10 pubertals) aged 5.5 to 16 years (mean 12.6 years), body weight from 16 to 45 kg (mean weight 26.2 $\pm$ 4.4 kg), body height from 115 to 152 cm (mean height 131.2 $\pm$ 10.2 cm). The arterial pH values amounted from 6.9 to 7.3 (mean 7.1) and serum bicarbonate levels from 2.2 to 16 mmol/L (mean 9.8 mmol/L). The control group consisted of 30 normal children and adolescents (16 females and 15 males; 13 prepubertals and 17 pubertals) aged between 6 to 14 years (mean 10.1 years), body weight from 14 to 55 kg (mean 33.3 $\pm$ 15.0 kg), body height from 105 to 165 cm (mean 135.0 $\pm$ 15.0 cm).

All the subjects examined were clinically euthyroid. After an overnight fast, cannulation of an antecubital vein blood was taken for hormone estimations. The thyroid hormones were measured by specific radioimmunoassay. Total serum triiodothyronine (T_3) and thyroxine (T_4) levels were measured with reagents provided by the Institute for Nuclear Sciences "Boris Kidrič" in Vinča. Serum free thyroxine (FT_4) was determined by RIA-cot (Mallinckrodt Diagnostica,

Dietzenbach) and reverse T_3 (rT_3) was detected by Reverse T_3 kit (Biodata, Roma). The RIA-h-TBG set and RIA-h-TSH set (INEP, Zemun) were used for detecting the TBG and the TSH. HbA_{1c} values were determined with chromatography on a BIO-RAD column. Total biocarbonate and pH were measured by standard Technicon Autoanalyser methods.

The statistical analysis was performed by student's t-test. Correlation coefficients were calculated by regression analysis. All data were represented as the mean $\pm$ 1 SD.

RESULTS

The serum levels of thyroid hormones, TSH and TBG in the diabetic patients and normal controls are summarized in Table I. Patients in groups B (poor control) and C (diabetic ketoacidosis) had significantly lower T_3 and higher rT_3 levels ($p < 0.001$) compared to the matched control subjects. Serum TBG levels were significantly lower ($p < 0.01$) in the group A, B and C in comparison with those found in the control group. Patients in group C had significantly lower T_4 levels ($p < 0.001$) than normal subjects. A negative correlation was found between T_3 and HbA_{1c} in group B (poor control) with significance ($r = -0.546$; $p < 0.02$) (Fig. 1).

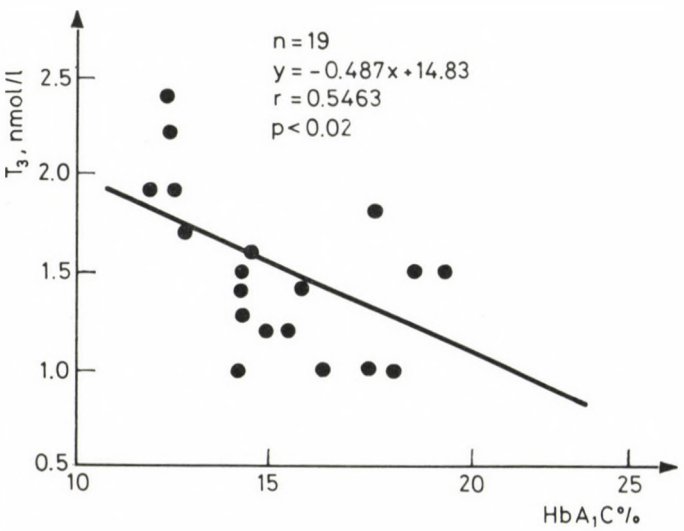

Fig. 1. Correlation between serum T_3 levels and the percentage of HbA_{1c} in diabetic patients with poor control

TABLE I

Serum hormone levels in euthyroid sick syndrome in type I diabetes mellitus in children and adolescents[1]

	T_3 (nmol/L)	T_4 (nmol/L)	FT_4 (nmol/L)	rT_3 (nmol/L)	TSH (nmol/L)	TBG (nmol/L)
Healthy subjects (n = 30)	2.2 (± 0.5)	108.0 (±29.0)	20.1 (± 4.9)	0.3 (± 0.1)	2.4 (± 1.5)	24.2 (± 3.1)
Group A: good control (n = 27)	2.0 (± 0.5	103.0 (±23.8)	22.4 (± 4.4)	0.3 (± 0.2)	2.0 (± 1.3)	19.5 (± 4.3)[x]
Group B: poor control (n = 19)	1.5 (± 0.4)[*]	108.0 (±24.4)	20.4 (± 4.0)	0.58 (± 0.2)[*]	2.3 (± 2.8)	20.3 (± 3.3)[x]
Group C: ketoacidosis (n = 16)	0.9 (± 0.3)[*]	81.9 (±24.2)[*]	18.6 (± 8.8)	0.6 (± 0.1)[*]	2.6 (± 1.8)	18.0 (± 3.8)[x]

[1] Results are expressed as mean ± SD

[x] $p < 0.01$

[*] $p < 0.001$: compared to healthy subjects

DISCUSSION

The values for T_3 and rT_3 found in diabetic patients with poor control and diabetic ketoacidosis are similar to those obtained in other studies in adults and juvenile type I diabetes /4,6,7,8,10,11/, and provide further evidence for an impairment in 5 monodeiodinase activity which controls the peripheral conversion of T_4 into T_3 and catabolism of rT_3 /12/. The inverse relationship between T_3 and HbA_{1c} (low T_3 and high HbA_{1c} values) shows that changes in thyroid hormone peripheral metabolism are related to the degree of impaired glucose utilization. These results are consistent with those of Dorchy et al /4/ and Salardi et al /11/ who found a significant negative correlation between T_3 and HbA_{1c} in the poorly controlled juvenile type I diabetic patients.

We found no difference between the serum T_4 and FT_4 levels in patients with good and poor control in comparison to the control group. Other studies of poorly and good controlled diabetics have shown serum T_4 concentrations to be low /10/ or not different from the control group /4/. However, our results for FT_4 are consistent with those of Radetti et al /10/ and Dorchy et al /4/ who found no difference between diabetic patients and controls.

The significant decrease of T_3 and T_4 serum concentrations as well as the significance increase of serum rT_3 levels in our patients with diabetic ketoacidosis are similar to those obtained in other studies /8,11/. These findings suggested that diabetic children and adolescents with ketoacidosis present an euthyroid sick syndrome. Therefore, we support the assumption of Castels /3/ that ketoacidosis has an inhibitory effect on peripheral conversion of T_4 to T_3, being thus responsible for disturbances in thyroid hormone blood level in these states.

Despite significant decrease of serum T_3 levels, basal TSH concentrations in our patients were normal. This suggests that circulating T_4 by its free fraction may have an important impact on the regulation of TSH secretion /6/, to which our FT_4 findings are confirmatory.

In conclusion, this study has shown that children and adolescents with poorly controlled diabetes and ketoacidosis exert an euthyroid sick syndrome. Thus, the thyroid hormones may be an important indicator of metabolic status in the diabetic children and adolescents.

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Acta Paediatrica Hungarica 31 (1), pp 75-81 (1991)

POSTOPERATIVE ECHOCARDIOGRAPHIC STUDY OF PATIENTS FOLLOWING VALVULOTOMY FOR CRITICAL VALVULAR AORTIC STENOSIS

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Received 28 April 1989

Authors report on long term follow up of 12 patients operated with critical valvular aortic stenosis. They could perform control echocardiography in 11 patients 4-83 (mean 29) months after valvulotomy. The size and function of the left ventricle was found to be satisfactory, with elevated ejection fraction. The cause of the significant pressure gradient between the left ventricle and the aorta is discussed emphasizing the importance of echocardiography in determining the optimal time for valve replacement or homograft implantation.

INTRODUCTION

Valvular aortic stenosis gives about 4-7 % of congenital heart diseases /1, 3/. Looking at the morphology and also the clinical signs of the disease two separate forms can be distinguished. In case of the childhood form the valves are usually soft, semi-transparent. Calcification develops later, depending on the severity of the stenosis, possible endocarditis, calcium metabolism, exercise, and also on sex.

In a few patients (about 5-10 %) the valves are very thick cartilaginoid, with excentric opening occurring already in infancy. This is the characteristic picture of the critical valvular aortic stenosis of infants; it occurs very rarely later in childhood or adolescents; mostly only after valvulotomy. In some cases myxomatous vegetations can be seen on the valves /1, 2, 4, 8/.

Patients of this group usually do not have the classical signs of aortic stenosis; ejection murmur is absent. The pale colour, the weak pulses, dyspnea, tachypnea, the signs of low

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cardiac output are the characteristic features /1, 4, 8/. The prognosis of the patients without surgery is very poor.

Earlier the diagnosis and indication of surgery were possible only on the basis of cardiac catheterization, but most of the patients didn't survive the invasive procedure. Other centers reported small number of operated infants with critical aortic stenosis /9/ too. In the last few years it became possible to operate after non-invasive investigation, too. Since 1984 we have been operating these patients after echocardiography /4/.

PATIENTS AND METHODS

We have had the possibility for infant cardiac surgery with cardiopulmonary bypass since 1979. Between 1979-1981 no baby has reached the operating theatre with aortic stenosis. There are two patients from the period Sept 1981 - June 1984 and 10 from July 1984 - June 1988 under postoperative control. In the latter group the diagnosis was based upon echocardiography (performed partly in the Hungarian Institute of Cardiology, partly in our hospital). One of them had cardiac catheterization without further information, too.

The aim of the study was to investigate the late postoperative state of these 12 patients. In 7 patients the typical clinical signs were discovered in the first few weeks; the youngest baby was 4 days old at the time of the operation, further 6 were under the age of 4 months. Some of them were operated following resuscitation, but all of them were critically ill. In a little better condition have reached the operating theatre 3 other babies at the age of 6-8 months, who were classified in this group on the basis of the valve morphology. 2 other patients were operated at 12 and 24 months of age respectively; the first had pulmonary banding, plastic of the aortic isthmus and ligation of the duct at 1 mth and total correction (valvulotomy, closure of a VSD and debanding) at 12 mths of age. The other patient could not be operated because of repeated respiratory tract infections caused by IgA deficiency before the age of 2 years.

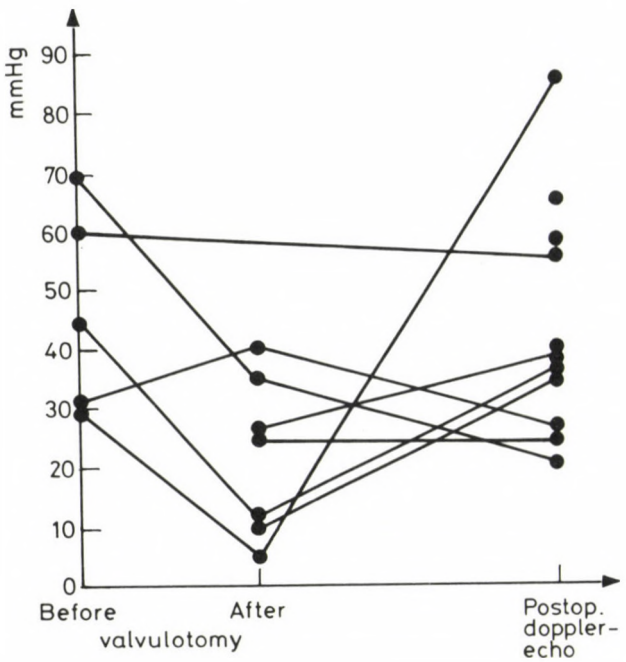
The postoperative echocardiography was performed at the outpatient department of our hospital, using a Picker SE 150 Cardiac Imager. The patients were sedated with chloralhydrat (0.05 g/kg body weight rectal), 4-83 (mean 29) months after surgery.

From the measurements the most important parameters are the following: the diameter of the left atrium and the aorta, the enddiastolic diameter of the left ventricle, the ejection fraction from the M-mode and from 2-dimensional picture, the fractional shortening; and the pressure gradient between the

left ventricle and the aorta calculated on the basis of the Bernoulli equation from continuous wave Doppler study.

RESULTS

The most important parameter of the severity of the valvular stenosis is usually the pressure gradient between the left ventricle and the aorta. In our cases we compared the gradient measured before valvulotomy on the table,(measurement was possible only in part of the patients because of the very poor clinical condition), immediately after valvulotomy, and the late postoperative gradient measured with continuous wave Doppler echocardiography (Fig. 1).



From the M-mode measurements we consider as important the size of the previously small left ventricular enddiastolic diameter (EDD), the left ventricular function and the development of the left atrium and the aorta. Table I shows

TABLE I

Left ventricular enddiastolic diameter (EDD)

	BSA (m^2)	EDD (mm)	% of normal mean value	MI	AI
1.	0.9	35	92.6	-	-
2.	0.6	38	114	-	+
3.	0.72	38	108	+	-
4.	0.7	37	106	-	-
5.	0.75	31	87	+	-
6.	0.75	32	90	-	+
7.	0.55	28	90	-	-
8.	0.5	33	108	+	-
9.	0.4	25	86	-	-
10.	0.35	24	86	-	-
11.	0.4	23	79	-	-

MI: mitral incompetence
AI: aortic incompetence

the left ventricular EDD of the patients in mm and in per cent of the normal values according to body surface area, (indicated whether mitral or aortic insufficiency are present or not) investigated with pulsatile Doppler.

Table II contains the results of left ventricular function measurements: the fractional shortening (FS) in percent and the ejection fraction (EF) calculated from the 2-D picture using planimetry and from the M-mode picture using the mean of the calculations according to Gibson, Pombo and Teichholz. Table III shows the diameter of the left atrium in mm and in per cent of the normal value and the ratio of the diameter of the left atrium and the aorta /2, 7/.

DISCUSSION

From the results of the gradient measurements we can establish, that these values are not easy to appreciate. Before valvulotomy the patients are in very bad condition, the left ventricle is unable to eject properly through the critical

TABLE II

Left ventricular function at the postoperative control

	FS (%)	EF (2D) (%)	EF (M-m) (%)
1.	40	65.4	75.4
2.	55.3	65.0	88.4
3.	44.7	74.8	80
4.	40.5	57.0	75.1
5.	48.4	69.0	83.3
6.	53.1	-	85.4
7.	50.	-	82.9
8.	36.4	62.6	71.2
9.	44.0	68.5	78.5
10.	50.	-	83.9
11.	52.2	-	87.1

FS: fractional shortening
EF: ejection fraction

TABLE III

Diameter of the left atrium (LA) and aorta (AO) (in mm and % of the normal mean value)

	BP	%	AO	%	BP/AO
1.	24	104	22	105	1.1
2.	21	101	18	97	1.2
3.	23	105.5	22	113	1.0
4.	23	106.5	19	98.5	1.2
5.	23	104.5	20	101.5	1.15
6.	23	104.5	20	101.5	1.15
7.	15	74	19	105.5	0.8
8.	24	120.5	17	96.5	1.4
9.	19	100	18	108	1.05
10.	18	97.5	14	86	1.3
11.	15	79	17	102	0.9

stenosis and to maintain normal pressure. Therefore the measured preoperative gradient in some cases did not reach the value of indication for surgery in classical terms. After

valvulotomy both the left ventricular output and pressure rises, and the gradient will not necessarily be less than before the operation. At the late postoperative Doppler-echo control the majority of the patients have a gradient above 30 mmHg. Other teams have reported on similar results /5, 10/. However the general condition of the patients is perfect, usually do not need medical treatment.

The most important question is the future of the patients. The operation resulted in survival of the critically ill patients being now in a good condition. The previously hypoplastic left ventricles are now usually of normal size, the EDD is above 100 % of the normal mean mainly in patients with mitral or aortic incompetence. The ratio of the left atrium and aorta is also near normal in all patients, neither the aorta is hypoplastic. We have to mention that we could have performed control investigation only in late surviving patients.

The left ventricular EF was in all cases above the normal value. Other left ventricular function measurements, e.g. Doppler-echo we were not able to perform on the restless patients at the outpatient clinic, however they could have given more reliable results /6/, than the EF values calculated from M-mode and 2D-methods with rather great differences.

It is still not clear what and when should be done with our patients. A second valvulotomy on these severely pathologic valves which are already incompetent in some cases will not give a good result. We suggest that some patients will need valve replacement or homograft implantation. To determine the optimal time for a second operation is a very difficult problem. Echocardiography can help to follow up these patients. We have to beware of valve replacement in childhood, but we have to define the time when the operation cannot be delayed any more because of the increase of left ventricular hypertrophy or dilatation, endocardial fibroelastosis or pressure gradient.

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Acta Paediatrica Hungarica 31 (1), pp 83-101 (1991)

VALUE OF BRAINSTEM ACOUSTIC EVOKED POTENTIALS IN POSTERIOR FOSSA TUMOURS IN CHILDHOOD

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Received 23 May 1989

Brainstem acoustic evoked potentials (BAEPs) were measured in 14 children with different type of posterior fossa tumours several times during the clinical course, in order to assess the value of this simple and non-invasive method in the diagnosis and follow-up of posterior fossa tumours in childhood. Eight children had midline medulloblastoma, three children had lateral astrocytoma, three had intrinsic brainstem glioma. Different BAEP patterns could be detected in different tumour's type: bilateral symmetrical or slightly asymmetrical I-V. IPL prolongation in midline medulloblastomas, unilateral or markedly asymmetrical I.-V. IPL prolongation or wave V. depression on the contralateral side in lateral astrocytomas, and severely distorted asymmetrical waveform in intrinsic brainstem gliomas. The BAEPs were abnormal earlier than CT scan in a case of craniospinal astrocytoma. BAEPs were useful in the follow-up: the effect of the preoperative chemotherapy or the progression of the inoperable tumours could be as well documented by this method, as by the CT scan. BAEPs proved effective in the assessment of postoperative neurological complications: bilateral symmetrical IPL prolongation and wave V. depression with clinical signs of increased intracranial pressure occurred in a case of postoperative occlusive hydrocephalus, unilateral IPL prolongation occurred during irradiation or chemotherapy after medulloblastoma removal as signs of cerebral oedema.

INTRODUCTION

Brainstem acoustic evoked potentials (BAEPs) are measures of electrical events generated along the auditory pathway that can be recorded from the scalp by far-field averaging methods. BAEPs were first described by Jewett in 1970, /9/ who

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registered in animals, later in humans five vertex positive deflections of submicrovolt amplitude during the initial 5 msec following a click. In 1971, Jewett and Williston /10/ described further two deflexions in the second five msec. Subsequently, a number of studies were published dealing with the theoretical and practical importance of BAEPs.

The basis of the clinical application of BAEPs is that BAEPs reflect the progressive activation of the auditory nerve and the brainstem auditory tracts and nuclei: Wave I.: acoustic nerve, Wave II.: acoustic nuclei, Wave III.: trapesoide body in the caudal pons, Wave IV.: lateral lemniscal pathway in the pons, Wave V.: inferior colliculi in the midbrain (Fig. 1).

The BAEP waveform becomes abnormal if there is a lesion along the central auditory pathway. The localisation errors are probably in the order of 1 cm, at worst, an accuracy level which is more than sufficient for most clinical purposes /4/. Giesser in 1986 /6/ reported that this method is more sensitive than the NMR to reveal a brainstem lesion in multiple sclerosis. Among posterior fossa tumours, the acoustic neurinomas result BAEP abnormalities earliest, because these affect earliest the central auditory pathway. Tumours larger than 1 cm will be detected using BAEP method /5/. This method precedes the CT scan and the routine audiological test in the diagnosis of acoustic neurinomas. In the diagnosis of posterior fossa tumours of childhood the CT scan is the most effective tool at present /1/. The posterior fossa, however, because of its bony artifacts and small structures is the brain area least amenable to visualisation by CT scan, especially postoperatively and after irradiation /3/ .

Recently, NMR has become a new powerful modality for assessing and quantitating the posterior fossa tumours in childhood /2/.

Both CT scan and MNR are morphological approaches to posterior fossa, and do not provide any functional information about its structures.

At present there are very few reports about the use of this simple and non-invasive method in the differential diagnosis, follow-up and assessing the effectiveness of chemotherapy and irradiation of posterior fossa tumours in childhood.

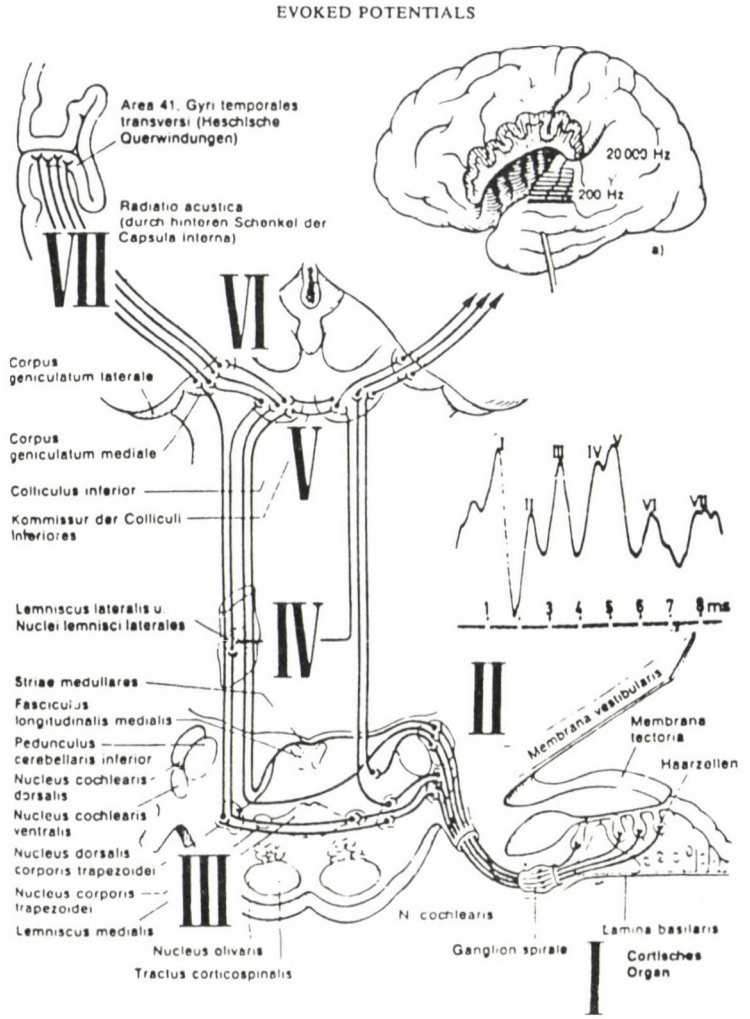


Fig. 1. Origin of BAEP (from Maurer)

METHODS AND PATIENTS

A complete otologic and audiologic examination was performed in each patient before the BAEP testing. The BAEPs were obtained in awakeness, in the few very young patients chloralose narcosis was used.

Brainstem acoustic evoked potentials (BAEPs) were measured by Amplaid MK-6 system. Clicks of 100 μsec in duration were delivered monoaurally at a rate of 10 per sec through earphones. Clicks of 0, 20, 40, 60, 80 dB above the mean normal threshold were used. BAEPs were recorded from the vertex electrode using ipsilateral earlobe reference. The objective

audiograms were plotted by X-Y recorder. BAEPs at 60 and 80 dB HL were analyzed, interpeak latencies (IPL) of I.-III., III.-V., I.-V. and amplitude ratios of wave I.-V. and III.-V. were measured. BAEPs were judged to be abnormal if one of the above values was beyond the age-matched normal mean $\pm$ 2.5 SD. Two control age groups were formed: children of 1-4 years, and 4-14 years (Table I).

14 children with posterior fossa tumours were tested by BAEP several times during the clinical course (Table II). Three children had brainstem glioma, three had astrocytoma (ponto-cerebellar, cerebellar, and cranio-spinal), and eight had medulloblastoma.

RESULTS

Preoperative BAEPs

Brainstem gliomas

All of our patients had right side pontine symptoms. All had deformed BAEPs: on the right side waves after the wave III. are difficult to recognize, on the left side wave IV.and V. have low amplitude, and I.-V. IPL is prolonged (Fig.2).

Medulloblastomas

All of our patients were first tested after shunting. The shunting operation was followed by preoperative chemotherapy. The direct tumour surgery was performed thereafter. All of our patients had bilateral symmetrical, or slightly asymmetrical I.-V. IPL prolongation. Two patients had III.-V. IPL prolongation, and one had additionally I.-III. IPL prolongation. Besides the IPL prolongations, three patients had wave V. amplitude depression on one side, or on both sides (Fig. 3).

(see p. 87)

Fig. 2. BAEPs and CT scans in a 3.5-years-old child with brainstem glioma.
a: at the beginning
V-A_2: right side: I.-III. IPL prolonged, wave IV. and V. are difficult to recognize
V-A_1: left side: I.-V. and III.-V. IPL prolonged, wave V. depressed
b: in clinical progression
V-A_2: right side: BAEP is depressed at 80 dB HL intensity
V-A_1: left side: BAEP waveform is distorted at 60 dB HL intensity

K.E. a
I
II
III
80dB
V-A2
V-A1
V-A2
60dB
V-A1
0.1uV
10msec

K.E. b
I
II
III
80dB
V-A2
V-A1
I
II
60dB
V-A2
V-A1
0.1uV
10msec

Astrocytomas

All of our patients had unilateral or markedly asymmetrical BAEP abnormalities: V/III. amplitude reduction in the cerebellar astrocytoma's patient (Fig. 4), I.-V. IPL prolongation in the craniospinal astrocytoma's patient (Fig. 5), when CT scan was still normal, and unilateral I.-III. IPL and I.-V. IPL prolongation with contralateral wave V. depression in the cerebello-pontine astrocytoma's patient (Fig. 6). In this last case, the CT scan suggested a medulloblastoma, and the BAEP finding was the first clinical sign of a possible astrocytoma, proved postmortem pathological.

Effect of the preoperative chemotherapy

Two patients were followed by BAEP and CT during the preoperative chemotherapy. The BEAP abnormalities improved in both of them. CT scan proved the tumour size's reduction (Fig. 3/b).

Postoperative follow-up

Six patients were followed by BAEPs after the tumour surgery between the 14th day and 10th months. Two patients had normal BAEPs in the 4th and 5th postoperative months.

They had only minimal cerebellar residual symptoms. One patient 3 months after the surgery had markedly abnormal BAEP, he had marked cerebellar and pontine symptoms, too. Three patients 1.5 months after surgery had abnormal BAEPs, while they had only cerebellar residual symptoms. In one of them BAEPs became normal 3 months after the surgery. Three patients had neurological complications in the postoperative period. One patient had cephalea 10 months after the surgery and irradiation. On his BAEP: I.-V. IPL prolongation and wave V. depression were seen on both sides. Together with the clinical features these abnormalities were judged as signs of increased intracranial pressure. CT scan proved the occlusive hydrocephalus caused by postoperative adhesions (Fig. 7).

(see p. 89)

Fig. 3. BAEPs and CT scans in a 1.5-year-old child with medulloblastoma
a: at the beginning, BAEP after shunting on both sides: I.-V. IPL and III.-V. IPL prolonged
b: after preoperative chemotherapy markedly reduced I.-V. IPL and III.-V. IPL

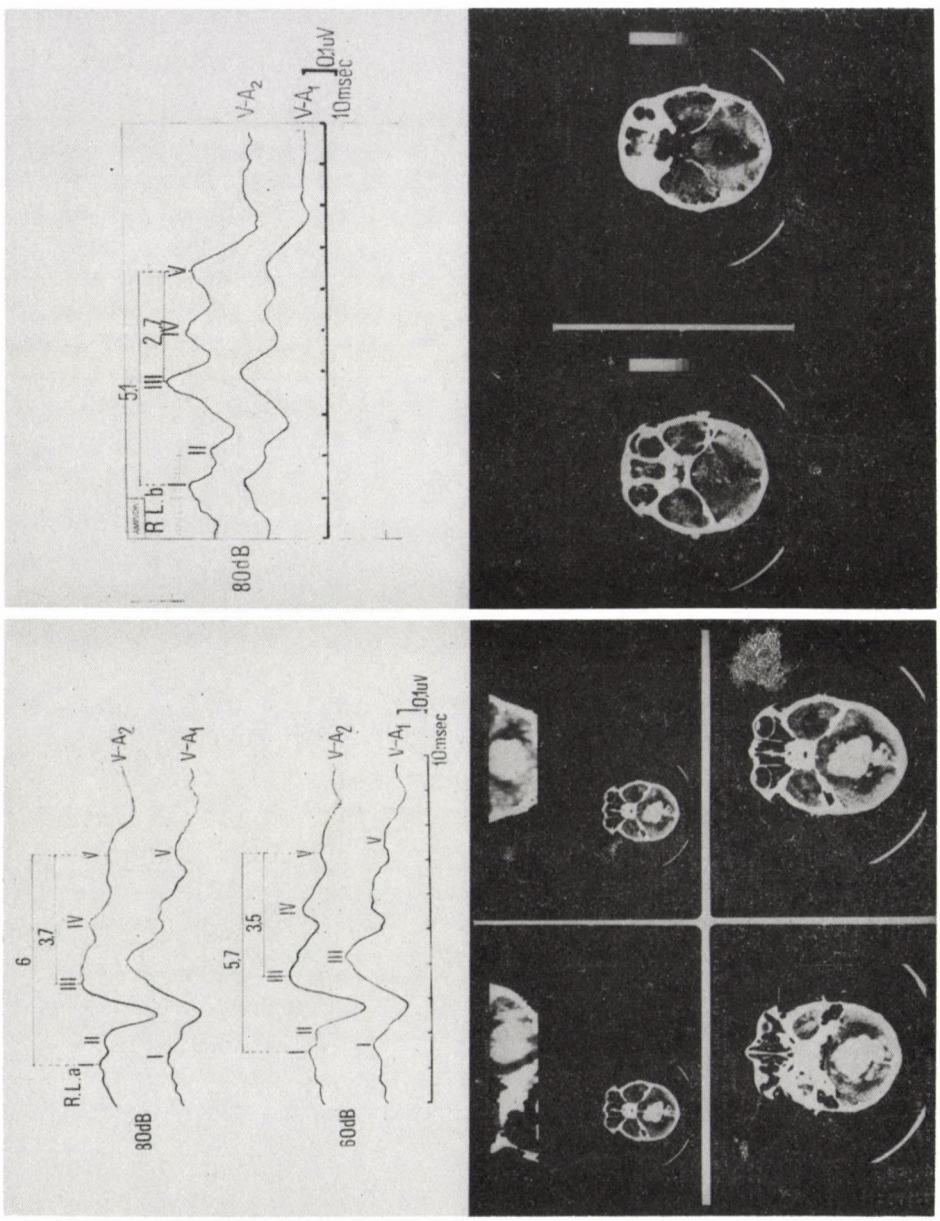

R.L.a
6
3.7
I
II
III
IV
V
V-A2
V-A1
80dB
5.7
3.5
60dB
0.1uV
10msec
R.L.b
5.1
2.7
80dB

TABLE I/a

Amplaid MK-6
Normal control groups- BAEP values
80 dB HL intensity

	4-15 years (N:21)			1-4 years (N:16)		
Latency	Mean	SD	Upper limit (mean+2.5SD)	Mean	SD	Upper limit (mean+2.5SD)
I.	1.39	0.09	1.62	1.39	0.12	1.69
II.	2.38	0.08	2.55	2.23	0.20	2.73
III.	3.50	0.12	3.80	3.68	0.22	4.23
IV.	4.43	0.20	4.93	4.58	0.20	5.08
V.	5.22	0.16	5.62	5.64	0.25	6.26
IPL ("Interpeak latency")						
I.-II.	0.93	0.25	1.55	0.91	0.16	1.38
I.-III.	2.12	0.12	2.42	2.31	0.19	2.78
III.-V.	1.71	0.13	2.05	1.95	0.14	2.30
I.-V.	3.84	0.16	4.24	4.29	0.25	4.91
Amplitude ratio						
I./V.	93.5 %	37.5 %	178 %	60 %	33 %	142 %
III./V.	80.0 %	28.0 %	150 %	60 %	35 %	148 %
IILD ("Interear interpeak latency difference")						
I.-II.	0.14	0.1	0.4	0.09	0.07	0.3
I.-III.	0.06	0.05	0.2	0.07	0.04	0.2
III.-V.	0.17	0.1	0.4	0.13	0.11	0.4
I.-V.	0.13	0.1	0.4	0.11	0.08	0.4

TABLE I/b

AMPLAID MK-6
Normal control groups-BAEP values
60 dB HL intensity

	4-15 years (N: 21)			1-4 years (N: 16)		
Latency (msec)	Mean	SD	Upper limit (mean+2.5SD)	Mean	SD	Upper limit (mean+2.5SD)
I.	1.61	0.17	2.04	1.57	0.18	1.83
II.	2.66	0.22	3.21	2.37	0.16	2.77
III.	3.71	0.25	4.34	3.71	0.19	4.18
IV.	4.45	0.15	4.83	4.73	0.35	5.60
V.	5.45	0.21	5.97	5.64	0.28	6.34
IPL ("Interpeak latency")						
I.-II.	0.97	0.15	1.35	0.83	0.11	1.10
I.-III.	2.11	0.20	2.61	2.24	0.18	2.69
III.-V.	1.79	0.29	2.51	1.93	0.19	2.40
I.-V.	3.83	0.16	4.23	4.23	0.22	4.78
Amplitude ratio						
I./V.	69.8 %	42.8 %	176 %	60 %	39 %	157 %
III./V.	70.0 %	25.0 %	133 %	55 %	32 %	135 %
IILD ("Interear interpeak latency difference")						
I.-II.	0.18	0.1	0.4	0.13	0.09	0.35
I.-III.	0.09	0.06	0.25	0.09	0.06	0.25
III.-V.	0.14	0.15	0.5	0.10	0.10	0.35
I.-V.	0.1	0.08	0.8	0.17	0.12	0.45

TABLE II

BAEP testings in 14 children with posterior fossa tumours

Diagnosis	Number of patients	Number of patients with BAEP testing					
		Preoperative		Postoperative			Follow-up
		After or before dg.	After shunt	After chemoth.	After op.	Compl	of inop. tu.
Medulloblastoma	8	-	8	2	6	3	
Astrocytoma	3	3	-	-	-	-	2
Brainstem glioma	3	3	-	-	-	-	3

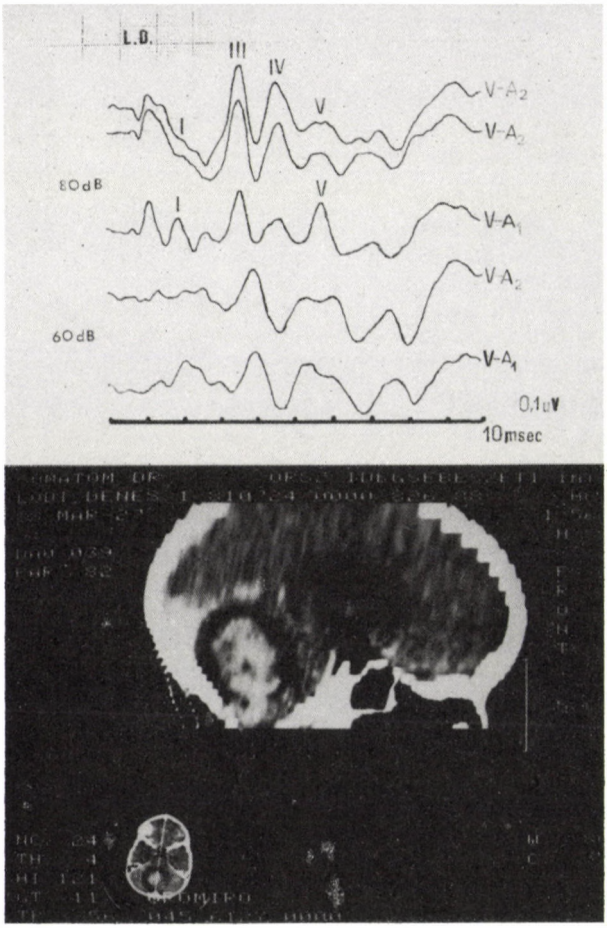


Fig. 4. BAEP and CT scan in a 6.5-year-old child with left cerebellar astrocytoma
$V\text{-}A_2$: right side: wave V. depressed, III./V. increased

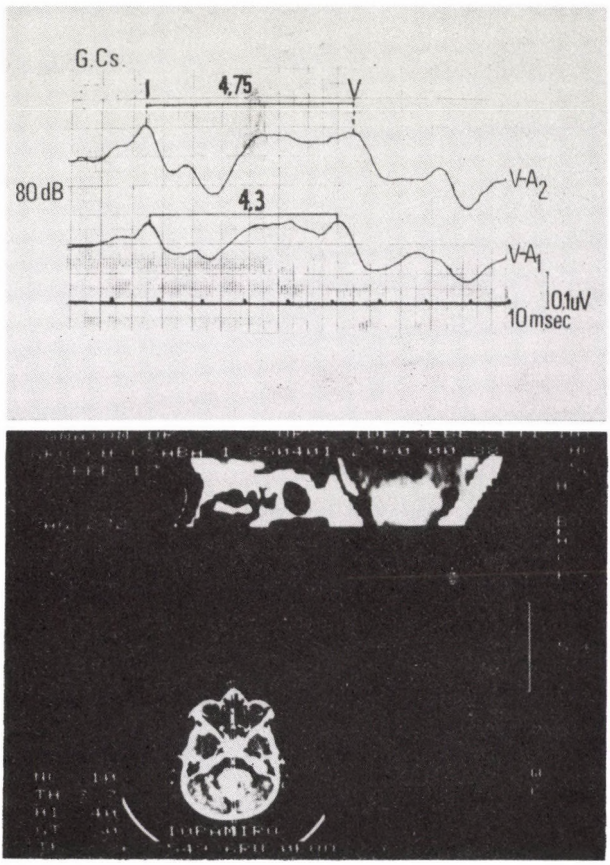
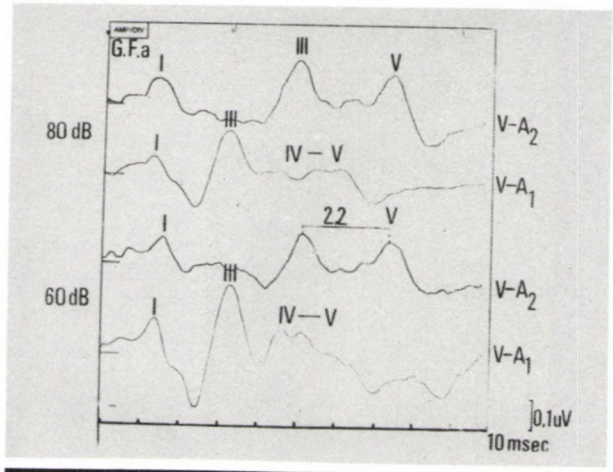
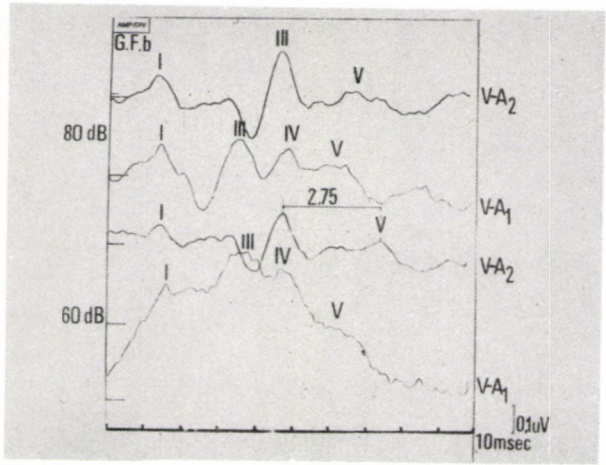


Fig. 5. BAEP at the beginning and CT 1 year later in a 1.5-year-old child with craniospinal astrocytoma $V\text{-}A_2$: right side: I.-V. IPL near the upper limit, and the IILD is increased





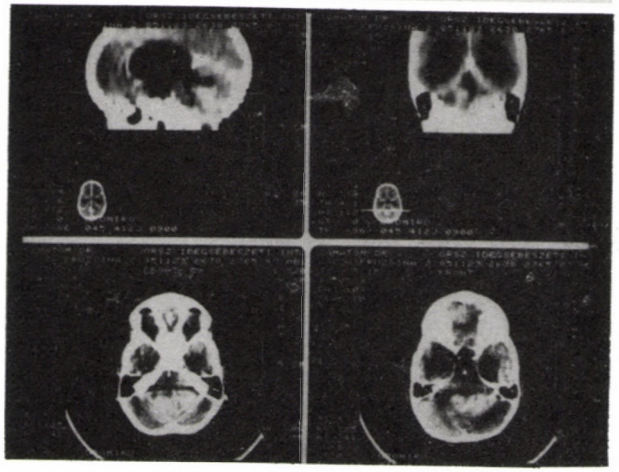


Fig. 6. BAEPs and CT scan in a 2-year-old child with right cerebello-pontine astrocytoma
a: at the beginning, BAEP after shunting
$V\text{-}A_2$: right side: I.-III. and I.-V. IPL prolonged
$V\text{-}A_1$: left side: wave V. depressed, III.-V. IPL prolonged
b: in clinical progression
$V\text{-}A_2$: right side: wave V. depressed, and at 60 dB HL intensity III.-V. IPL increased
$V\text{-}A_1$: left side: at 60 dB HL intensity the waveform is distorted

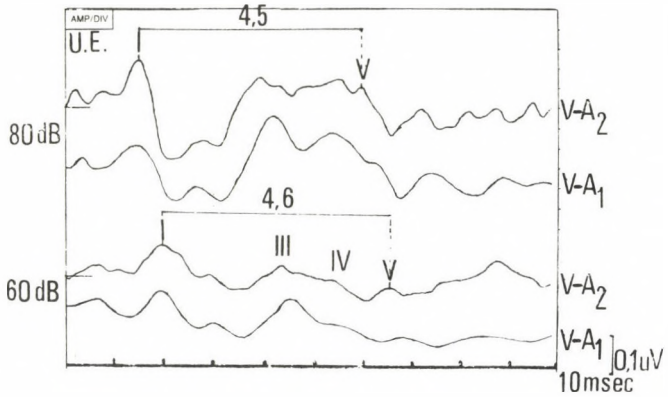


Fig. 7. Postoperative BAEP in a 5 year-old child 10 months after the tumour removal and irradiation. On the previously normal BAEP the I.-V. IPL is prolonged on both sides and wave V. is depressed. Occlusive hydrocephalus.

Another child had cephalea and vomitus during the irradiation. BAEPs became normal previously, but this time unilateral III.-V. IPL prolongation appeared. We justified this abnormality as a sign of local cerebral oedema (Fig. 8).

Another child had vomitus and papilla oedema during the postoperative chemotherapy. His BAEP which previously became already normal, turned again to abnormal that time: unilateral I.-V. IPL prolongation was seen. CT scan excluded passage disturbance or recidiva. The BAEP abnormality could indicate toxic brain oedema (Fig. 9).

Follow-up of inoperable tumours

Three brainstem glioma, the cerebello-pontine astrocytoma and the craniospinal astrocytoma's patients were followed by BAEPs, and CT scan. The deterioration of BAEPs could be observed according to the clinical progression. The CT scan and the postmortem pathological findings proved the increase of the tumour's size (Fig. 2/b, Fig. 6/b).

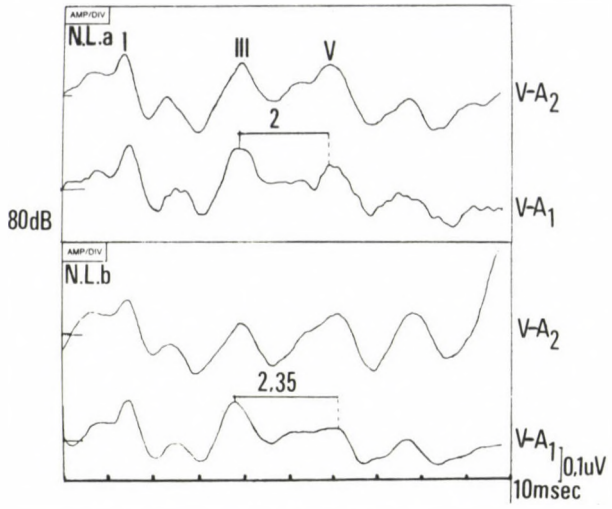


Fig. 8. Postoperative BAEP in a 2-year-old child after medulloblastoma removal during irradiation.
a: postoperative BAEP: normal
b: during irradiation: left side III.-V. IPL increased ($V-A_1$) Cerebral oedema.

DISCUSSION

There are very few reports concerned with the possible specificity of BAEP in different tumour types of posterior fossa in childhood.

Rotteveel et al. in 1985 /13/ followed 8 children with posterior fossa tumours by BAEP. Bilateral BAEP changes were found in medulloblastomas, while asymmetric recordings were obtained in three cerebello-pontine tumours. Goldie et al. in 1987 /7/ reported BAEP results in 12 children with medulloblastomas, 6 children with cerebellar astrocytomas, 9 children with brainstem gliomas, and 4 children with ependymomas. Nine of the 12 children with medulloblastomas had normal BAEPs, three patients had delayed I.-V. IPL. Five of the six children with astrocytomas had I.-V. IPL prolongation, or wave V. depression. This report does not deal with any side difference, asymmetry or symmetry of BAEP abnormalities.

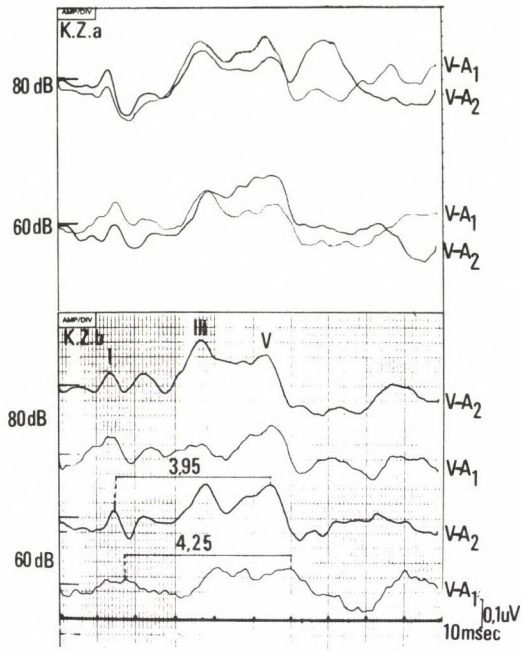


Fig. 9. Postoperative BAEP in a 6-year-old child after medulloblastoma removal during the chemotherapy.
a: postoperative BAEP: normal
b: during chemotherapy: left side I.-V. IPL prolonged ($V-A_1$). Cerebral oedema

In our material different BAEP patterns were documented in midline medulloblastomas, lateral astrocytomas and intrinsic brainstem gliomas.

In medulloblastoma bilateral I.-V. IPL prolongations were found, which could be produced by direct pressure of the tumour on the brainstem. The effect of the increased intracranial pressure was excluded by the fact that the testings were performed after preoperative shunting.

In astrocytoma situated laterally from the midline, the BAEPs were always asymmetrical. Contralateral BAEP abnormalities could be detected in two of our three patients, similarly to Rotteveel et al's findings /13/. These findings are consistent with the observation of Nodar and Kinney (1980) /12/ in adult patients with acoustic neurinomas larger

than 2 cm concerned to the contralateral effects on BAEP test. In brainstem gliomas most of the authors agree in the significance of the BAEP pattern: the waveform is severely distorted with asymmetry /7,8,12,13/. The sensitivity of the BAEP testing method is high enough to indicate posterior fossa tumour earlier than CT scan in some cases: a craniospinal astrocytoma in our material and a medulloblastoma in Rotteveel et al's material /13/.

BAEPs seem to be useful in the follow-up of posterior fossa tumours in childhood. The effect of the preoperative chemotherapy could be well documented by this method in our patients. The postoperative BAEPs remained abnormal longer than the neurological status in most of the cases suggesting that the BAEP abnormalities are more sensitive signs of the brainstem dysfunction than the clinical symptoms (Kálmánchey et al 1986). In the inoperable tumours BAEP testing was just as useful tool as the CT scan to objectivate the tumour's progression.

BAEPs proved to be useful, too, in the assessment of the postoperative neurological complications: after removal of medulloblastoma the previously normal postoperative BAEP became bilaterally prolonged with wave V. depression in a case of postoperative occlusive hydrocephalus. Unilateral IPL prolongation during irradiation or chemotherapy indicated cerebral oedema.

CONCLUSION

14 children with posterior fossa tumours were studied by BAEP method. The results were compared with the CT scan findings, postmortem pathological findings and the clinical features. 8 children had midline medulloblastoma, 3 had brainstem glioma, 1 had ponto-cerebellar astrocytoma, 1 had cerebellar astrocytoma, 1 had craniospinal astrocytoma. Different BAEP patterns were observed in the different types of tumours: 1. In midline

medulloblastomas: bilateral, symmetrical, or slightly asymmetrical I.-V. IPL prolongation was found in all of the cases. In some cases III.-V., or I.-III. IPL prolongation and wave V. depression could be seen. 2. In lateral astrocytomas: asymmetrical BAEP abnormalities were found. In cerebellar astrocytoma unilateral wave V. depression, in pontocerebellar astrocytoma besides the contralateral wave V. depression, ipsilateral I.-III. and I.-V. IPL prolongation and in craniospinal astrocytoma unilateral I.-V. IPL prolongation was observed. 3. In brainstem gliomas: BAEPs were severely distorted and asymmetrical, waves after the wave III. were depressed, and later in the clinical course disappeared. These findings suggest that BAEPs are useful in the differential diagnosis of posterior fossa tumours in childhood, and can be necessary even besides the CT scan. This method is sensitive enough to suggest a posterior fossa lesion earlier than CT scan positivity in some cases. In such cases repeated CT scanning will be necessary. Finally BAEP testing is an appropriate method in the follow-up of posterior fossa tumours in childhood; it can document the tumours's progression or therapeutic regression, it is non-invasive, simpler and cheaper that the CT scan.

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Acta Paediatrica Hungarica 31 (1), pp 103-112 (1991)

EFFECT OF PARENTAL SMOKING ON WHEEZY BRONCHITIS AND BRONCHIAL HYPERREACTIVITY

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Received 20 June 1989

In the course of the follow-up of 206 previously obstructive bronchitis children, the effect of parental smoking upon the occurrence of respiratory diseases, the yearly frequency of wheezing episodes and the age until the obstructive episodes used to return have been investigated. Familial and maternal smoking was more frequent in this group compared to the control group. In spite of this, however, no correlation could be detected between familial smoking and frequency of respiratory diseases, as well as the above mentioned characteristics of obstructive bronchitis. The familial smoking did not seem to influence the bronchial hyperreactivity challenged with acetylcholine, although the prevalence was higher.

INTRODUCTION

In the literature the influence of smoking on the development of chronic respiratory diseases in adults is widely discussed. Special attention is paid to the carcinogenic effect, as well as to changes in lung function parameters. By the progress of time the lung function parameters of smokers deteriorate faster than those of adults with chronic respiratory disease /3/. In young adult smokers there is no considerable alteration in lung function parameters yet, a change used to appear rather later at an age above 40 years /3/. Although the respiratory symptoms and coughing can be manifest much earlier /2, 5/.

In childhood the effect of passive smoking can primarily be considered, however, according to Wuthe and coworkers 62 % of children report on their smoking already at age of 16 years, boys try the first cigarette at 9, while girls in 13 years

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/18/. Considerable proportion (6-30 %) of teenage boys smoke regularly daily more than one cigarette /1, 2, 4/. The stimulation for smoking comes partly from the parents, partly from the surroundings of the young people (clubs, gangs, etc.)

High number of children of smoking parent(s) exhibit chronic coughing as a significantly more frequent symptom compared to those of non-smokers /4, 6, 9/. Respiratory diseases (bronchitis, tracheitis) occur also considerably more frequently in children of smoking parents and especially if more than one of the family members do smoke /4, 6, 7/. According to Pedreira and coworkers the incidence of these respiratory diseases is higher under age of one year /12/. It has to be noted that children living with family-members suffering from chronic respiratory disease and excreting sputum were found to have respiratory diseases in higher proportion than those under healthy conditions /6, 12/. The investigations of Lebowitz and Burrows carried on at Tucson with several thousands of children showed similar correlation between familial smoking and respiratory symptoms, however, independently of the smoking habits, these symptoms showed significant correlation with familial respiratory diseases, as well /9/.

Maternal smoking was observed to cause more frequent respiratory symptoms in children /4, 12, 13, 14, 15/. This correlation was, however, not experienced by Liard and coworkers /10/. Maternal smoking yielded significantly frequent wheezing in children as reported by several authors /7, 11, 13/. Weiss and coworkers registered wheezing more often even in the case of familial smoking /16/. In contrast, the prospective study of Horwood and coworkers does not show any correlation between familial smoking and wheezing until 6 years of age /8/. The postnatal mortality, as well as the number of respiratory diseases manifested until 5 years of age were found to be higher in children whose mothers did smoke during pregnancy /14/.

The impact of familial smoking upon the lung function values of children and upon bronchial hyperreactivity was also studied. In a prospective study of Tager and coworkers the FEV_1

values of children aged 5-9 years of smoking parents and especially of smoking mothers, did not show normal progress /15/. In their view, the development of alveoli in the infantile lung at this age - alveolization period - is impaired by the smoke. In contrast, Wuthe and coworkers in their cross-sectional examinations found no difference in lung function parameters (FEV_1, FVC, PEFR, R_t) even in the case of smoking children /18/. Woolcock and coworkers following infantile and childhood respiratory diseases observed lower flow-volumes with familial smoking /17/. 10 years after RSV (respiratory syncytial virus) infection Pullen and Hey showed lower lung function values and increased bronchial reactivity relative to the average, however, these differences seemed to have no correlation with familial smoking /13/.

Our study performed 10 years after obstructive bronchitis of children under the age of 2 years investigates the influence of maternal and familial smoking on the trends of the disease and on bronchial hyperreactivity, as well.

PATIENTS AND METHODS

The follow-up examination involved children treated with obstructive bronchitis at the I. Department of Paediatrics 10 years earlier. Criteria of obstructive bronchitis were as follows: wheezing accompanied by symptoms of airway infection, prolonged expiration and hyperinflated lungs to be detected also with X-ray, as well as by physical examination at age under 2 years.

206 children aged between 9-13 years (mean 11 years), boy: girl ratio 2:1, were enrolled in the study. The child's own and familial history including maternal and/or familial smoking, date of the first obstructive episode, yearly frequency of episodes, the age until these episodes occurred, frequency of other respiratory diseases were registered on questionnaires. The incidence of pneumonia, bronchitis, otitis, tonsillitis, laryngitis and angina was scored with marks 0 - 3 resulting in Σ value. The term "frequent respiratory disease" was applied if the Σ value was over 8 and exceeding the mean + 2SD of that of control group.

Bronchial hyperreactivity was examined by acetylcholine challenge. Acetylcholine solution of 0.5 % concentration nebulized by ultrasonic nebulizer (TUR-USI 50) was inhaled by children for 3 minutes, and PEF (peak flow) and FEV_1 (forced expiratory volumen in the first sec) were measured in the 3.,

5. and 10. minutes thereafter. In the case of negative results, the challenge was repeated with a solution of 1 % concentration again for 3 minutes. Positive response, i.e. bronchial hyperreactivity was considered when the PEF and/or FEV_1 values decreased by more than 20 % compared to the initial ones. At the time of the examination the children were symptomfree and so were they in the 4 weeks prior to the examination, as well.

Data of 82 healthy children of similar age and sex distribution were registered on questionnaires serving as controls.

The examination data were processed by a computer. In the mathematical statistical calculations the level of significance was $p < 0.05$.

RESULTS

In 73 % of the study group familial, while in 43 % maternal smoking was registered (Fig. 1). The control group contained

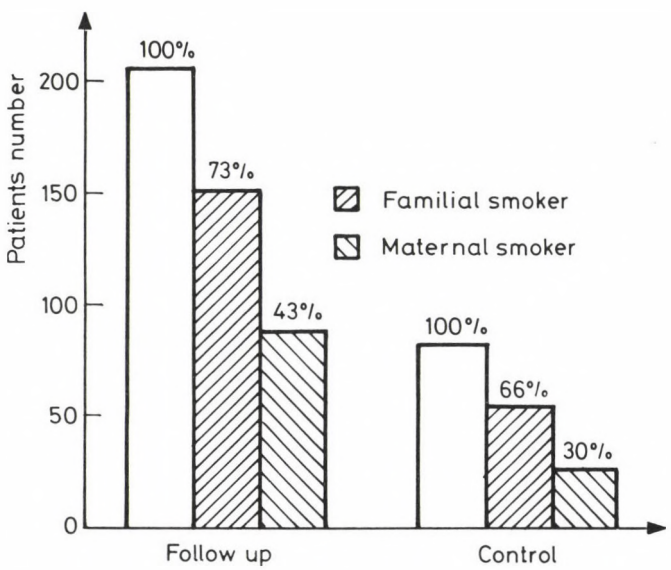

Fig. 1. Familial and maternal smoking

children living with familial smokers in 6 %, and with smoking mothers in 30 %. The frequency of familial smoking was high,

although not significant, in both groups, while maternal smoking differed greatly in the two groups, but this was not significant either ($p < 0.10$).

80 % of the children had their first wheezy episode before the age of one year. Appearance of the first episodes divided to age: 0-6 months, 7-12 months and later than 12 months, no correlation could be found between the familial and/or maternal smoking and point of time of the first episode ($p=0.20$ and $p=0.63$ resp.) (Table I).

TABLE I

The first episode set up

	0-6	7-12 months	over 12		0-6	7-12 months	over 12
no smoker	23	18	15		23	18	15
familial smoker	82	40	28	mother	43	26	19
	$p = 0.20$				$p = 0.63$		

40 % of the children had only one obstructive episode, but 60 % of them had several ones. Similarly no significant correlation was found between the familial and/or maternal smoking and the frequency of episodes $p=0.28$ and $p=0.23$, resp. (Table II).

Obstructive episodes occur after 3 years of age in 29 % of the children, while after 7 years of age only in 8 %. Smoking family members or mothers were present in both age groups in similar ratio: 75-75 % (Table III) and no significant correlation could be established between the time of setting in of obstructive episodes and familial smoking ($p=0.65$) and maternal smoking, resp. ($p=0.35$).

The yearly number of obstructive bronchitis episodes is a good marker for the severity of the disease. 20 % of the

TABLE II

Frequency of wheezy episodes

	only once	2-3x	4 or more		only once	2-3x	4 or more
no smoker	20	13	23		20	13	23
familial smoker	62	21	67	mother	34	11	43
	p = 0.28				p = 0.23		

TABLE III

The wheezy episodes disappeared

	till 3 years	over 3 years		till 3 years	over 3 years
no smoker	41	15		41	15
familial smoker	105 (75 %)	45 (75 %)	mother	58	30
	p = 0.65			p = 0.35	

children had yearly 3 or more times episodes. No significant correlation was found between the familial and/or maternal smoking and severity and yearly frequency of the obstructive episodes, p=0.95 and p=0.45, resp. (Table IV).

TABLE IV
The episodes per year

	1-3 times	over 3		1-3 times	over 3
no smoker	45	11		45	11
familial smoker	120	30	mother	66	22
	p = 0.95			p = 0.45	

Children having undergone obstructive bronchitis suffer more frequently from respiratory diseases compared to the controls, mean 6.63 vs. 3.71 (Table V). Respiratory diseases occur

TABLE V
The frequency of respiratory tract infections

		Patients	Controls
Respiratory tract infections			
	mean	6.63	3.71
	SD	2.31	1.97
Frequency			
$\sum$	0 - 8	158	81
	over 8	48 (23%)	1 (1%)
		$p < 0.001$	

significantly more frequently - Σ value over 8 - among the follow-up children than in controls ($p < 0.001$). The familial

and/or maternal smoking shows no correlation with the frequent respiratory diseases, p=0.27 and p=0.42 resp. (Table VI).

TABLE VI

Respiratory tract infections

	till Σ 8	over Σ 8		till Σ 3	over Σ 8
no smoker	40	16		40	16
familial smoker	118	32	mother	68	20
	p = 0.27			p = 0.42	

Bronchial challenge with acetylcholine solution of 0.5 % concentration resulted in PEF and/or FEV_1 decrease corresponding to bronchial hyperreactivity in 31 children (15 %), while none of the control children. No significant correlation could be established between bronchial hyperreactivity and familial smoking (p=0.29).

DISCUSSION

In the course of the follow-up of 206 children having been treated for obstructive bronchitis under 2 years of age, familial smoking was found in high proportion: 73 %. Within this, the maternal smoking reaches also high ratio: 43 % compared to the control group. Pedreira and coworkers examined 1143 children in the neighbourhood of Washington and found only 36 % smoking frequency in the families /12/.

Our follow-up examinations showed that respiratory diseases occurred more frequently in the obstructive bronchitis group compared to the control group. At the same time, however, we were unable to observe significant correlation between the frequency of respiratory diseases and familial or maternal smoking, in contrast to several other investigators /4, 6, 7,

9, 12/. Our results agree with those of Liard and coworkers /10/.

Weiss and coworkers described the wheezing "inducing" effect of familial smoking, while maternal smoking yielded wheezing more frequently as reported by several authors /7, 10, 11, 13/. On the other hand, orwood and coworkers in their prospective study, did not find similar correlation. In our follow-up examinations the familial and/or maternal smoking did not influence the yearly frequency of wheezy episodes, their first appearance and the age until they are repeated.

The ratio of bronchial hyperreactivity was higher among children having undergone obstructive bronchitis compared to the healthy controls. Similarly to the data of Pullen and Hey /13/ no correlation could be detected between familial smoking and bronchial hyperreactivity.

Although our examination results do not seem to support the findings of several authors cited above, i.e. the deteriorating effect of passive smoking, the hazard of the high familial smoking should be emphasized.

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Acta Paediatrica Hungarica 31 (1),pp 113-118 (1991)

PROSTACYCLIN AND THROMBOXANE LEVELS OF CHILDREN OF PARENTS SUFFERING FROM EARLY ISCHEMIC HEART DISEASE

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Received 20 February 1989

Offsprings of parents who had acute myocardial infarction before age of 45 years were investigated. The aim of this examination was to obtain information whether the variation in the balance of prostacyclin/thromboxane ratio is a common cardiovascular risk factor in children.
In children whose parents have had early myocardial infarction, a significant decrease was shown in 6-keto-prostaglandin $F_{1\alpha}$ level while the thromboxane B_2/6-keto-prostaglandin $F_{1\alpha}$ ratio increased in these children. Plasma tromboxane B_2 levels hardly differed from those of the control in that group of children whose one parent and at least one of the grandparents or uncles or aunts suffered from coronary heart disease. Plasma thromboxane concentration was lower in another group of children whose "only" one parent had myocardial infarction. It may be supposed that this is a compensatory mechanism in the offspring of parents suffering from early coronary heart disease.

INTRODUCTION

Coronary heart disease (CHD) mortality of Hungarian middle-aged men is highest in Europe. There is an urgent need to know more of the early stages of its development and of its various risk factors /1/.

As part of a comprehensive cardiovascular prevention program in Hungary a study of risk factors in children, whose parents have had an acute myocardial infarction (AMI) before the age of 45 years, was carried out.

The present work was aimed at the measurement of concentrations of circulating stable hydration products of prostacyclin (PGI_2) and thromboxane (TXA_2)- namely 6-keto-

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prostaglandin - $F_1\alpha$ (6-keto-$PGF_1\alpha$) and thromboxane B_2 (TXB_2). We examined whether the variation of TXA_2/PGI_2 ratio was a common cardiovascular risk factor in children.

MATERIALS AND METHODS

68 children were investigated ranging in age from 3-14 years, and divided into 2 groups. Group I consisted of 31 children, whose one parent had CHD. Group II consisted of 27 children, whose one parent and at least one of the grandparents or uncles or aunts suffered from CHD. We chose a control group of 28 healthy children without any history for CHD. For prostaglandin (PG) analysis venous blood was collected into tubes containing 0.1 volume of 7.4 x 10^{-2}M EDTA and 2.8 x 10^{-5}M indomethacin solution. The blood was centrifuged for 10 min at 1200 g 4°C to obtain plasma. (Plasma samples were stored at -30°C). For PG extraction octadecylsilyl (ODS) cartridges were used (SAMPLEX C 18 Bio-Separation Technologies, Budapest). Plasma samples were acidified to pH 3 by citric-acid and were applied to the cartridges. The columns were washed with 6 ml petroleum-ether and were eluated with 8 ml ethylacetate. The eluate was dried under vacuum and resuspended in assay buffer. The 6-keto-$PGF_1\alpha$ and TXB_2 were determined by radioimmunoassay Izinta RIA KIT, Budapest.
Extraction efficiency calculated from extracted control plasma was 75.6 $\pm$ 15.2 % for 6-keto-$PGF_1\alpha$ and 81.5 $\pm$ 13.2 % for TXB_2. Statistical significance between group means was assessed by Student's t-test.

RESULTS

Plasma 6-keto-$PGF_1\alpha$ concentrations were lower in both groups of affected children. The difference is statistically significant. Plasma TXB_2 levels were lower in group I compared with the controls, and hardly differed from the control in group II. TXB_2/6-keto $PGF_1\alpha$ ratios were elevated in both groups of the examined children. There was a statistically significant difference between the ratio of TXB_2 to 6-keto-$PGF_1\alpha$ in group II (Table).

TABLE

Plasma levels of prostaglandin metabolites and TXB_2/6-keto-$PGF_{1\alpha}$ ratio in examined children compared with controls

	N	6-keto-$PGF_{1\alpha}$ ± SEM (pg/ml)	Thromboxane B_2 ± SEM (pg/ml)	TXB_2/6KPGF$_{1\alpha}$ ± SEM
Control	28	80.9 ± 9.3	325.3 ± 24.0	5.9 ± 0.9
Group I.	31	32.5 ± 7.2^{xx}	197.5 ± 41.4^{x}	29.5 ± 13.2
Group II.	37	14.6 ± 2.5^{xx}	332.8 ± 79.4	56.3 ± 21.8^{x}

^{x}: $p < 0.05$

^{xx}: $p < 0.001$

Group I : one parent had CHD

Group II : one parent and at least one of the grandparents or uncles or aunts suffered from CHD.

Control : healthy children without any history for CHD.

All values are presented as means ± SEM

DISCUSSION

Platelet aggregation and formation of thrombi are important in the pathophysiological mechanism of the development of myocardial ischemia and infarction. The arachidonic acid metabolites thromboxane A_2 and prostacyclin have been recognized to play important roles in platelet function and in the development of the atherosclerotic processes and of CHD /3, 5, 7, 8, 9-11, 19/. PGI_2 inhibits platelet aggregation by stimulating adenylate cyclase, leading to an increase in cAMP levels in the platelets /9, 11/. PGI_2 is a strong hypotensive agent and a vasodilator of all vascular beds. In contrast to PGI_2 the TXA_2 is a vasoconstrictor and platelet aggregator. Balance between formation of PGI_2 by the vessel-wall and of TXA_2 by platelets is important for the control of hemostasis /10/. A number of diseases have been related to an imbalance in the PGI_2 - TXA_2 system /9, 12/. Platelets from patients with hypercholesterolemia have been shown to produce abnormal amounts of TXA_2 /11/. Increased release of TXA_2 has been described in rabbits made atherosclerotic by high-cholesterol diet and patients who survived AMI, and also an elevated level of TXB_2 in blood of patients with Prinzmetal's angina and vasotonic angina /6, 13, 16, 18, 20/. The precise role of TXA_2 in cardio-vascular disease is still unclear. It is possible that multiple factors may be involved in the pathologic etiology.

The results of this study suggest that there is a PGI_2/TXA_2 imbalance in children whose parents had early CHD. A significant decrease was shown in 6-keto-$PGF_1\alpha$ levels while TXB_2/6-keto-$PGF_1\alpha$ ratio increased in both groups of examined children. Plasma TXB_2 concentration in group II hardly differed from that of controls, but it was lower in group I compared with the controls. This may be due to compensatory mechanism in the offspring of parents suffering from early CHD.

Because PGI_2 generation by atherosclerotic arterial tissue has been shown to be lower than by a normal one /15/, our results suggest that childhood is an important prevention stage

of atherosclerotic processes in children with highrisk families.

This PGI_2/TXA_2 imbalance in affected children may contribute to the initiation of atherogenic processes in the blood vessels in childhood and shows the importance of screening examination and of dietary measures that consist of feeding polyunsaturated fatty acids (e.g. eicosapentaenoic acid) to stimulate PGI_2 production of the vessel-walls (2, 4, 14, 17).

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Acta Paediatrica Hungarica 31 (1), pp 119-129 (1991)

PHYSICAL HEALTH AND BEHAVIORAL PROBLEMS IN TWO HIGH SCHOOLS IN HOLON, ISRAEL

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Received 10 April 1989

1320 students of two high schools were examined in Holon. Their physical and behavioral problems are summarized. These data serve to build the model of the medical profile of the adolescent in Israel, as a background to medical screening in school, which should be done at least every two years.

INTRODUCTION

Physical and behavioral maturity occurs between the age of 12-17 years. The "adolescent" has his own characteristic features and is not to be considered as "older child" or "young adult". In Israel he is examined and treated by pediatricians. During the high school they are examined twice as it is recommended. The morbidity during that period is low in comparison with pediatrics age /1/.

The purpose of this study is to provide information about the medical and behavioral problems of the adolescent in Israel.

MATERIALS AND METHODS

1320 students from two high schools in Holon were examined (Eilon and Yavneh High School). Their age was between 14-18 years. There were 972 in the first and 348 in the second. Holon is the 4th greatest city in Israel and there are 5.000 pupils learning in six high schools. This city served as the site of

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our study. The data were collected by informative questionnaires completed by their parents and all the pupils had been physically examined by senior pediatricians during 1987-1988.

RESULTS

The results of our study indicate a ratio of boys to girls 47 to 53 %. The socio-economic composition of the population in these two schools is in the upper mean level of the society in Israel.

TABLE I

The professions of the parents

	No	%
Academic professions (university graduates)	713	27 %
Technical and teaching professions	898	34 %
Mechanical and commercial work	1029	39 %

65 % of the mothers worked during this year at full or part-time jobs.

TABLE II

The origin of the parents

	No	%
Ashkenazic Jews	1162	44
Sephardic	1082	41
Mixed	231	8.8
Yemenite	158	6
Shomronic	16	0.2

TABLE III

The characteristics of the pupils

No. of children in the family	No. pupils	%
1	132	13
2	502	38
3	462	35
4	198	15
5	26	2

TABLE IV

The familial situation

Married parents	1032	78 %
Divorced	238	18 %
Orphanage (at least 1 parent)	52	4 %

TABLE V

The prevalence of chronic disease (with duration of 3 weeks or more) versus acute illness among the pupils during 1987.

Illnes	No. of pupils	%
Acute	977	74
Chronic	211	16
Without	132	10

80 % of the pupils with chronic disease had good compliance with medication.

TABLE VI

Diseases and accidents leading to hospitalization of the pupils during 1987

Disease	No. of pupils	%
Acute	66	5
Chronic	53	4
Accidents	40	3

The mean absence from school because of acute medical problems was 4.7 in comparison with mean of 14.3 days for pupils with chronic disease.

Each pupil had an average of 2.7 acute or exacerbation of chronic medical problems during the year, even though 57 % of the pupils did not have any complaint concerning their health.

Dealing with adolescent girls the average of menarch was 12.8 years according to the pupil's answers: in comparison with the average age of onset in girls in U.S. of 12.5 years /2/.

TABLE VII

The data of the medical pathological finding

Pathology	No. of pupils	%
Hypertension	53	4
Obesity	158	12
Bronchial Asthma	132	10
Diabetes Mellitus	2	0.15
Epilepsy	3	0.23
Abdominal pains	92	7
Cepelalgic (recurrent)	198	15
Kyphosis/Scoliosis	92	7
Acne	515	39
Dental Carries	858	65
Visual problems Myopia	304	23
Strabismus	30	2.3
Daltonism	18	1.4
Hearing loss	34	2.6
Stuttering	9	0.7
Undescended testes	2	0.15

TABLE VIII

The prevalence of psychological disturbances

Psychological disturbances	No. of pupils	%
Behavioral problems (all grades)	106	8
School Refusal (pholic)	40	3
Nocturnal Enuresis	53	4
Anorexia Nervosa	40	3
Suicidal Attempts	5	0.5

TABLE IX

Smoking habit among the pupils

Age	No. of pupils	%
14-15	92	7
15-16	211	16
16-17	356	27
17-18	501	38

DISCUSSION

In Israel pupils in high school are examined by the school physician (pediatrician) whose work is highly diversified. He is doing a screening and verifies that the pupil with the chronic disease is under surveillance, and he knows the medication with the appropriate dose that he has to take during his illness. Besides the physical examination and the screening the physician gives lectures in preventive medicine in classes. The subjects include topics as the damage of smoking, the danger of drug addiction and sexual education.

The adolescent disease profile is given in Table VII. There were not any cases of rheumatic fever or rheumatic heart disease.

Dental cavities worsen with age /3/. An average of 5 cavities were present in the permanent teeth between the age of 14-16 years and 9 between the ages of 16-18 years. 35 % of the adolescents in this study were free of cavities. A possible solution to this problem involves vaccination against acidogenic bacteria, streptomutans and lactobacilus.

There was a familial incident of hypertension in most cases found. The data of 40 % is less than the margin of 6-10 % mentioned in the literature.

Chronic diseases interfere with the adolescents acquiring independence and choosing a profession /4/. The adolescent with chronic disease can have a diminished self image, be frustrated and have sentiments of guilt. This is often expressed by absence from school and poor achievement in his studies /5,6/. 16 % of pupils suffered from chronic diseases.

The two schools are located between the agricultural field and industrial zones of Holon. This can be the reasons that 24 % of the pupils suffered from allergic symptoms including urticaria, angioneurotic edema, allergic rhinitis, allergy to food and medication.

Bronchial asthma was found among 10 % of the pupils and the number increased if tests of provocation are used (with Histamin, for example).

The number of absent days of the asthmatics was influenced by the severity of the asthma and the social conditions at home /7/. In this group the mean absence days is 14.3 but the pupils were asked to bring with them to school inhalators (salbutamol or turbutalin) and to use them in case of bronchospasm crisis occurred at school.

60 % of the asthmatic pupils suffered from exercise induced asthma. They were instructed to take slow release theophyllin on the morning of gymnastics.

Concerning kyphoscoliolis there were discrepancies between the objective finding and the percentage of back pains. Only approximately a third of these pupils had pains. There were more pupils with scoliosis than with kyphosis. The back pains increased during physical activity or after prolonged standing /8/.

Two boys with cryptorchidism who have been found for the first time during the school medical screening: certainly they should have been diagnosed and treated at an earlier stage, since there is a higher risk of atrophic testis and malignancy (Seminoma). They were sent without delay for ultrasound examination and surgical treatment.

40 pupils were involved in accidents during the year. The accidents occurred at school, at home or on the way to school. The type of accidents in order of frequency were: falls, road accidents, burns and accidental poisoning. Accidental injury is the leading cause of childhood handicaps and in this group 3 children suffered from severe handicaps (all 3 were involved in road accidents) /9-12/.

Behavioral problems.

The adolescent have specific psychological attributes. 8 % of the pupils had behavioral difficulties and problems in all grades of severity. Psychological problems were expressed by lack of concentration, chronic fatigue or by agression towards other children /13/. But there is no certainty about the prevalence of these symptoms as for example, enuresis nocturnal

or sleep disturbancies, the data depend upon the revealing of the child and/or his parents.

The percentage of children whose parents were separated rose in some classes to 25 % (one pupil out of four in this class). In 78 % of these cases the child lives with the mother.

90 % of pupils have mean absence of 9.5 days per year. 40 % of the absences from school were explained by medical certificates (as permitted up to absence of 3 days). 25 % were explained by parents and the remaining third of absences were explained by the students. The smoking habit is first acquired at 14-15 years. The reasons stated by the pupils are: family habit, influence of friends, a drive towards independence and need to reduce tension. Most of smokers had non productive cough. The data concerning the psychological problems were given by the educative advisor and the teachers. Most of them were under psychological treatment and surveillance.

The attempted suicides were by means of taking medication. All cases required and were treated by stomach lavage in the regional hospital. They returned to school after short hospitalization and several days of ambulatory treatment. All were under psychiatric ambulatory treatment and surveillance.

SUMMARY AND IMPLICATION

The purpose of this survey was to obtain information about the health problems of the pupils and to use it in organizing the health program in school. The data is a pilot study as they were obtained only from 2 schools out of 6 in the city. The results have the following implication for the adolescent's health in school, and our conclusions are the following:

1. The main purpose of the medical examination in school is to do a screening of all pathologies and to explain the findings to the student and to verify that he is followed up and is under control.

2. The most serious medical problems of this period are chronic diseases such as bronchial asthma, inflammatory bowel disease and lower back pain (kyphoscoliosis). The school physician can try, together with the specialist, to reduce the absence from school in this category of disease.

3. Everytime that the absence rate from school is higher than the average, the school physician has to check if there are medical reasons and if not, to collaborate with the school educational advisor to find the reasons.

4. School achievement is influenced by medical problems /14/. In each case that happened in acute failure in studying the physician has to verify and culminate medical reason for it. Therefore the physician has to participate in assessments sessation and to be their advisor concerning absence of the pupil from school and from gymnastic lessons for short or long periods.

5. The school medical examination is usually the last systemic chance to screen these youngsters and to inform them about their medical situation. We have to bear in mind that these adolescents will in a few years be adult citizens. Their medical, intellectual and behavioral level will set the future standard of the country.

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Acta Paediatrica Hungarica 31 (1), pp 131-140 (1991)

INFLUENCE OF SOME SOCIAL AND MATERNAL FACTORS ON BIRTH WEIGHT IN HUNGARY

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Received 26 June 1989

In this study, we have examined the impact of some social and maternal factors on birth weight in the two regions Hungary, in the capital and in one county. Although many of the variables were analyzed in both regions, significant relationships were found between LBW and smoking habits, birth order and mother's age. The incidence of low birth weight was higher in Budapest than in Vas. Smoking habits were found as a common factor of higher significance in both regions. Birth order and mother's age were found as other factors of higher significance in the Budapest sample as compared to Vas.

INTRODUCTION

Birth weight is a major determinant of an infant's potential for survival and future development /6/. Perinatal and infant mortality, as well as increased risk of morbidity are highly correlated with birth weight /4/. For this reason, frequency of LBW is accepted as a general indicator of health status of population groups.

It is estimated that about 21 millions infants with LBW are born each year in the world. The vast majority of these infants are encountered in the poorest part of the developing world /9/.

Previous studies have shown that the most important factor leading to the higher infant mortality rate in Hungary (19 ‰)

[x] A. Dindar was supported for this study by a scholarship of the Hungarian government.

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as compared to other European countries is the relatively high rate of LBW. The frequency of LBW in Hungary was reported as 9.8 % in 1986 /5,8/.

This study is a data analysis of some factors such as maternal age, parity, birth interval, education, occupation, marital status, smoking which are known to influence outcome of pregnancy and LBW in Hungary as they relate to two regions which show differences in their perinatal mortality and LBW rates.

MATERIALS AND METHODS

Budapest and Vas, which represent two regions with different LBW and perinatal mortality rates were selected for the study.

Reported perinatal mortality rates for the two regions were 10.9 % and 7.8 %; frequency of LBW in the two regions being 23.3 % and 16.1 %. The number of pregnant women living in these two regions were 1043 and 947 in respective order at the time when the demographic data were collected. The total number of pregnancies in the two regions (1990) corresponds to 0.24 % of the national figure /4/.

The data used in this study were taken from the longitudinal survey program, "Health and demographic study of pregnant women and infants", carried out in eight regions of Hungary (Demographic Research Institute, Department of Population Statistics of the Central Statistical Office, National Institute of Child Health and Department of the Maternal, Child and Youth Welfare of the Ministry of Health.) The first stage of this survey started in November 1979 and ended in August 1983, data on 8800 pregnant women and on outcome of all pregnancies were collected (corresponding to a representative sample of 1.2 % of the national figure) /4/. For this purpose detailed questionnaires were filled in by the health visitors during pregnancy starting at around the 9th week of pregnancy and repeated at the 20th, 27th and 34th weeks and after delivery. Gestational age was taken as the number of completed weeks from the first day of the last normal menstruation to the date of delivery. All newborn babies were examined and weighed immediately after delivery. For the purposes of this study, the results of this survey as they relate to the two regions, Budapest and Vas were evaluated by an IBM PC/XT computer with SPSS/PC statistical package. Chi-square test was used in statistical analysis. Multiple regression was also applied for the simultaneous analysis of these factors (Table I).

TABLE I

Factors related to birth weight (by multiple regression analysis)

Total Group	R^2	F	Budapest	R^2	F	Vas County	R^2	F
1) Smoking habits	0.016	34.1^{xx}	1) Birth order	0.020	21.2^{xx}	1) Smoking habits	0.016	15.8^{xx}
2) Birth order	0.023	24.3^{xx}	2) Smoking habits	0.032	17.4^{xx}	2) Educational level	0.019	9.4^{xx}
3) Mother's age	0.029	19.7^{xx}	3) Mother's age	0.039	14.2^{xx}	3) Marital status	0.021	6.9^{xx}
4) Employment status	0.030	15.4^{xx}	4) Educational level	0.041	11.2^{xx}	4) Mother's age	0.022	5.4^{xx}
5) Educational level	0.032	13.2^{xx}	5) Employment status	0.043	9.5^{xx}	5) Birth interval	0.023	4.4^{xx}
6) Marital status	0.033	11.3^{xx}	6) Birth interval	0.044	8.0^{xx}	6) Employment status	0.023	3.7^{x}
7) Birth interval	0.333	9.7^{xx}	7) Marital status	0.044	6.9^{xx}	7) Birth order	0.023	3.2^{x}

^{x} $p < 0.01$

^{xx} $p < 0.001$

RESULTS

Gestational age

There was a difference between the two regions in the rate of prematurity among low-birth-weight infants. In Budapest, 52.3 % of all babies with LBW were preterms with gestational ages less than 37 completed weeks while this proportion was much higher (75 %) in Vas.

The overall prematurity rate was also higher in Vas being 11.9 % as compared with 7.0 % in Budapest. Fifty five (55.1 %) percent of these preterm babies were born with normal birth weight (2500 g or higher).

The incidence of small-for-dates was more than twice higher in Budapest as in Vas (4.3 % and 1.8 %).

For all infants, the increase in birth weight by gestational period was statistically significant ($p < 0.001$) in both regions.

Age of mothers

In the Budapest group, birth weight distribution was found to be influenced by maternal age ($p < 0.001$). A higher incidence of low-birth-weight infants was noted among the offsprings of young mothers and also for infants born to mothers over 40 years of age (Figure 1). The incidence for LBW increased from the low 8.8 % for 20-29 year old mothers to 19.0 % in babies born to mothers under the age of 20 years and to 40.0 % in babies born to 40-49 year old women.

The incidence of low-birth-weight babies varied from a low 6.4 % for those born to mothers aged 20-29 years, to 9.2 % for infants born to 30-39 year old mothers and to 10.5 % for infants born to women under the age of 20 years in Vas. No significant correlation was found between birth weight distribution and mother's age.

Number of previous pregnancies and birth interval

The incidence of LBW was low in first pregnancies (6.7 %) in Budapest and in third (3.0 %) and second pregnancies (5.6 %) in Vas. It has risen sharply for three and more pregnancies in both regions, being higher in Vas (21.0 %) than in Budapest (13.3 %).

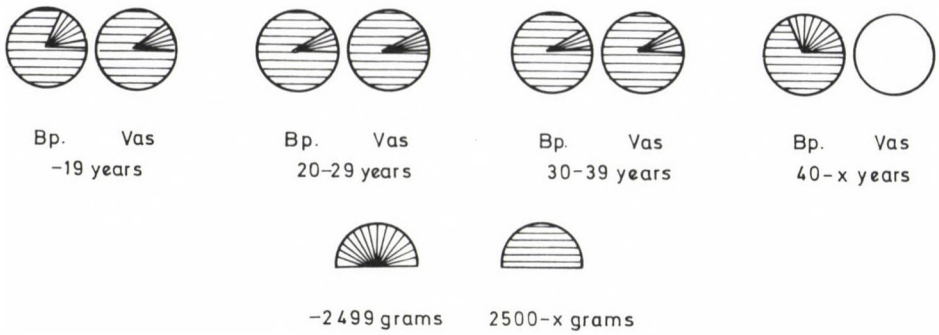

Fig. 1. Low birth weight rate of live births by age-group of mothers in Budapest and Vas county

Birth weight distribution by interval between the actual and previous pregnancy is shown in Figure 2. A higher incidence of low birth weight for very short and very long intervals was found in Budapest. The optimum interval was between 2 and 3 years.

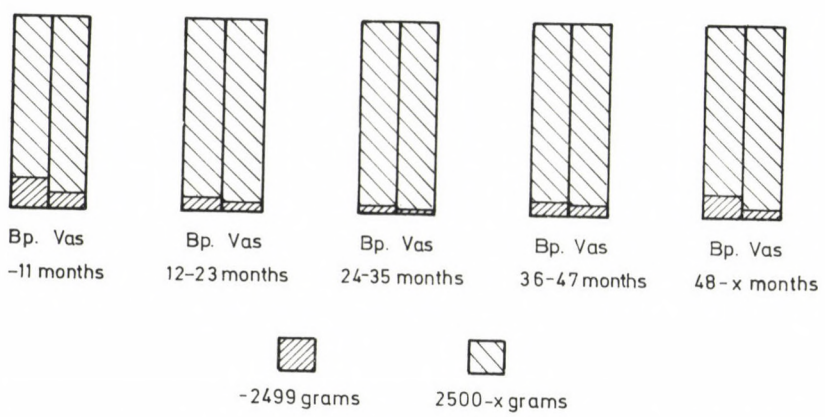

Fig. 2. Low birth rate of live births by interval between previous pregnancies in Budapest and Vas county

Although birth weight distribution by number of previous pregnancies was statistically significant in both regions ($p < 0.001$), the correlation by birth interval was evident only in the Budapest group ($p < 0.001$).

Marital status

The frequency of unmarried women was higher in Budapest than in Vas (10.5 % and 7.9 %). A higher rate of low birth weight was observed for out-of wedlock infants. At all ages, unmarried mothers were more likely to deliver a low birth weight infant than married mothers (14.7 percent compared with 8.1 percent in Budapest and 15.5 percent compared with 6.4 percent in Vas). However, the difference in low birth weight rate between married and unmarried mothers is the highest in the group of women under the age of 20 years. The ratio of this age-group is much higher among unmarried than married mothers. (8.9 % and 4.3 % in Budapest; 39.4 % and 7.0 % in Vas).

Educational level

A relationship between the educational level of the mother and LBW rate was found in both regions (Figure 3). This relationship was statistically significant ($p < 0.01$) in Budapest.

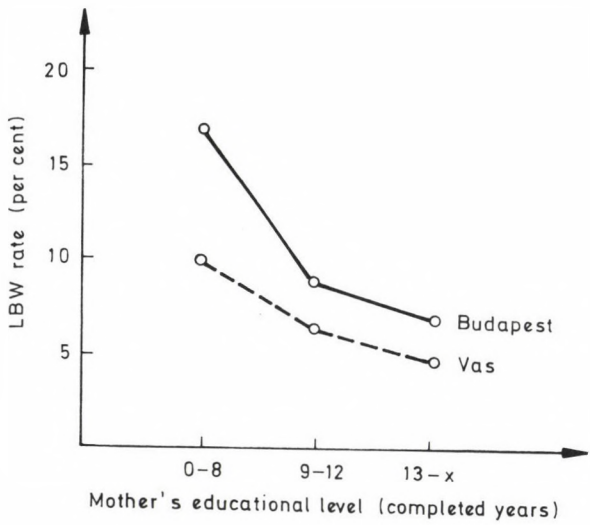

Fig. 3. Low birth weight rate of live births by mother's educational level in Budapest and Vas county

Smoking habits

In Budapest, the prevalence of smoking before pregnancy was the highest among females under 20 years (48 % in both regions) decreasing gradually with age. A similar trend was also noted in Vas. Thirty-four percent of the smokers in Vas and 25.3 % in Budapest stopped smoking during pregnancy.

Smoking before and during pregnancy was directly related to birth weight distribution. In both regions the incidence of low birth weight was higher among smoking mothers and increased with the number of cigarettes smoked daily (before pregnancy, from 9.1 % to 14.3 % in Budapest and from 8.2 % to 42.9 % in Vas; during pregnancy, from 8.3 % to 16.7 % in Budapest and from 16.0 % to 16.7 % in Vas). Decline in birth weight related to smoking was statistically significant in both regions ($p < 0.01$ in Budapest, $p < 0.001$ in Vas).

Employment status

Distribution by type of employment showed differences in the two regions.

The LBW incidence in case of women of manual occupation was higher than in case of non-manual workers in both regions (12.8 % compared with 7.7 % in Budapest and 9.4 % compared with 5.5 % in Vas). Difference in birth weight distribution between the workers' groups was statistically significant ($p < 0.01$ in Budapest, $p < 0.04$ in Vas).

DISCUSSION

This study was carried out in an effort to contribute to the available information on the aetiology of LBW by investigating the influence of some social factors and maternal variables on birth weight in two different regions in Hungary. It is known that differences exist in incidence of LBW related to mother's residence place in Hungary, the incidence being higher in rural areas than in urban areas. One example to this is Budapest, the capital, which shows a high rate of LBW and which is one of the

two regions selected for this study. The proportion of small for date babies in the LBW group was twice as high in Budapest as in Vas (47.7 % as compared to 25 %).

Gestational age determined as the number of completed weeks from the first day of the last normal menstruation to the date of delivery was accepted as reliable in this large sample.

Our analysis in this study included some social and maternal factors known to have an impact on birth weight. In each category analysed the effects of single and multiple factors have been studied. In this analysis of many factors known to be correlated with birth weight we concentrated on some social factors which showed differences in distribution in the two regions included in the study. One important difference between the two regions is that one of them is the capital and the other a county.

According to the data derived from the total group including both the Budapest and Vas county samples, the incidence of low birth weight varies by mother's age, number of previous pregnancies, birth interval, marital status, educational level, smoking habits and employment status. Multiple regression analysis was carried out to compare the effect of these inter-related factors (Table I). Smoking habit was found as the most significant variable related to birth weight. Smoking during pregnancy is widespread in Hungary. Its relationship to LBW, especially in the group of babies weighing 1500-2000 g at birth had been reported in previous studies /8/.

Mother's age, marital status and birth order were also among the major factors influencing birth weight. The incidence of LBW was higher among very young and older mothers, and, at all ages, among unmarried mothers.

The lower birth weight in third or further deliveries is well-known. Two-child families prevail in Hungary and a higher birth order is characteristic of mothers of a low educational level. Higher educational level of the mother was associated with a reduced incidence of low birth weight. Employment status was also a determinant of birth weight in both regions. Low birth weight rate was higher among manual workers as compared to the non-manual workers or to house-wives.

The effect of the various factors influencing the weight of the infant at birth was differently distributed in the two regions. Birth order, smoking habits and mother's age were found as the leading factors in the Budapest sample, while smoking habits, educational level and marital status were the factors of highest significance in Vas. Independently of these factors, LBW rate was higher in Budapest than in Vas.

This analysis was useful in bringing out the risk factors for LBW and in showing that the order of importance of these factors is not uniform in all societies.

ACKNOWLEDGEMENT

I am extremely grateful to Dr. O. Neyzi and to Dr. D. Schuler for their helpful advice in the design and realization of this study.

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Index: 26.021

Acta Paediatrica Hungarica

VOLUME 31, NUMBER 2, 1991

Akadémiai Kiadó, Budapest

ACTA PAEDIATR HUNG HU ISSN 0231-441X

ACTA PAEDIATRICA

HUNGARICA

A Quarterly of the Hungarian Academy of Sciences

Editor

M. MILTÉNYI

Acta Paediatrica publishes original reports of studies in paediatrics and borderline fields.

Manuscripts and editorial correspondence should be addressed to

ACTA PAEDIATRICA HUNGARICA

Bókay J. u. 53.
H-1083 Budapest, Hungary

Subscription information

Orders should be addressed to

KULTURA Foreign Trading Company
P.O. Box 149,
H-1389 Budapest

or its representatives

Acta Paediatrica Hungarica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ
Publishing House of the Hungarian Academy of Sciences
Prielle Kornélia u. 19–35.
H-1117 Budapest

Acta Paediatrica Hungarica is abstracted/indexed in Biological Abstract, Chemical Abstracts, Excerpta Medica, Index Medicus

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PRINTED IN HUNGARY

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Acta Paediatrica Hungarica 31 (2), pp 141-147 (1991)

ÜBER KINDERHEILKUNDE UND KINDERÄRZTE*

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Ich will hier und heute nicht etwa einen kurzen historischen Überblick über die Entwicklung der Kinderheilkunde geben - so reizvoll dies auch sein könnte. Es sollen vielmehr lediglich einige allgemeine Aspekte der Pädiatrie angesprochen werden, zugleich mit Blick auf die Jünger dieses Teiles der Heilkunde.

Wir Pädiater halten unser Fach mit Überzeugung für eine ars et scientia amabilis. Wenn ich dieses sage, hoffe und glaube ich natürlich, dass dies bei Vertretern anderer medizinischer Disziplinen im Prinzip entsprechend ist. Wir aber meinen, die Kinderheilkunde sei das anmutigste der klinischen Fächer.

Einige Charakteristika unseres Faches

Die Pädiatrie betreut und begleitet den Menschen von der Geburt bis in die Adoleszenz. Sie bringt ihre Adepten also wesensgemäss mit dem sich entwickelnden, wachsenden, reifenden und sich differenzierenden Menschen in dessen Gesamtheit zusammen. Über dieses Hauptpunkt könnte natürlich Viel und Detailliertes ausgeführt werden. Ich muss darauf verzichten, will aber betonen, dass wir Pädiater diese Aspekte immer wieder als unerhört faszinierend und stimulierend empfinden. Unser Fach hat hohe ästhetische Reize (und mehr oder weniger gegenteilige Erlebnisse und Eindrücke treten relativ ganz zurück).

*Vortrag gehalten anlässlich der Ehrendoktor-Promotion an der Medizinischen Universität, Pécs, Ungarn, 5. Oktober 1990.

Akadémiai Kiadó, Budapest

Unser Fach bringt uns - vor allem bei jungen Kindern - so gut wie immer auch mit den Müttern zusammen, und was gäbe es menschlich-Schöneres und auch Wichtigeres als speziell das Duo Mutter und Säugling?!

Als mein späterer klinischer Lehrer Jussuf Ibrahim (er entstammte der glücklichen Ehe eines ägyptischen Paschas und Hochschullehrers der Medizin mit einer Berlinerin) während des ersten Weltkriegs auf den Lehrstuhl für Kinderheilkunde in Jena berufen wurde, hielt er - 1917 - seine berühmt gewordene Antrittsvorlesung "Über die Mütter"[+]; sie enthält wohl eine der schönsten Lobpreisungen der Frau und Mutter[*], die es gibt. "Mehr als anderen Menschen ist dem Kinderarzt vergönnt, Blicke in das Wesen und die Tiefe der Mutterliebe zu tun. Alle Unterschiede der Bildung und des Standes schwinden und die reine Menschlichkeit tut sich ihm auf" (J. Ibrahim).

Nahezu selbstverständlich erscheint uns Heutigen jene Erkenntnis, die die erste Generation der Kinderärzte gefunden und immer wieder betont hat, dass nämlich das gesunde und das kranke Kind nur verstanden werden kann in seinen sozialen Bezügen, in seinen Bindungen und Wechselbeziehungen zur Mutter, zur Familie und zu den weiteren Menschen und den Dingen seiner sich immer mehr erweiternden Umwelt (H. Patzer, Jena-Erfurt).

Und dennoch wird eine Frau, die sich - wie es selbstverständlich sein sollte und überall möglich sein sollte - in dem Zeitabschnitt, in dem sie jüngere Kinder hat, ganz diesen ihren Kindern und ihrer Familie widmet (unter Verzicht auf Geldverdienst bzw. sog. "Selbstbestätigung" ausserhalb des Hauses) heute in manchen Ländern und von mancherlei "Zeitgeist" abschätzig beurteilt: als "unmoderne" Frau, als eine Frau, für die es angeblich "nur Kinder, Küche und Kirche" gibt.

Es obliegt also auch der heutigen Pädiatrie, immer wieder und mit grösstem Nachdruck darauf hinzuweisen, welch' unerhört wichtige soziale und gesellschaftliche Leistung gerade diese Frauen und Mütter erbringen. Der Prager pädiatrische Senior

[*] im Druck erschienen 1919; Nachdruck in dieser Zeitschrift 15. Jg. /1984/ 4: 518 - 522 und 5: 679 - 864.

Prof. Josef Švejcar hat bis in die allerjüngste Zeit immer aufs neue und mit bewundernswertem Elan die überragende Bedeutung der "Mutter-Kind-Dyade" hervorgehoben.

Die Kinderheilkunde bringt ihre Adepten mit Schwestern - älteren und erfahrenen und einer Vielzahl an jüngeren - zusammen, die ihr Leben oder doch einen Abschnitt ihres Lebens der Säuglings- und Kinderkrankenpflege gewidmet haben. Von seiner erfahrenen ersten Stationsschwester lernt der junge Pädiater oft Entscheidendes. In meinen Augen (und demgemäss an den von mir geleiteten Kliniken) hat die bewährte Kinderschwester immer als echte und unersetzliche Partnerin neben (also nicht etwa unter) dem Arzt gestanden. Dasselbe gilt natürlich sinngemäss für Physiotherapeutinnen u.ä. Fachkräfte. Es sollte natürlich überall so sein -, aber es ist wohl noch nicht überall so.

Säuglings- und Kinderkrankenschwestern sind im schönsten Fall - und diese Situation strebt die gute Schwester immer an und versucht sie zu erhalten - Vizemütter im umfassenden Sinne für die ihnen anvertrauten Kinder. Ich könnte ein hohes Loblied auf viele, viele solcher Schwestern singen, mit denen ich habe zusammenarbeiten dürfen: Welche Pflegefähigkeit, wieviel tröstende, stärkende und heilende Kräfte sind in weiblichen Menschen geborgen!

Dieser letzte Satz soll gleich für den folgenden Punkt mitgelten: Die Pädiatrie ist die klinische Disziplin mit dem höchsten Anteil an Ärztinnen und es ist nun schon ausser Zweifel, dass ich darin ein grosses Positivum sehe - das keiner weiteren Erläuterung mehr bedarf.

Übrigens haben das jugendliche Alter der pädiatrischen Patienten und auch von deren Müttern, die relativ hohe Zahl in der Kinderheilkunde arbeitender junger Schwestern und Schwesternschülerinnen sowie der hohe Anteil - er kann 50 Prozent und mehr erreichen - in Kinderkliniken neben den Assistenten arbeitenden Assistentinnen zwei "Nebenwirkungen", über die, obwohl ich es ernsthaft meine, gern gelächelt werden darf:

Zum einen erhält die Kinderheilkunde, insbesondere die klinische Pädiatrie, die in ihr Wirkenden nach vielfachem

Eindruck über-durchschnittlich lange jung,
und zum anderen erliegt ein relativ hoher Anteil der in pädiatrischen Kliniken arbeitenden jungen Menschen den Reizen des mit ihnen am Kinde zusammenwirkenden anderen Geschlechts: Kinderärzte heiraten überdurchschnittlich häufig sei es eine Conassistentin, eine Kinderschwester, eine Physiotherapeutin oder eine medizinisch-technische Assistentin oder sonstige Fachkraft der gleichen Klinik. Ich will keineswegs behaupten, dass alle diese Ehen in den Himmel führen; aber mehr-minder gleichgerichtete Interessen und vermutlich auch aus gleicher Freude an Kindern ein beiderseitiger Wunsch nach eigenem Nachwuchs sind ja wohl gute Voraussetzungen. Wo aber könnten ein junger Kinderarzt und eine junge Ärztin oder Schwester näher und gründlicher Kinder beobachten und an ihnen weiter lernen als an eigenen Kindern?!

Aufgaben der Pädiatrie

Das Spektrum der Aufgaben der Pädiatrie kann in verschiedenen Ländern sehr verschieden sein. Es ist aber immer breit - und es ändert sich laufend. Herrn Prof. Méhes und mich hat die medizinische Genetik miteinander verbunden. Wir sind uns aber gewiss ganz einig, dass z.B. die Prävention exogener Kinderschädigungen durch den häufigen Alkohol- und den immer häufiger werdenden Nikotin-Abusus von Mädchen und Frauen nicht minder wichtig sind.

Wahl der Pädiatrie als Beruf

Lassen sich einige Voraussetzungen für die Wahl der Pädiatrie als Beruf aufführen?

Wer Kinderarzt werden will, sollte sich zu Kindern hingezogen fühlen, sich für Kinder interessieren, ja, Kinder lieben. (Ich schaue noch jetzt nach Möglichkeit in jeden Babywagen, versuche ein kurzes Gespräch mit der Mutter und

bewundere ihr Kind.)

Er sollte aber auch eine "Hand" besitzen vor allem für junge Kinder. (Ich erlebte einen Assistenten, einen sonst vortrefflichen jungen Mann, der mit Säuglingen einfach nichts anzufangen wusste; er hatte Angst, sie in die Hand zu nehmen und musste schliesslich ausscheiden.)

Der Adept sollte auch ein gewissen pädagogisches Interesse haben oder entwickeln; denn - spätestens seit Adalbert Czerny (Breslau, Berlin) - soll der Pädiater sich (im Rahmen des Möglichen und Erlaubten) auch als ein Erzieher des Kindes fühlen und diesen wichtigen Bereich nicht völlig an den Klinikpsychologen abtreten.

Er muss "Herz haben". Denn er hat sich ja auch vielen schwerkranken oder behinderten Kindern und deren Angehörigen zuzuwenden; sein Wissen und Können sollen also an die Kräfte des Menschlichen gebunden sein. Er muss anzuerkennen bereit sein, dass es in dieser Welt immer auch Schwergeschädigte, Fehlgebildete und Schwerbehinderte geben wird und dass deren Lebenssinn nicht zuletzt darin liegt, unter uns sog. Gesunden Mitleid, Barmherzigkeit und Aufopferung als edelste menschliche Eigenschaften hervorzurufen.

Der eine leitende klinische Funktion anstrebende Pädiater muss sich für geeignet halten und verpflichtet fühlen, für einen guten Geist des freundlichen Miteinander aller Mitarbeiter - also für ein gutes seelisches Klima des Hauses - zu sorgen. Ein solches ist an Kinderkliniken ganz besonders wichtig, weil eine Atmosphäre blosser Sachlichkeit oder gar teilweiser Unfreundlichkeit und Intrigen sich auf Kinder als besonders hilflose Patienten ausserordentlich ungünstig auswirken würde. Man wird feststellen dürfen, dass in den allermeisten pädiatrischen Kliniken und Abteilungen ein solcher guter Geist tatsächlich herrscht!

Einige Punkte zum Abschluss

Wer ein hohes Einkommen anstrebt, sollte nicht Kinderarzt werden (zumindest in der Bundesrepublik Deutschland liegen die

Pädiater im unteren Drittel der Verdienstskala der medizinischen Disziplinen). - Wenn, sei es in Auswirkung äusserer Ereignisse, sei es durch Einflüsse des "Zeitgeistes", die Geburtenziffer drastisch zurückgeht - so gab es in den siebziger Jahren in Westdeutschland infolge verminderter Motivation zum Kinde ein Minus von reichlich 1/2 Millionen Säuglingen -, dann werden die Pädiater dies rasch und intensiv spüren (bei uns musste damals der eine oder andere seine Praxis aufgeben). - Natürlich werden der tüchtige Kinderarzt, die tüchtige Kinderärztin aber in aller Regel ihr wirtschaftliches Auskommen sehr wohl finden!

Bei jeder entsprechenden Gelegenheit heisst es, modificatis modificandis, wie folgt: "Die Gesundheit unserer Kinder ist unser höchstes Gut, daher gilt unseren Kindern von Geburt an unser höchstes Augenmerk!" So sprechen die Herren Minister, die Herren Oberbürgermeister und andere Inhaber hoher Funktionen und Stellungen. Wir Pädiater kennen solche schönen Worte zur Genüge und ich will auch nicht etwa sagen, dass sie Heuchelei seien. Indessen: Wenn es darum geht, ob eine Einrichtung für Erwachsene oder für Kinder geschaffen werden soll bzw. ob zuerst für Kinder gebaut oder in sonstiger Hinsicht Geld ausgegeben werden soll, dann siegen in aller Regel die Anliegen der Erwachsenen - der Erwachsenen, die sich selbst eben doch am nächsten sind. "Betrachtet man im Blick auf die entscheidungswirksamen Wertmass-stäbe die heutige Situation der Kinder, so rangiert deren Wohl und Wehe weit hinter einer langen Reihe von anderen Werten" (B. u. H. Hassenstein*). Mit anderen Worten: Kinder - noch keine politischen Wähler! - sowie Kinderärzte und Pädiatrie können leider keine kräftige Lobby aufweisen.

Alles hier und heute Gesagte gilt wie für die interne Pädiatrie und die in ihr wirkenden Pädiater praktisch genau so auch für die Kinderchirurgie und unsere Kollegen, die Kinderchirurgen. -

* "Was Kindern zusteht", Piper/München 1978, S. 157.

Wir Ärzte gehören zu denen, für die es mit Johann Wolfgang v. Goethe heisst "Wer beschützet und erhält, hat das schönste Los gewonnen". Dieses und teilweise ohne unser besonderes Verdienst zuteilgewordene freundliche Los und die damit gegebene Bevorzugung und Verpflichtung wollen wir dankbar immer wieder in unser Bewusstsein heben.

Für die Kinderheilkunde möchte ich abschliessend ein kleines Gedicht verlesen:

Kinder in ihrer Gesundheit stützen
Kinder nach Kräften vor Krankheit schützen
Kinder - erkrankt - zur Gesundung leiten
immer aber sie treulich begleiten

Hüten - fördern - bewahren - erhalten
raten und trösten und sorglich walten
Handeln für Kinder Wirken fürs Leben
kann es schönere Aufgabe geben?

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Acta Paediatrica Hungarica 31 (2), pp 149-158 (1991)

THE CARE OF INFANTS AND CHILDREN*

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Received 5 May 1990

The growth and development of pediatrics took place principally in the twentieth century. One result has been the reduction in deaths in the first year of life 165/1000 live births to 10/1000 live births in 1987. The birth rate was reduced by one-half during the same period. The challanges of the future are to consider the needs of American children in the context of the world's children, since isolation from global problems associated with logarithmic population growth in the developing countries and maldistribution of food is no longer possible. The time has come to consider the limits to application of our ever more sophisticated technology to support life at both ends of the spectrum of human life, the most immature and the most debilitated elderly. Human behavior continues to be unnecessarily destructive for children. Congenital AIDS and drug-abusing parents are catastrophic for the infant. Accidents remain the leading cause of death of children in America. Deaths from diarrheal diseases and malnutration are the main causes of death of children in developing countries. Nearly all of these problems are preventable with the application of current knowledge. How could we have let the best interests of children slip so far down on our national list of priorities in health care?

Thank you for the honor of participating in this celebration and sharing my views (prejudices) about the seminal events in doctoring infants and children that took place through the past century, and to reflect on circumstances that will influence what we need to do in the next century.

*Presented at the centennial of Johns Hopkins Medicine Baltimore, Maryland
Symposium: Doctoring America: The last 100 years and the next 100 years (February 23-24, 1990)

Akadémiai Kiadó, Budapest

Past century

Pediatrics emerged as a distinct branch of medicine in the United States during the second half of the nineteenth century. The scientific underpinnings of knowledge about infants and children began under Holt in New York at Babies Hospital and was given an enormous boost when John Howland and McKim Marriott moved to Johns Hopkins and established a department of pediatrics by full-time clinicians and investigators /1/. Let me illustate with a few of the contributions of pediatricians in the 20th century. For example, in 1916 the demonstration by Howland and Marriott that the acidosis that accompanied diarrhea resulted from bicarbonate loss in the stools, led to the insight into the Chemical Anatomy of Extracellular Fluid by Gamble in 1939, which opened the way to safe and effective intravenous therapy /2,3/. The extension of that knowledge, largely by Darrow and Harrison, has made possible the more recent nearly universal application of oral rehydration therapy, which promises to do for the developing countries what was achieved many years ago in this country, is to decrease dramatically deaths from the dehydration that accompanises diarrhea.

The evolution of subspecialties within pediatrics has been an important advance necessitated by the ever-increasing knowledge made possible by pediatric research. One of the first of the subspecialties was pediatric cardiology, which made possible cardiac surgery. The many contributions of the surgeons were in turn helped by development of extracorporeal oxygenators, intravenous alimentation, ever-better antibiotics, and the availability of better imaging, and interpretation made possible by pediatric radiologists /4/.

To mention just a few examples of pediatric research the increased understanding of nutritional needs of infants and children, including the role of vitamins and the subsequent elimination of rickets, was largely achieved by pediatricians, including Drs. Martha Eliot and Edwards Park.

Of course, all of mankind has profited from the increased awareness of the role of infectious diseases and the control of

some of them, at least, by appropriate antibiotics and immunization. Children have been the major beneficiaries of effective vaccines against polio, measles, diphteria, tetanus, rubella, mumps and in some instances, meningococcemia and pneumococcal disease.

The most dramatic application of existing knowledge was accomplished under the leadership of Dean Henderson and the Health Organization in the eradiction of smallpox from planet earth. Never again will humans have to be vaccinated since the virus does not exist in the wild state. The lack of an animal reservoir of infection, the ability to make correct diagnosis, and the availability of effective immunization, is also possible for polio and measles. WHO/UNICEF have embarked on a program for universal immunization, with the expectation of eliminating these diseases which remain leading cripplers and killers in developing countries.

The discovery of major blood groups by Landsteiner made possible Hart's first successful exchange transfusion in an infant affected by severe jaundice in 1925. Understanding of the pathogenesis of neonatal icterus gravis awaited the discovery of the Rh antigens by Landsteiner and Weiner in 1940. It was not until 1946 however when Diamond and colleagues introduced the technique of umbilical vein catheterization that the procedure gained wide usage /1/.

Perhaps the most important discovery of the century took place in 1953, when Watson and Crick discovered the structure of DNA and introduced modern medicine to the whole new world of molecular biology. Another major step forward was achieved within the past few years in the use of reverse genetics to identify the gene product which are responsible for Duchenne muscular dystrophy /5/ and cystic fibrosis /6/. (Formerly, knowledge of the gene product was needed to point the way to localization of the gene). Insights into hereditary disease are most important for the professions of obstetrics and pediatrics since identification of carriers and prenatal molecular diagnosis is now possible for a number of conditions that can be prevented by abortion; on the horizon is gene replacement therapy for at least some diseases.

The list of major advances in pediatrics must include the introduction of aminopterin by Farber and Diamond in 1948, which was the first time chemotherapy was effective for cancer. Advances in the treatment of leukemia in particular have been dramatic and cancer chemotherapy for all ages is now undergoing extensive evaluation with ever-increasing efficacy.

In neonatology, there has been an increasingly agressive approach to diagnosis and therapy of specific conditions made possible by new knowledge of adaptations to extrauterine life pioneered by the English physiologist, Sir Joseph Barcroft. New technologies including micromethods for measurement of blood constituents and equipment designed to meet the needs of ever-smaller infants have made monitoring of changing conditions possible. Better incubators, ventilators, and new drugs have been life saving. Understanding of nutritional needs and meeting them with intravenous alimentation has made it possible for ever-smaller infants to survive and expect a normal lifespan.

Other examples include the reduction in deaths from hyaline membrane disease, with approximately 10 000 per year during the years 1969-73 in the United States reduced to 5 000 per year 1979-83 and an estimated approximately half that number when pulmonary surfactant replacement therapy becomes widely available within the next few years (Table I).

The reduction in mortality in low birthweight infants has been achieved not only by new technologies, but also by regionalization of intensive care, with the establishment of centers staffed by trained personnel and stage-of-the-art equipment to which babies born in outlying hospitals can be transferred safely. The package of services provided by neonatal intensive care has made possible the major reduction in mortality and morbidity among low-birth weight infants (Table II). It is unfortunate that so little effort has been expended on the most desirable goal of reducing rates of low-birth weight.

We must acknowledge that accidents account for about half the deaths of children in North America. Although poisonings

TABLE I

Annual RDS/HMD specific mortality
(U.S. vital statistics)

	1969-73	1974-78	1979-83
White	7880	5945	3837
Black	1989	1897	1345
Total	9993	7962	5271
Rates/1000 live births	2.89	2.47	1.46
% change in rates within 5-year period	+2.7	-9.4	-8.8

TABLE II

Survival by weight group
July 1, 1987 to June 30, 1988

No.	Weight groups	% Home from hospital
3	501-600	13
6	601-700	62
12	701-800	66
13	801-900	74
18	901-1000	91
25	1001-1100	> 90

Total births 9808

% Births under 2.5 kg 10 %
under 1.5 kg 4 %
under 1 kg 1 %

Data from Brigham and Women's Hospital, Boston Courtesy of Drs. M. Epstein and E. Lieberman

have been reduced through systematic health education and poison control centers, maternal substance abuse, one of the worst forms of poisoning is now reaching epidemic proportions particularly in our inner cities and especially with cocaine /7/. Approximately 15-20 % of infants delivered at Boston City Hospital have metabolites of cocaine identifiable in their urine. The infants are often born prematurely; they are usually somewhat small for gestational age, often irritable, occasionally have a high-pitched piercing cry, and they may have central nervous system problems that occur while still in utero /8,9/. Coupled with the wide use of intravenous drugs, especially, heroin, is the increase in promiscuity and the excess risk of HIV infection imposed on that same group of mothers and infants /10/. To be born addicted to cocaine and have AIDS as well, with predictable mortality within the first years of life, remains a totally preventable catastrophe.

Future century

It is clear that the world's population is growing logarithmically especially among those with the least opportunity for access to good medical care or education.

The world's population was approximately 3 billion in 1960, and exceeded 5 billion in 1985. Most of this increase was in the developing world. It is estimated that enormous numbers of children in the developing world are inadequately fed, and deaths from malnutrition continue to occur in the wake of man-made disasters such as war, but also in the presence of drought and a failed food distribution system. In developing countries in the 1980s, one death in every 3 was a child under age 5 years.

There is ample evidence of complacency or lack of awareness of the magnitude of these problems, particularly as they affect children. For example, one important solution is the urgent need to have widely available safe birth control. Fortunately, the "pill of choice" RU 486 is available, an estimated 25 000 women have taken it in France, where it is found to be 96 % effective /11,12/. It is specific for inducing termination of pregnancy by completely blocking a receptor for progesterone

and can be most effective within the first 9 weeks of pregnancy. The research for the development of this compound was supported by the Ford Foundation and the French government, as well as the pharmaceutical industry, but it is not available in the United States except by the black market through China. There are no U.S. funds to support the WHO special pogram on human reproduction and minimal U.S. funds available for study of RU 486 in this country. All of this is in the context of an estimated 200 000 maternal deaths per year worldwide, half of which are estimated to be from unsafe abortions /13/.

It is worth considering what is being done to improve child health, and thus in due course reduce the numbers born. I already mentioned that UNICEF is active in eradicating polio and measles. Perhaps as important as their program for immunization is their ongoing advocacy of oral rehydration therapy in countries where deaths from dehydration remain prominent. The teaching of maternal and child health with respect to the use of growth charts and the benefits of breast feeding and family spacing are making headway around the world. Fortunately, decrease in fertility rates usually follows the reduction in infant mortality, where as an example, Japan in 1940 had 90 deaths per 1000 live births and parents had on the average of 4 children; whereas in 1988 the comparable figures are 5.5 per 1000, and the average number of children are 1.8 per couple /14/ the same has been the case in the U.S. (Table III).

TABLE III

Year	Birth rate (Per 1000)	Births/woman (Fertility rate)
1790	55	8
1900	30	-
1940	20	2.3
1950	22	3.2
1957	22	3.7
1970	19	2.5
1985	15.5	1.8

While we acknowledge the enormously important needs of children around the world, we in the developed world at the same time are focusing considerable attention on the provision of intensive care for ever more immature infants. One of the advances only recently made available in the United States, (but used for several years in Japan) pulmonary surfactant replacement therapy, promises to produce ever more survivors of 25- and 26- week gestational age. This poses a problem with respect to definition of the lower gestational age or weight limit for which intensive care should be mobilized. Complex, ethical dilemmas surface with regularity in nurseries. For example, is the preterm infant of 25 weeks of a couple with a history of infertility, long awaited, a better candidate for surfactant replacement than a similar preterm infant born after an unwanted pregnancy in a 16-year old? Who will care for either infant? What are the chances of a normal outcome, or does that matter? Should every infant born alive at 24 weeks be given surfactants, regardless of other problems that may be present? Making the lung function precociously does not ensure normal brain development for example. In a society where we are not prepared to provide optimal supports for the child after discharge from the hospital, let alone assurance that the child will achieve education sufficient to become some kind of a competitor in society, does it make sense to provide intensive care to infants under 26 weeks or 25 weeks or 24 weeks gestational age?

What do we do? Many neonatologists try to come to some kind of consensus by taking into consideration the specific facts surrounding the given child in consultation with parents and their advisors. But we remain concerned about the paradox of extraordinary effort for the borderline viable infant in a world that allows so many infants to die of neglect.

In conclusion, what will "doctoring" become in the next 100 years? As Leon Eisenberg has noted "at the root of it is the need to recognize that doctoring is but one element in the envelope of care that should surround every child? The health of infants and children will be dependent on massive societal efforts to eliminate war and other forms of violence, to reduce

poverty, to promote birth control, and to foster education. I believe it also mandates a continued quest for more knowledge so that babies will not only be well-born, but well-cared for and supported thereafter. We are experiencing, in neonatal intensive care at least, "a half-way technology", to quote Lewis Thomas, and the only way ahead, I beleive is for more new knowledge and a hope that society will use that knowledge wisely. On issues of the sort I just addressed, I tend to recall Mr. James Rouse's comment on one occasion. When I asked how he managed to convince the residents and land owners of the need for inner city rejuvention, for which he has been so responsible, his answer was, "there is no such thing as an opponent; only an ill-advised citizen".

Walt Whitman had some wise words on these issues:

Now understand me well - it is provided in the essence of things that from any fruition of success, no matter what, shall come forth something to make a greater struggle necessary.
(from Song of the Open Road)

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Acta Paediatrica Hungarica 31 (2), pp 159-168 (1991)

NEONATAL ABDOMINAL CYSTIC MASSES: SPONTANEOUS REGRESSION DEMONSTRATED WITH ULTRASOUND

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Received 4 January 1990

Abdominal cyst was diagnosed in 14 babies by ultrasound examination (9 multicystic dysplastic kidneys, 4 ovarian cysts, 1 bowel duplication). The cysts were followed in 6 patients by ultrasound and marked reduction of multicystic dysplastic kidneys was observed in 2 patients, and 1 ovarian cyst fully disappeared. Their experience in agreement with the literature data suggest that conservative management with sonographic reevaluation is an acceptable alternative to surgical therapy in uncomplicated cases.

INTRODUCTION

With the increased use of sonography prenatal and postnatal detection of neonatal cystic masses has extended, and asymptomatic cystic masses are being discovered. In one part of the cases the serious complications can be prevented by an early surgery, on the other hand, the mass may spontaneously disappear and conventional treatment is satisfactory.

Neonatal abdominal cystic masses are: ovarian cyst, mesenteric and duplication cysts, cystic teratoma. In the cases of renal cystic masses one has to distinguish multicystic kidney, hydronephrosis, polycystic kidney disease. The basic condition of the sonographic follow-up is the correct diagnosis.

PATIENTS AND METHODS

14 patients with abdominal cystic masses were admitted to the I. Department of Pediatrics between November 1987 and August 1989. At the time of the first investigation the patients' age ranged from 6 days to 3 months. There were 8 females and 6 males. Sonography was performed with a 5 MHz real-time mechanical sector scanner (Combison 310, Kretz). The diagnosis was multicystic kidney in 9 cases, ovarian cyst in 4 cases, enteric duplication in 1 case. The sonographic findings of the multicystic kidney include great number of cysts varying in size and shape, absence of connection between cysts, absence of renal parenchyma.

The sonographic finding was echo-free in one case of ovarian cyst, 3 cysts contained internal echos, by a fluid-debris level, internal septation.

The sonographic appearance of the duplication cyst was echo-free, mobile, surrounded by an echogenic wall.

RESULTS

In 7 neonates, the multicystic kidney was discovered in utero (Table I). All of the patients presented a unilateral flank mass, 4 patients had undergone surgical exploration because of renal insufficiency/or intestinal obstruction caused by the very large tumor mass. 1 patient had undergone surgery at age of 1 year, because the extremely great size of the cysts did not change during the follow-up period. Clinical, ultrasound and surgical findings in 4 cases of multicystic kidneys without surgery are summarized in Table I. The cases are illustrated in Figs. 1-2. In two patients by the age of 1 year and by the age of 18 months, respectively instead of the earlier several large cysts only one small cyst in each could be observed.

Clinical, ultrasound and follow-up findings in 4 cases of ovarian cysts are summarized in Table II.

In two cases the cysts were detected during postnatal sonography. Both had asymptomatic, palpable masses, the sonographic appearance supposed torsion. They underwent surgery which proved twisted ovarian cysts. 1 patient was examined by ultrasound at the age of three months, because of the

TABLE I

Clinical, US and follow-up findings in 9 cases of multicystic kidneys

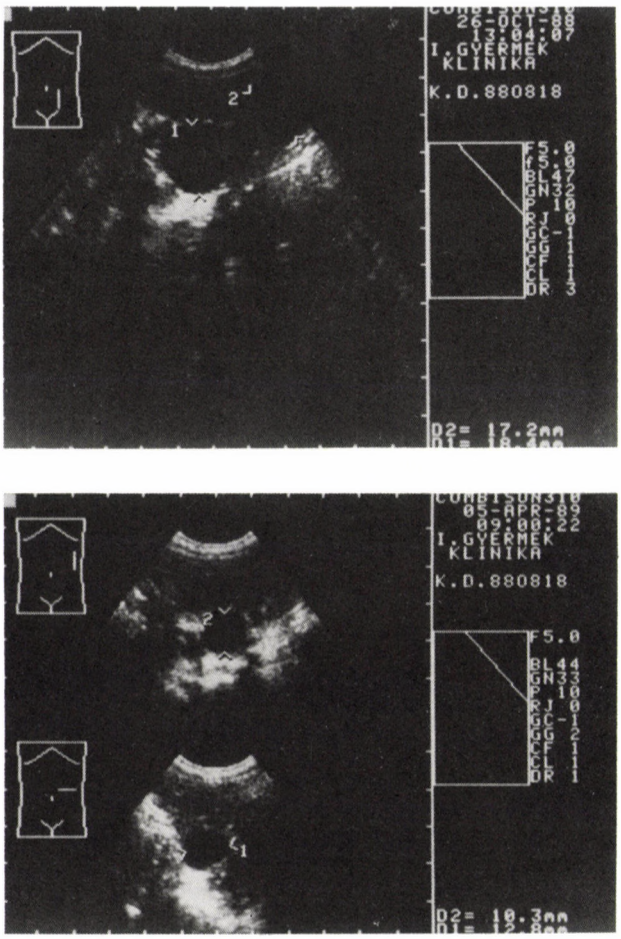
Case	Age at discovery/ Sex	Clinical presentation	US Findings Initial	US Findings Subsequent	Follow-up Findings
1.	prenatal, male	palpable mass	multiple large cysts, right side	same	surgery at age 1 year
2.	prenatal, male	palpable mass	multiple large and small cysts, left side	at age 1 year: small cysts	at age 2 years: one small cyst
3.	newborn, female	palpable mass	multiple cysts, right side	-	surgery
4.	prenatal, male	palpable mass	multiple cysts, left side	at age 5 months: small cysts	at age 18 months: one small cyst
5.	prenatal, female	palpable mass	multiple cysts, right side		surgery at age 3 weeks/intestinal obstruction/
6.	newborn, male	palpable mass	multiple cysts, left side	at age 7 months: no change	
7.	prenatal, male	palpable mass, renal insuff.	4 cm cyst, left side	-	surgery
8.	prenatal, female	palpable mass	multiple cysts left side	at age 2 months: no change	
9.	newborn, male	palpable mass	5 cm cyst right side	-	surgery

TABLE II

Clinical, US and surgical findings in 4 cases of ovarian cysts

Case	Age at discovery	Clinical presentation	Diameter in cm	US findings	Follow-up findings
1.	intrauterin	asymptomatic	3	echo-free	at age 6 months -3 cm at age 1 year -6 mm
2.	1 week	asymptomatic palpable mass	6	fluid-debris level, septa	surgery - twisted cyst
3.	3 months	side-finding	3	echo-free internal echos, septa	ureter obstruction, hydronephrosis surgery-twisted cyst
4.	1 week	asymptomatic palpable mass	4	fluid-debris level, septa	surgery-twisted cyst

possibility of right side diaphragmatic hernia (patient 3. Table II). She had relaxation of diaphragm, and in addition a



Figs. 1-2. 1./ left parasagittal section of the abdomen: large cysts, no renal parenchyma
2./ at the age of 1 year: left sagittal sonogram shows one 10 mm cyst

left side hydronephrosis connected to a cystic mass with internal echos was seen under the left kidney (Fig 3.). The investigation was reapeated on the next day, the hydronephrosis was moderated and the cyst was located in the right pelvis. The

child was operated on, a twisted ovarian cyst was removed, which previously by the compression of the ureter caused the left side hydronephrosis.

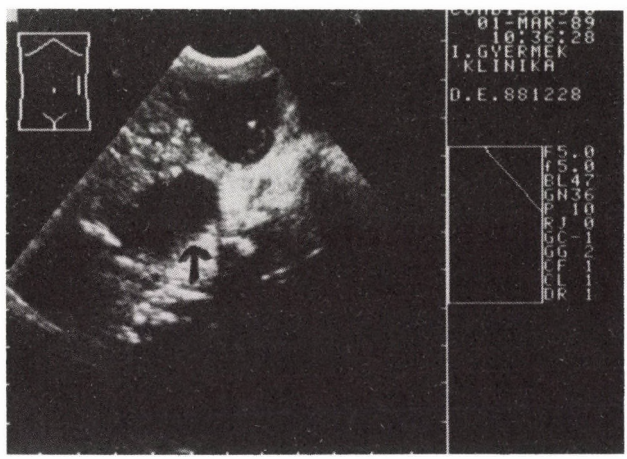


Fig. 3. left parasagittal section of the abdomen: hydronephrosis /↑/, under the kidney echo-free ovarian cyst, with internal echogen ring

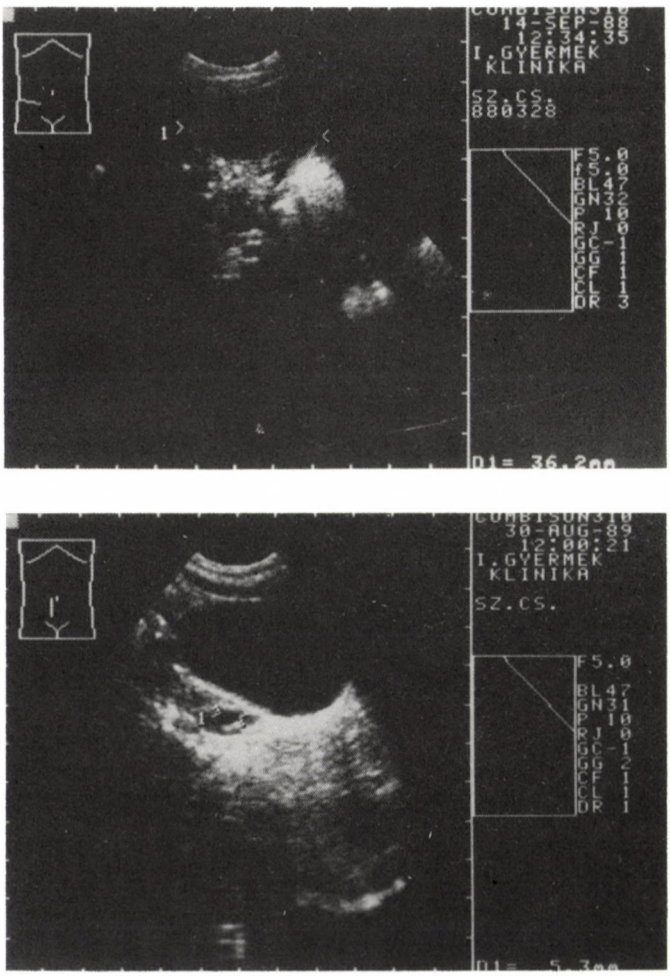
In 1 infant the ovarian cyst (3 cm in diameter) was discovered in utero. She was asymptomatic, followed by ultrasound examinations, the cyst disapeared within 1 year of discovery (Figs 4, 5.). The infant diagnosed to have intestinal duplication has undergone surgery.

DISCUSSION

Multicystic dysplastic kidney is usually discovered on the first day of life. In some cases the condition may however, be undetected until adulthood, when it is diagnosed incidentally. Usually it is unilateral; when bilateral, the outcome is lethal.

Two types of multicystic kidneys have been identified: a dysplastic type with non-communicating cysts, and a

hydronephrotic-obstructive type, with possibly communicating cysts /2, 4, 6, 11/. Previously the therapy was nephrectomy. In most cases the diagnosis can be established by ultrasound, followed by nuclear scintigraphy. One has to differentiate it from hydronephrosis, Wilms tumor, mesoblastic nephroma, polycystic kidney. Once the diagnosis has been set up, the subsequent treatment ranges from nephrectomy to conservative observation. According to the literature, nephrectomy should be reserved for those patients, whose multicystic dysplastic



Figs. 4-5. 1./ newborn girl, ovarian cyst in diameter 3 cm
2./ at age 1 year: 6 mm cyst

kidney causes complaints (pain, pressure, infection). In asymptomatic cases a conservative therapy is recommended /10, 12/. Vinocur et al. reported about 30 patients, 19 cases were followed up without surgery, in two patients the mass disappeared /12/. They did not notice infection and hypertension either. Pedicelli et al. reported on 9 patients, followed up by ultrasound, in 6 patients the cysts disappeared /10/. They speculate that some cases of renal agenesis may represent a complete resorption of multicystic kidney.

In some adults malignant transformation of multicystic kidney is occurring. Considering the frequency of multicystic kidney and the extreme rarity of its malignant transformation, surgery is not proposed unless other reasons (mechanical obstruction, infection) indicate it, rather an observation time of at least 1 year is suggested. Our cases also support this observation, in two of our nine cases the cysts have become smaller or fully disappeared, and in 3 the cysts were early removed because of renal insufficiency/or intestinal obstruction.

The most common abdominal cysts in newborn girls are the ovarian cysts. Earlier it was considered to be a rare disease, only 71 cases have been reported in the literature until 1976 /8/. Ovarian cysts were discovered only if they were palpable, caused symptoms because of torsion or rupture, or were associated with intestinal obstruction.

With the increased use of sonography, asymptomatic cystic ovarian masses are being discovered more often. One part of them are not clinically significant, and may involute, their conservative treatment after birth is satisfactory /1, 3, 5, 8, 9/. The differential diagnosis includes mesenteric and duplication cysts, cystic teratoma, dilated bowel loops. The size of a neonatal ovarian cyst varies considerably from some millimeters to more centimeters: it could be asymptomatic, but a large cyst can be associated with torsion, vomiting, intestinal obstruction, pain, fever. One of our cases caused hydronephrosis by the compression of the ureter. Most of them can be mobile and situated on the contralateral side. In some cases, mobility of the cyst was evident for us during real-time

sonography /9/. The sonographic appearance of an ovarian cyst varies, primary depending on whether the cyst is uncomplicated or complicated by torsion or hemorrhage. An uncomplicated cyst is echo-free, its wall is thin. A complicated cyst contains a fluid-debris level, retracting clot or septa and often has an echogen wall. From the time that to spontaneously regress, small, uncomplicated cysts have been reported, sonographic distinction between twisted and non-twisted cysts may be of value in therapeutic management /8/.

One of the potential risks in conservative management is that the cystic lesion is not an ovarian cyst. Malignancy is extremely rare in infancy, and usually does not take the form of fluid-filled mass. Mesenteric and duplication cysts may be sonographically indistinguishable, however, because this masses are benign, conservative management is not hazardous. Another risk is the possibility for an uncomplicated cyst to undergo torsion during the period of observation. In this case, however, the sonographic caracter of the masses will change, and thus detection of the complication will be possible /8, 9/.

According to the literature data, as well as, to our experience the conservative management with the sonographic reevaluation is an acceptable alternative to surgical therapy in uncomplicated cysts measuring less than 5 cm in diameter. The spontaneous regression of these cysts can be expected by all probability. For larger cysts no conventional treatment, rather surgery is suggested /8/.

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Acta Paediatrica Hungarica 31 (2), pp 169-186 (1991)

THE DIAGNOSIS AND TREATMENT OF THE NONIMMUNE HYDROPS FETALIS

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Received 14 November 1989

Fetal hydrops is associated with two distinct pathophysiologic situations. The isoimmune hydrops fetalis is a well understood disorder, and as the result of medical advances and prophylactic therapy its frequency is diminishing. The nonimmune hydrops fetalis is a poorly understood disease with a bad prognosis. The two disorders can be differentiated with the indirect Coombs test. In both cases the ultrasound examination plays an important role in the diagnosis, prognosis and management.

Examination of the fetal blood sample gives recently a possibility to approach the disease. In NIHF the examination of fetal blood sample would give a relatively quick and effective diagnosis but its value for the treatment is limited.

Although with the present technology it is impossible to diagnose all cases of NIHF, the early recognizing, the careful and step by step investigation, the active perinatologic management mostly can show the etiology and can help the perinatal team at the treatment of the disease.

Abbreviations
NIHF = nonimmune hydrops fetalis
IIHF = isoimmune hydrops fetalis
TORCH = toxoplasma, rubeola, cytomegalis herpes

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INTRODUCTION

The latin term "hydrops fetalis" means an excessive fluid accumulation into the extravascular compartment and the body cavities, leading to the development of anasarca, ascites, pleural or pericardial effusion. The hydrops is called "nonimmune" if there is no feto-maternal blood group incompatibility, therefore the haemolysis of fetal red blood cells and the anaemia of the fetus are not the result of a maternal IgG antibody response.

Though Ballantyne wrote about the heterogeneity of the conditions of fetal hydrops about 100 years ago /2/, Potter was the first who made acquainted in details with the nonimmune hydrops fetalis (NIHF) disease /30/.

Because in Hungary the description of this disease has not been described until now - probably because of the rare occurrence - we aimed to survey the material of the Department and review the experiences with the NIHF.

PATIENTS AND METHOD

In the Department of Prenatal Diagnostic and Therapy of the University Bonn 402 cases were diagnosed prenatally in the last 9 years. After the ultrasound examination the first step was to determine the maternal anti-D titers (indirect Coombs antibody screen) to exclude the diagnosis of the isoimmune hydrops fetalis (IIHF). After this step in a logical sequence (later detailed) from the noninvasive methods to the use of the invasive techniques, it would be possible to reach the correct diagnosis in most of the cases.

Frequency

Until recently the disease was very rare. However, with the introduction of the anti-D IgG gamma globulin the cases of

isoimmunization decrease parallel with an increasing percentage of hydropic patients from other causes /1/. With the general use of the ultrasound examinations a lot of NIHF cases were diagnosed earlier registered as unexplainable intrauterine death. In Norfolk (USA) the ratio of the NIHF to IIHF was 9 to 1 in 1986 /36/. In the Australian population the incidence of NIHF is one out of 3538 newborns /24/, while one newborn out of 2566 deliveries in the Los Angeles area /25/.

By the examination of the etiology of the nonimmune hydrops fetal cases, Holzgreve et al classified as idiopathic 50 percentage /16/, while others classified 80-85 percentage as idiopathic /15, 16, 18/.

DISCUSSION

Etiology

Numerous causes of NIHF have been described /16/. The possible causes of NIHF in the literature data as well as in our own experiences are summarized in Table.

Diagnosis

The usual presentation of the NIHF is polyhydramnion or a decreased fetal body movement leading to an ultrasound examination and discovery of NIHF. Most frequently the diagnosis is made by ultrasound requested for routine or another cause.

The most striking sign is the ascites. The most characteristic picture of the ascites is a large amount of intraabdominal fluid and the free floating or compressed bowel (Figure 1). With a good equipment it is possible to detect the fetal ascites as little as 100 ml /8/. The judgement of the punction of the ascites is ambiguos. According to the general opinion the punction of ascites during pregnancy is unlucky, because of a rapid reaccumulation. However, just before the

TABLE

Diseases playing role in the developing of nonimmune hydrops fetalis

A./ Maternal disease
- diabetes mellitus
- EPH gestosis
- severe anaemia

B./ Placental causes
- umbilical vein thrombosis
- chorionangioma
- true cord knots

C./ Fetal causes

1. Cardiovascular
   - severe congenital heart disease (atrial septal defect, interventricular septal defect, hypoplastic left heart, pulmonary valve insufficiency, Ebstein's anomaly, aortic stenosis)
   - premature closer of foramen ovale
   - myocarditis
   - large atrioventricular malformation
   - tachyarrythmias: atrial flutter, supraventricular tachycardia
   - bradyarrythmias
   - tumor of the heart
2. Haematologic
   - homozygous alpha thalassaemia
   - chronic feto-maternal transfusion
   - twin-to-twin transfusion
   - multiple gestation with parasitic fetus
3. Chromosomal
   - trisomy 21
   - Turner's syndrome
   - triploidy
   - mosaicism

Table continued

4. Pulmonary
   - cystic adenomatoid malformation of the lung
   - pulmonary lymphangiectasia
   - pulmonary hypoplasia
   - congenital chylothorax
5. Renal
   - congenital nephrosis
   - renal vein thrombosis
   - spontaneous bladder perforation
6. Intrauterine infections
   - syphilis
   - toxoplasmosis
   - leptospirosis
   - Chagas disease
   - congenital hepatitis
   - herpes simplex
7. Congenital anomalies
   - achondroplasia
   - thanatophic dwarfism
   - sacrococcygeal teratoma
   - Francois's syndrome (Tip.III.)
   - artogryposis multiplex congenita
   - McKusik-Kaufmann's syndrome
   - Smith-Lemli-Opitz's syndrome
8. Miscellaneous
   - meconium peritonitis
   - fetal neuroblastomatosis
   - tuberous sclerosis
   - small-bowel volvulus

D./ Idiopathic cause

delivery it would be useful to decompress the fetal abdomen and to allow vaginal delivery. This procedure would be useful for

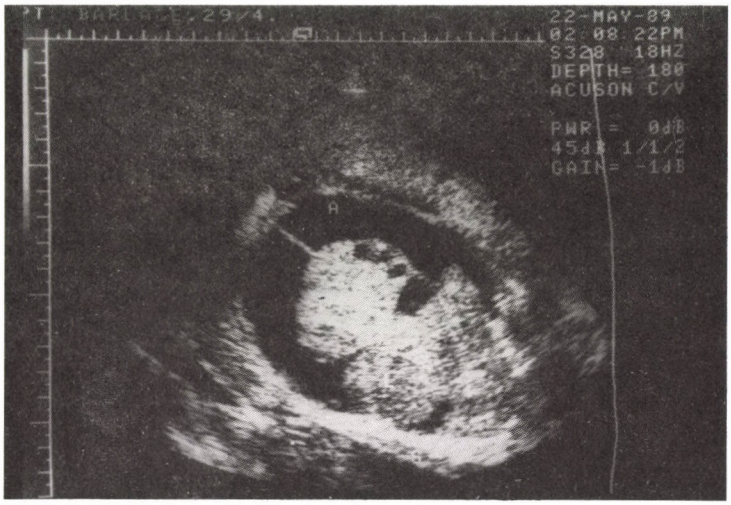

Fig. 1. Ascites in the 29th week of pregnancy.
A cross section of fetal abdomen can be seen on the picture. Under the liver (L) the compressed bowels (I) can be observed. Abdominal cavity is filled up with ascites (A) (S=spine)

the newborn after the delivery, too, as to impair diaphragmatic motion and lung compression, and to achieve spontaneous respiration in the newborn. Ascites and hydrothorax are frequently a common symptom of NIHF (Figure 2).

The pericardial effusions (Figure 3) and the hydrothorax (Figure 4) can also be easily visualized, and can occur isolated or as a part of the generalized hydrops.

With a fetus with generalized hydrops, the outer margins of the edematous tissue over the fetal head, neck, thorax and abdomen are thick, sometimes giving a halo in sonographic cross sections (Figure 5). Sometimes it is possible to diagnose the skin edema in the first trimester of pregnancy (Figure 6).

By experience the increasing ascites and the generalized edema are very bad signs, the fetal outcome is very bad. When at the examination of the fetal anasarca the generalized skin thickness is 5 mm or more, it is in most cases an ante finem stage.

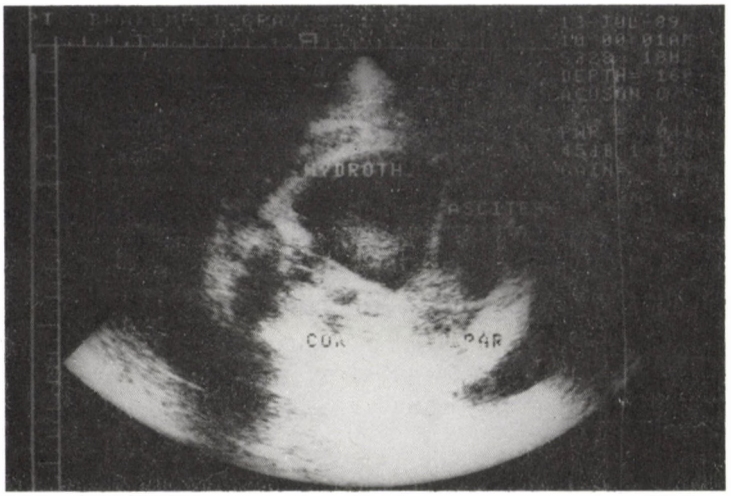

Fig. 2. Hydrothorax and ascites in the 31st week of pregnancy. Excessive fluid can be observed both in the thorax and in the abdominal cavity (hydroth., ascites). The compressed lung and the fetal heart (cor) can be observed beside the hydrothorax and the liver (hepar) can be observed beside the ascites

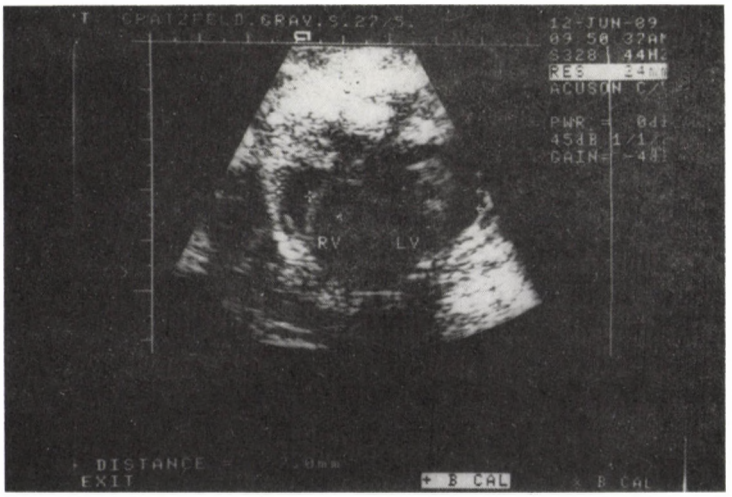

Fig. 3. Pericardial effusion in the 27th week of pregnancy. The right ventricule (RV) and the left ventricule (LV) can be observed on the picture. The crosses indicate 7 mms thick fluid on the right side and on the left side marked by arrow there is a thicker effusion

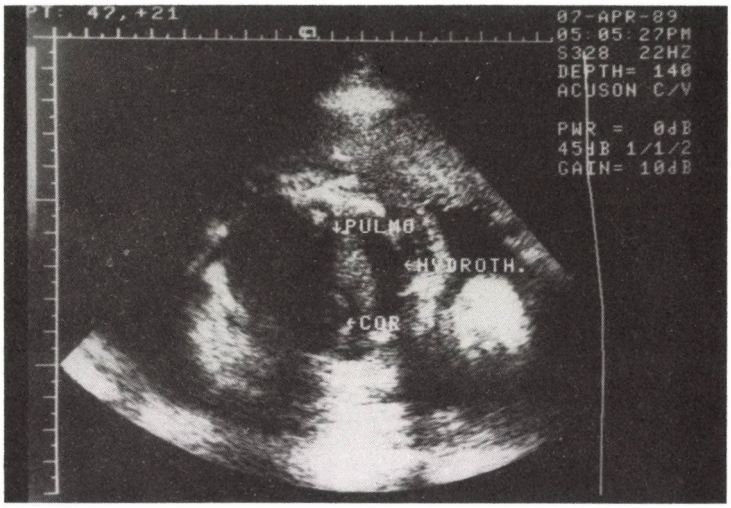

Fig. 4. Two sides hydrothorax in the 25th week of pregnancy. The compressed lung (pulmo) on both sides of the cross section of the thorax and on the left side the fetal heart (cor) can be seen. The pathological fluid volume (hydroth.) can be seen very well on the both sides

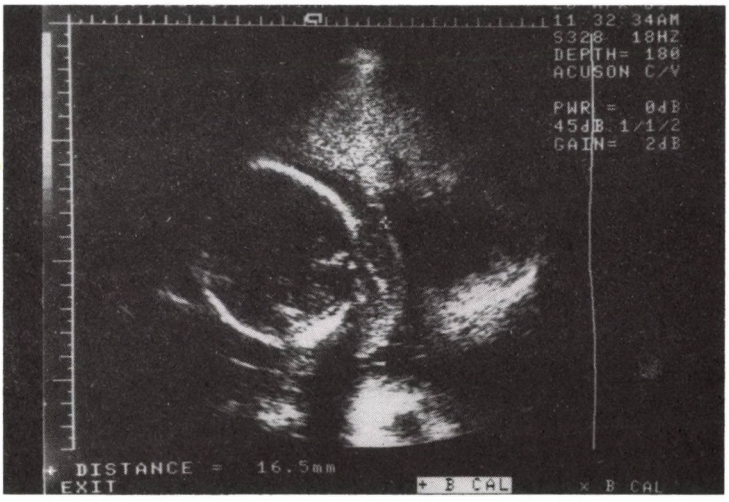

Fig. 5. Skin edema in the 30th week of pregnancy. Around the fetal head like a "halo", about 1.5 cm thick edema would be seen (sign with +)

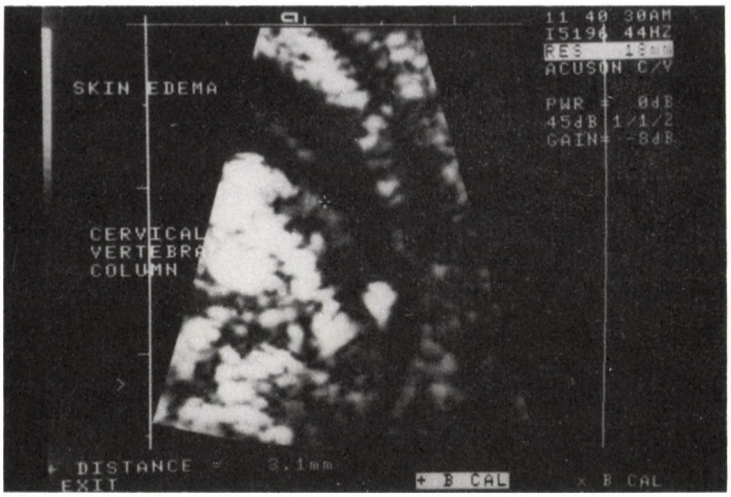

Fig. 6. Skin edema in the 13th week of pregnancy.
3 mm edema can be observed at the cervical cerebral column of the fetus (sign with +)

Though it is impossible to differentiate between NIHF and IIHF by ultrasound alone, the cardinal symptoms of NIHF in the second trimester are the anasarca, ascites, hydrothorax. These symptoms occur by an IIHF only in the final stadium of the fetus.

If the hydrops is recognized, the first step is to clear up if the diagnosis is IIHF or NIHF. For this question the indirect antibody screening will immediately give answer. If it is negative, the possible causes must systematically be examined in a logical order. It is very important to go on from the less invasive methods to the more invasive ones emphasized also in the literature by the authors /14, 15, 39/.

The first step is the examination of the maternal blood sample. A complete blood count, haemoglobin electrophoresis, blood chemistry (serum glucose level for the exclusion of diabetes mellitus), Kleihauer-Betke test (exclude the fetomaternal transfusion), VDRL (because of the maternal disease can cause a fetal infection and this can be a cause of the NIHF), Torch titer examination (Toxoplasma, Rubeola,

Cytomegalia, Herpes), excluding the paravirus infection, specific enzyme deficiencies in the fetus can be suggested by determination of the glucose-6-phosphate dehydrogenase and pyruvat kinase levels of maternal erythrocytes. The examination of maternal serum AFP level is suggested, too.

A complete blood count with red blood cell indices should be obtained on both parents, to search for heterozygous alpha-thalassemia.

At those patients, where the NIHF is reccurent or it was impossible to detect the cause, parental HLA typing must be done. Warsof et al have written two cases of idiopathic NIHF in which the parents have similar HLA alleles /37, 38/. The idea is that in these cases the common fetal and maternal HLA haplotypes derange the normal immunologic responses and the NIHF devepoled secondary to an abnormal maternal immunologic response against her fetus.

Among the examinations the ultrasound can be counted as the most important noninvasive method. The fetal anatomical developing status must be determined very precisely. The congenital anomalies must be recognised. The fetal skin thickness has to be measured. Very important is the real-time echocardiography (2-DE examination, M-mode examination, Doppler colour-examination), to exclude arrhythmias, anatomical disorders of the heart and of the great vessels. The diagnosis of lethal (uncorrectable) anatomic heart lesions, or complete heart block gives the possibility of interruption of the pregnancy in the second trimester.

The placental thickness must be measured. Polyhydramnion may cause a thinning of the placenta which means, if in the third trimester of the pregnancy a normal thickness of the placenta could be measured (3.0 to 4.5 cm), the placenta is likely edematous /36/.

Invasive methods are the examination of amniotic fluid by amniocentesis, the examination of fetal blood sample by chordocentesis and the transabdominal placental biopsy.

By the examination of the amniotic fluid it is possible to do a chromosomal analysis (duration 2-4 weeks), clear up the aneuploidia, it is possible to exclude the specific enzyme

deficiency of amniotic fluid cells or the cytomegaly viral infection, respectively.

From the fetal blood sample the total blood count, the blood chemistry (albumin, total protein, enzymes and so on), the Torch specific IgM could be determined /17, 20, 24, 26, 28, 39/. Karyotyping can also be done in 2-4 days from fetal lymphocytes.

The fastest method for rapid karyotyping which can reveal cytogenetic results on the day of the sampling procedure is the transabdominal placenta biopsy with direct cytogenetic preparation /16, 17/.

According to the literature /16/ a general perinatal examination (maternal serological, ultrasound, echocardiography, amniocentesis, fetal blood sampling) and examination after delivery (genetics, X-ray examination of the skeletal system, to recognise an achondrogenesis, serologic and metabolic examination) lead in most of the cases (85 %) to a diagnosis /15, 16/. According to Hutchinson et al /18/ with a very profound examination, 80 % of the NHF cases could be diagnosed at about the 28th week of pregnancy.

Prognosis

Despite of the recent advances the fetal prognosis in the NIHF is generally poor, especially when we do not know the cause. In a recent study of the King's College Hospital (London) in 30 pregnancies, where fetal arrythmias were excluded, the survival was only 10 % /28/. Other reports have written 75-90 % perinatal loss /16/. The survival was more frequent in cases where it was possible to convert the cardiac arrythmia and the hydrops disappeared.

After 21 examinations Castillo et al found that the prognosis of the NIHF is especially poor when fetal malformation and/or pleural effusion can be detected with ultrasound /7/.

In the literature the perinatal mortality rate is between 50 % /10/ and 98 % /18/, which depends on the distribution of the etiologies in the different groups.

Recurrence of NIHF is fortunately very rare /10, 22, 23/. In

those cases in which a cause is determined, the recurrence risk is that of the disease. In the further pregnancies the survival has a good chance, in one case there were three /10, 32/ survivors.

Treatment

Management must be individualized. The most important point in the control of the treatment's efficiency is the repeated ultrasound examination. So, it is possible to check the progress or regress of the disease. Furthermore, it is very important to check the number of fetal body movements and to make cardiotocography. Medical and surgical treatment must be considered individually.

The arrythmia must be identified and treated. Digoxin, quanidin, and procainamid have been used frequently. Arrhythmias must be treated with digoxin, mostly, when the cause of the NIHF is unknown and the delivery is impossible because of the prematurity.

Furosemid would be helpful in the mobilization of excessive fetal fluid.

Ultrasound-guided para- or thoracocentesis would be justified, though the benefit of the procedure is unclear.

If the examination of fetal blood sample showed a hypalbuminaemia of the fetus it is possible to give an albumin infusion into the umbilical vein, though it would be also successful to inject albumin intraabdominally for the fetus /34/.

Preterm delivery may be indicated in some cases, though the treatment of the hydropic newborn has a lot of problems. In most cases, the death of premature infants is caused by pulmonal edema or pulmonal hypoplasy, so peritoneal dialysis can be used to eliminate the excessive fluid from the intravascular and third space caused by a congestive heart failure /6/.

Fetal conditions

According to Phibbs et al /29/ the decreased albumin syntesis and the low colloid osmotic pressure could play a role

in the mechanism of hydrops formation in erythroblastosis fetalis, but it is still uncertain whether the hypoproteinemia due to liver dysfunction or the congestive heart failure is the prime pathophysiologic process for the development of NIHF /19, 33/. Nikolaides and Rodeck found hypoproteinaemia in all 40 cases at the examination of fetal blood samples /27/. By the survivors of NIHF the total protein and albumin were in the normal range /21/.

One of the causes of NIHF could be the obstruction of venous return /12/ but also the obstruction of the lymph flow (e.g Turner's syndrome, congenital lymphedema).

In twin pregnancies as a result of the pathological blood flow situation between the fetuses (twin-transfusion syndrome), NIHF developed in the donor twin, because of the secondary anaemia that causes a congestive heart failure /3, 9/.

Maternal conditions

Holzgreve et al /16/ found after the examination of 103 cases the following maternal complications associated with NIHF: EPH gestosis (12 cases), severe anaemia (4 cases), hypalbuminaemia (7 cases), postpartum haemorrhage with difficult delivery of the placenta (6 cases).

According to one paper /13/, which overviews 26 cases, hypalbuminaemia was found in 67 % and pregnancy induced hypertension was found in 46 %.

The presence of polyhydramnion is very frequent /31/, it can be observed in 50 % of the cases /16/, but in one case oligohydramnion was found.

According to Holzgreve et al /16/ there was no teratogenic exposure in the examined groups.

Feto-maternal haemorrhage /35/, or a massive haemorrhage in the in utero closed space (e.g.premature placental abruption) could be the cause of the NIHF /4/.

Differential diagnosis

The diagnosis of fetal ascites and of the generalized edema with ultrasound is usually not difficult, but sometimes there are some differential diagnostic problems, e.g.hygroma colli or

a big intraabdominal cystic structure.

Cystic hygroma colli is easy to diagnose with ultrasound /11/, it is also described in Turner'syndrome, trisomy 21 and other chromosome aberrations. By Brock et al /5/ the mistakenly punctured cystic hygromas fluid can be distinguished from the amniotic fluid by the measurement of alkaline phosphatase (ALP) isoenzymes.

The persistent cloaca syndrome is an important differential diagnostic problem. The typical ultrasound picture of this disease is the multiple thin-walled cysts of different size which have to be differentiated from the ascites by visualising the free floating bowels. The most cases of fetal common cloaca are associated with other serious congenital anomalies.

RESULTS

On the basis of the analyses of 402 cases - similar to the literature data - extremely great number of possible causes of NIHF were found:

1./ Cardiovascular disease was the most frequent cause of NIHF (90 of 402 - 22.8 %). The survival in this group was generally poor (17 of 90), except where tachyarrhytmia was diagnosed and where it was possible to treat it (12 of 21).

2./ Chromosomal disorder was the cause of NIHF in 54 cases (11.7 %). In this group only 3 newborns survived, their diagnosis was set up after the 24th week of pregnancy. In 36 cases, in which pathological karyotypes were recognized before the 24th week of pregnancy, all of the pregnancies have been interrupted.

When the NIHF joined to hygroma colli (11.7 %) the survival was very poor, even when the karyotype was normal. 1 of 48 survived with a little hygroma colli and minimal ascites with a normal karyotype.

3./ If the cause of the NIHF was haematologic (either feto-maternal of feto-fetal transfusion) the survival rates were only 9.7 % (10 out of 39). The possible treatment is the

intrauterine transfusion and the transplacental digitalisation.

4./ In 11 cases (2.7 %) it was possible to identify the intrauterine infection with the examination of fetal blood sample. There were only 2 survivors.

5./ In 23 fetuses the diagnosis was hydro-, chylothorax. If there was no chromosomal disorder the survival rate was very high (13 of 16 newborns). In 7 cases the diagnosis was 21 trisomy (survival rate was 1 out 7). For facilitating the postnatal resuscitation it was in all these cases necessary to make a therapeutic thoracocentesies before the delivery.

6./ All of the 18 fetuses with isolated ascites (4.5 %) survived. In 15 cases the ascites spontaneously disappeared in utero.

7./ There were different etiologies in 98 cases (24.3 %). In this group the diagnoses were gastrointestinal, urogenital disorder or malformation syndromes. The survival rate was very poor, 12 out of 98.

8./ In 47 cases the diagnosis was idiopathic NIHF (11.7 %), and only 1 newborn has survived.

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Acta Paediatrica Hungarica 31 (2), pp 187-200 (1991)

EXAMINATION OF THE FETAL HEART WITH TWO-DIMENSIONAL ECHOCARDIOGRAPHY

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Received 14 November 1989

The methodology of the two-dimensional fetal echocardiography is described by the basic of the accepted international nomenclature. By their opinion it is necessary that on the 18-20th weeks of gestation the high risk pregnants in aspect congenital heart disease examined by well-trained specialists. The indications of the echocardiography are listed, too.

INTRODUCTION

The frequency of congenital heart disease is 0.3-0.6 % /5, 6, 8, 9, 11, 17, 21, 24, 25/. The development of the method of ultrasound examination gives more possibilities to discover the sick fetuses just on the 16th-20th weeks of pregnancy. So the parents have been informed about the status of their fetus in time and after a well-founded consideration they can make a decision about the future of the pregnancy. As the number of ultrasound equipments are increasing continuously and the routine screenings are made by more and more colleagues, it seems to be necessary to review the methodology of the examination of the fetal heart.

MATERIAL AND METHODS

As this Department is one of the examining centers - there are only four centers in the Federal Republic Germany - the number of patients is very large and varied. Within the district,

[1]Supported by the Alexander von Humboldt Foundation, Federal Republic Germany

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the colleagues sent all the "suspect" cases to our center for examination and in these cases the fetal heart has been studied, too. From the point of view of congenital heart disease (Table I) the high risk pregnancies must be examined by a colleague who worked earlier as a cardiologist.

TABLE I

Indication for fetal echocardiography

A. Familial cause:
1. Congenital heart disease
2. Genetic disease
3. Anomaly syndrome

B. Maternal cause:
1. Heart disease (congenital, acquired)
2. Drug exposure (narcotics, lithium, amphetamin, contraceptives, sexual hormones, trimethadion, hydantoin)
3. Alcohol, heroin
4. Viral infection (rubeola, cytomegalia, Coxsackie, mumps)
5. Metabolic disease (diabetes mellitus, phenylketonuria)
6. Rh isoimmunisation
7. Hydramnion (oligo-, poly-)
8. Collagen vascular disease (SLE)
9. Elderly gravida
10. EPH gestosis
11. High dose ionize radiation
12. Toxoplasmosis

C. Fetal cause:
1. Intrauterine growth retardation (IUGR)
2. Fetal arrhythmia
3. Somatic anomalies
4. Decreased fetal movement
5. Abnormal genetic screen (Patau-, Edwards-Turner-, Klinefelter syndrome)
6. Hydrops fetalis

All of the examinations were performed with a high resolution real-time sector scanner (Acusion 128, Acuson GmbH, Erlangen, GFR). If it was necessary M-mode, pulsed Doppler or colour Doppler ultrasound examinations were done, too. But this paper discusses only the two-dimensional echocardiography (2DE), because this method has been used most frequently.

Of course the procedure of the examination was the same as other obstetrical ultrasound examinations. We have been informed about the position of the fetus, the adhesion of the placenta, the quantity of amniotic fluid, the fetal kinetic activity and the biometrical measurements were performed (biparietal, frontooccipital, abdominal diameter, femur length).

2-DE echocardiography

Characteristics of the examination of the fetal heart

Fetal echocardiography cannot be directly compared with the newborn study /1, 3/. There are several reasons:

1. The fetal heart lies in a different position than after birth. The apex is pressed up by the large fetal liver, so it is in a more horizontal position.
2. The size of the right ventricle is equal to the left ventricle. The right ventricle lies just below the chest wall. In the newborns its position is more inferior.
3. It is possible to observe the patent foramen ovale and ductus arteriosus.
4. The lung fields are unaerated and fluid-filled.

Nomenclature

In 1980 "The Committee of the American Society of Echocardiography" recommended for the standards of the nomenclature used in the two-dimensional echocardiography /13/. The main point of this suggestion is that we get always the same information about the heart when transsecting the heart in the different planes (these planes are defined). Three main planes are distinguished (Fig. 1).

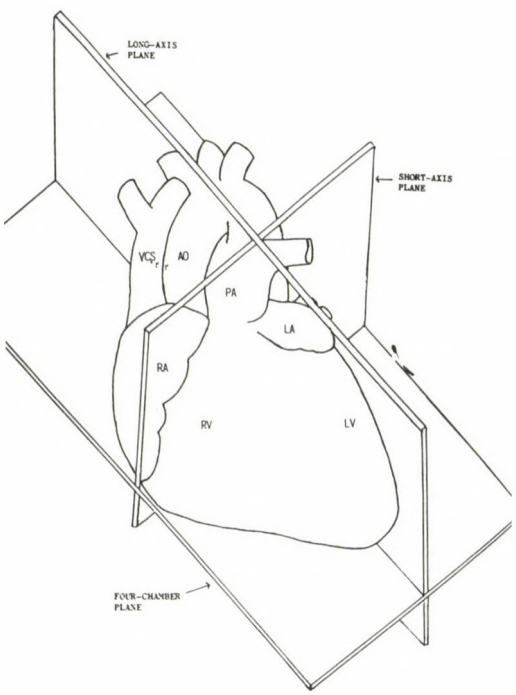

Fig. 1. Three main planes intersect the fetal heart
Explanation in detail see in the text.
(VCS=vena cava superior, AO=aorta, PA=pulmonary artery, RA=right atrium, LA=left atrium, RV=right ventricle, LV=left ventricle)

1. Long axis plane:
The plane that transsects the heart perpendicularly to the

dorsal and ventral surface of the body and parallel to the long axis of the heart.

2. Short axis plane:
   The plane that transsects the heart perpendicularly to the dorsal and ventral surface of the body, but perpendicularly to the long axis of the heart.
3. Four-chamber plane:
   The plane that transsects the heart approximately parallel to the dorsal and ventral surface of the body.

Of course with a minimal change of the main transectional planes it is possible to study the different parts of the heart, but the knowledge of main transectional planes is enough to diagnose the healthy heart.

Four-chamber view

This plane provides information about the fetal heart in a relatively easy way (Fig. 2). This view is achieved in a

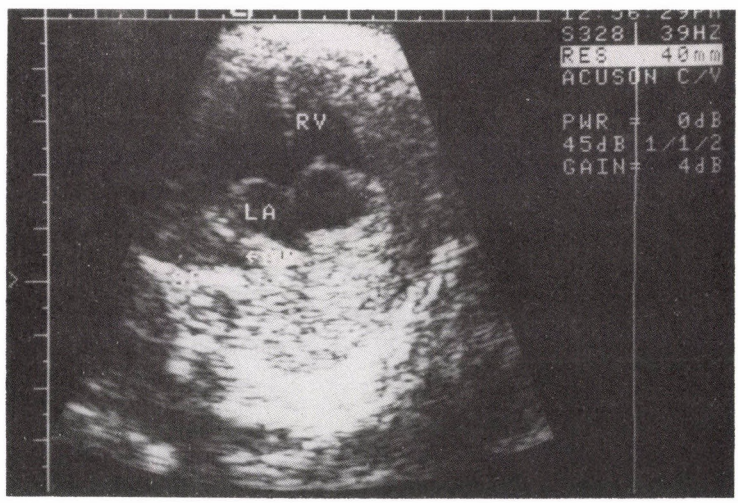

Fig. 2. Four-chamber view
Anterior to the spine (SP) is a relatively small circle is the aorta (AO). Directly opposite to it, one rib (R) should be seen and the underneath of rib is the right ventricle (RV). Between the aorta and the right ventricle the left atrium can be identified (LA). After this all of the other parts of the fetal heart are recognizable

straight cut across the fetal thorax at the base of the sternum. This section lies between the view of the abdomen and the head (the place of the measurement of the abdominal circumference and the biparietal diameter). Therefore the obstetric ultrasonographers offer to start from the well-known planes and move the transducer up and down so the four-chamber view of the heart can be achieved. The thorax appears as a circular structure and one of the points of this structure, the

spine, is easily recognized. To ensure that the section is not oblique at least one complete rib should be seen. The sternum can be located lying directly opposite to the spine in the centre of the anterior chest wall. The right ventricle lies underneath the sternum. The descending aorta, lying anterior to the spine can be seen as a circle in cross-section. It lies between the spine and left atrium. Afterwards all the other parts of the heart can easily be recognized (Fig.3).

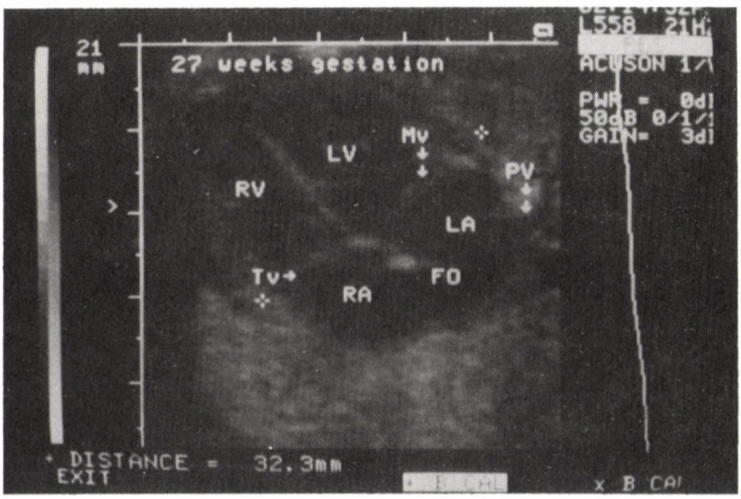

Fig. 3. Four-chamber view in the 27th week of pregnancy
Beside the main four-chamber of the fetal heart (RV=right ventricle, LV=left ventricle, RA-right atrium, LA=left atrium) it can be identified the tricuspid valve (TV), mitral valve (MV), and the patent foramen ovale, too. The pulmonary vein can also be recognized, running into the left atrium (PV). The distance of the meeting points of the tricuspid valves (TV) and bicuspid valves (BV) and the myometrium (marked with a " + " at the level of the anulus fibrosus), mean the cross section size of the heart

Studying the four-chamber view and making a decision that there is a healthy and normally developed heart, all of the following points must be noted:

1. The heart fills about 1/3 of the fetal thorax.
2. The right and left atrial chambers are similar in size.
3. To measure the right and left ventricular cavities just below the atrioventricular valve; they must be approximately equal in size.
4. The posterior walls of the right and left ventricles and the interventricular septum are approximately equal in thickness.
5. The atrioventricular valves open with each cardiac cycle.
6. The atrial and ventricular septa and atrioventricular valves

meet at the crux of the heart in an offset cross.
7. The normal atrial defect (foramen ovale) can be seen.
8. The ventricular septum appears intact.

Short axis plane

This view is important because it shows the relation of the great vessels to each other (Fig. 4). On the transsect, the right atrium (possibly with the streaming inferior vena cava), the tricuspidal valve, the right ventricle, the pulmonary valve, the main pulmonal artery, and the ductus arteriosus would be seen. The ductus arteriosus joins the descending aorta anterior to the spine. The main pulmonary artery and the right pulmonary artery wrap around the ascending aorta creating an image that looks like a "sausage". The middle of the picture, as it is written above could be compared to a "doughnut", too.

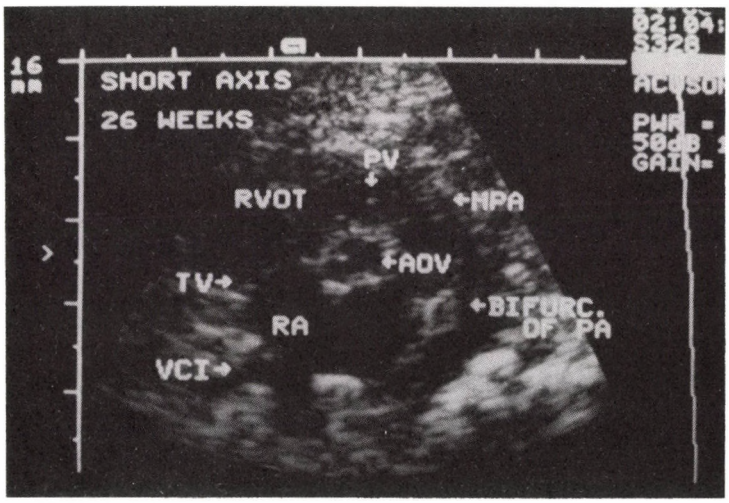

Fig. 4. Short axis plane
In the middle of the circular structure it can be seen the aorta and the aortic valve (AOV). It can be recognized on the right side of the picture the right atrium (RA) with the streaming inferior cava vein (CVI), the tricuspidal valve (TV), the right ventricle and the right ventricle outflow tract (RVOT), respectively. These structures together with the pulmonary valve, with the main pulmonal artery as well as the bifurcation of the pulmonary artery (Bifurc. of PA) make the picture look like a "doughnut".

Left ventricle outflow tract/long axis plane.

From the four-chamber long axis view, the transducer is angled slightly cephalad, revealing the anterior portion of the left ventricle, the aortic root, the aortic valve, and the ascending aorta (Fig. 5). A small portion of the right ventricle and right atrium is also seen. The interventricular septum is contiguous with the medial wall of the aorta. The

ascending aorta arises from the midportion of the heart and courses anteriorly and to the right within the fetal thorax.

An overriding aorta or narrowing of any portion of the ascending aorta can be determined from this view.

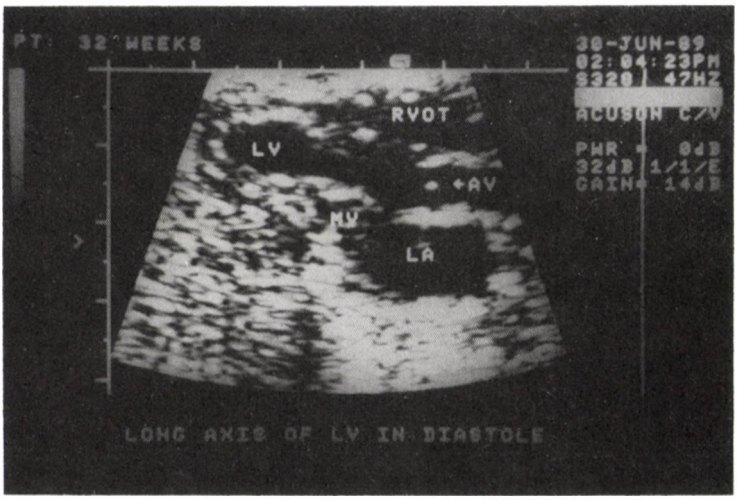

Fig. 5. Left ventricle outflow tract (LVOT)
This picture is obtained if from the four-chamber view the transducer is angled slightly cephalad. It would show the aorta - rising from the left ventricle - and the aortic valve AOV). (RVOT=right ventricle outflow tract, LA=left atrium, MV=mitral valve)

Right ventricular outflow tract/long axis plane

By sliding the transducer slightly from the position of the left ventricular outflow tract imagine towards the fetal head, the right ventricular outflow tract can be seen (Fig. 6). There can be observed the right ventricle, the pulmonary valve, the main pulmonary artery.

In both of these long axis views of the aorta and pulmonary artery, lying close to each other, it is possible to see their relation to each other by "rocking" the transducer back and forward. Transposition of the great vessels can be detected, using this maneuver. Double outler of the right ventricle, and aortic and pulmonary stenosis and atresia can also be detected by these views.

Five-chamber view

This view can be obtained by a slight caphalic tilt of the transducer from the apical view. The so-called "fifth chamber" is the aorta, separating the right and left atria (Fig. 7).

Aortic arch

The ascending aorta, arising from the left ventricle, crosses initially from left to right, passing behind the

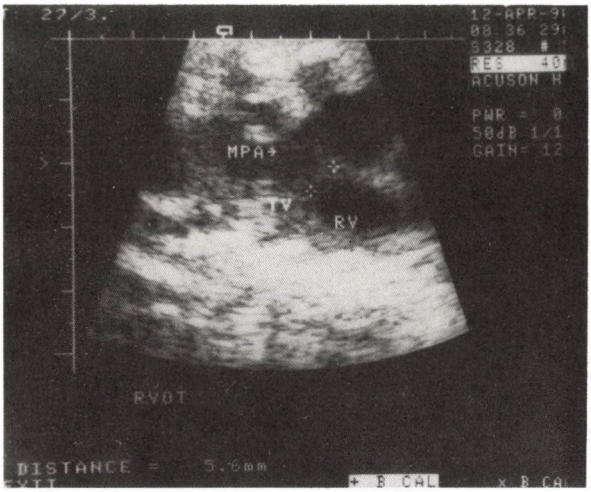

Fig. 6. Right ventricle outflow tract (RVOT)
By sliding the transducer slightly toward the fetal head from the position of the left ventricle outflow tract, the picture of the right ventricle outflow tract could be got. The main pulmonary artery (MPA) arising from the right ventricle (RV) is visible.
(TV=tricuspidal valve)

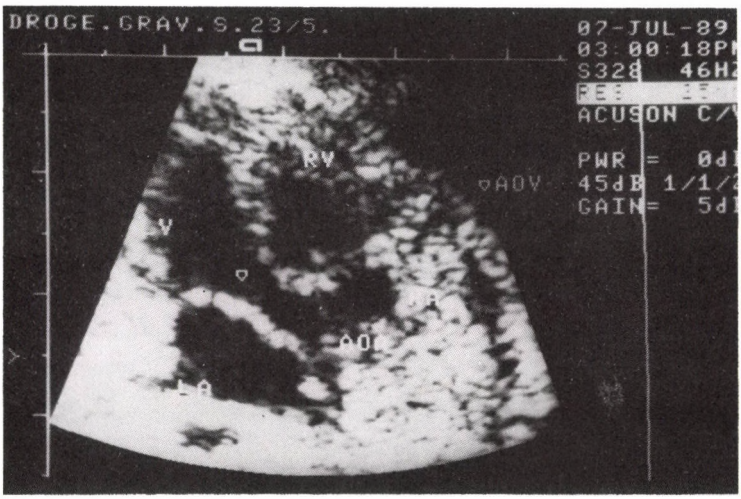

Fig. 7. Five-chamber view
Both ventricles (LV, RV) and both atria (LA, RA) would be seen on the picture. The ascending aorta (AAO) rising from the left ventricle gave the so-called "fifth chamber". (AOV=aortic valve).

pulmonary artery. It then bends from right to left and courses slightly anteriorly as it becomes the transverse arch. Then it curves posteriorly and inferiorly and becomes the descending portion of the aorta. Because of the somewhat tortuous course, the entire aorta from root to descending portion cannot be seen in one single view.

The easiest technique for examining the aortic arch and descending aorta is to place the transducer along the long axis of the fetus to the left of the fetal spine (Fig. 8). The left and right atria, and the short portion of the ascending aorta may be seen. The left subclavian artery, left common carotid artery, and the brachiocephalic trunk should be visible arising from the transverse arch.

The transposition of the great vessels and the coarctation of the aorta could be diagnosed from this view.

The image looks like a "walking stick"

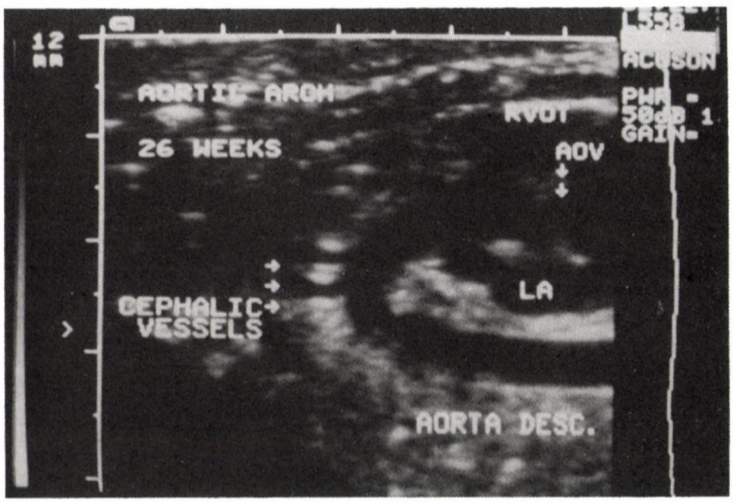

Fig. 8. Aortic arch
The aortic arch reminding of a "walking stick" can be seen in the 26th week of pregnancy with vessels arising from its cephalic vessels. The aorta would be followed from the arising (AOV=aortic valve) till the descending part of the aorta (aorta desc.). (LA=left atrium, RVOT=right ventricle outflow tract).

Ductal arch

The ductal arch looks more like a "hockey stick" than a "walking stick", because it curves much stronger than the aortic arch. The ductus arteriosus enters the descending aorta superior to the point where the transverse arch becomes the descending aorta.

The ductal arch should be focused from the ventral surface of the fetus in a longitudinal cross section (Fig. 9), since it is usually obscured by the fetal spine from the dorsal surface, especially late in gestation. The transducer is placed

in the way, that the beam enters the fetal thorax to the right of the sternum, and transsects the thorax slightly towards the left as it passes along the chest. The descending aorta is seen anterior to the spine and the ductus arteriosus enters it in a smooth curve. Of course there are no arising vessels from the ductus arteriosus.

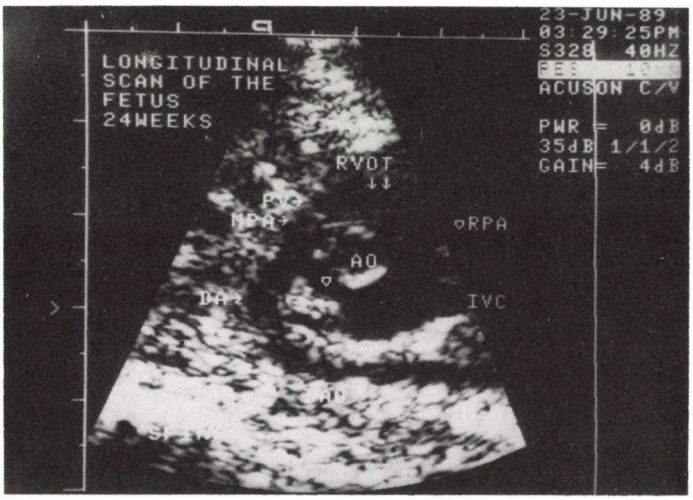

Fig. 9. Ductal arch in the 24th week of pregnancy
The ductal arch much more reminds of a "hockey stick" because of the much broader curve compared to the aortic arch. The ductus arteriosus connected the main pulmonal artery (MPA) and the aorta (DAO) are also seen.
(RVOT=right ventricle outflow tract, PV=pulmonary valve, RPA=right pulmonary artery, AO=aorta, IVC=inferior vena cava).

Factors, influencing on the examination

By Allan /1/ there are a lot of factors which have an influence on the result of the examination. The fetal cardiac structure can be visualized from the 10th weeks of pregnancy, but the most optimal time is about the 18-20th weeks of pregnancy. In this time it is possible to get an information about the structural details of the fetal heart. Sometimes the examination is impossible to achieve because of the strong fetal body or breathing movements or the disadvantageous position of the fetus for the examination (e.g.the fetal spine is between the transducer and the fetal heart). With maternal obesity the distance between the transducer and fetal heart increases and makes it difficult to get an orientation. The cause is similar in case of polyhydramnions. In the case of oligohydramnion the favourable effect of a fluid-filled sac for optimum transmission of ultrasound is lost.

DISCUSSION

The first articles about the examination of the fetal heart by ultrasound were published about ten years ago /4, 13, 15, 23/. These were followed by a large number of other works /14, 18, 19, 22, 26/ but the checking of the fetal heart did not become general. Looking over the indications of the examination of the fetal heart (Table I), a great number of the pregnant women should get a reassuring information about the cardiological status of their fetuses. This review calls attention that not only the obstetricians but all of the colleagues who are in contact with the pregnant women (see familial and maternal causes) must be very careful that all of the pregnants, for whom it is necessary, should be checked. The acquirement of the 4-chamber-view is important fundamentally /2/. Here, at the routine obstetrical examination raises the suspicion of the congenital heart disease. The exact diagnosis is the duty of a specialist, who has a special practice in the echocardiography and pediatric cardiology, too. Recently a paper was prepared after 545 examinations during 1 year. The result was as follows: 498 negative and 45 positive cases, 1 false negative (the finest diagnosis was an atrioventricular septum defect, with a small ventricular septum defect), 1 false positive (common atrioventricular canale, but the coarctation of aortae was not diagnosed). So, the sensitivity is 98 %, specificity 99 %, positive predictive value 98 %, negative predictive value 99 %. In all of the positive cases the diagnosis was confirmed with repeated examinations. As all of the examinations were performed with a high resolution colour Doppler equipment, so it would be one of the explanation of the very low false negative and false positive cases. In a lot of cases this method made easier the orientation on the fetal heart, which would be sometimes difficult with a black-white equipment /20/. By all means, it is reasonable that at the suspicion of fetal cardiac malformation, the obstetrical ultrasound examination be followed by a consilium with pediatric cardiologist /28/. Verifying the final diagnosis and the

judgement of the further work to be done must be the result of a conclusing obstetrics-pediatric cardiologist consilium, as in pathological cases, depending on the gestational age the tasks can be various (interruption of the pregnancy, operative delivery, per vias naturales delivery). Under the review of the methodology of the fetal heart's examination we wanted to point out the necessity that the examination of high risk pregnancies, especially in congenital heart disease, should be done by well-trained specialists.

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Acta Paediatrica Hungarica 32 (2), pp 201-213 (1991)

URINARY N-ACETYL-β-D-GLUCOSAMINIDASE ACTIVITY IN HEALTHY, POLYCYTHEMIC AND HYPOXIC NEONATES

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Received 14 November 1989

The authors investigated the urinary N-acetyl-β-D-glucosaminidase (NAG) activity in the case of 101 normal healthy and 20 polycythemic newborns and prematures, and 50 prematures suffering from hypoxia on the 1st, 2nd, 4th, 14th, and 28th day after birth. The obtained activities were referred to the creatinine concentrations of the urine samples and given as NAG index. There were no significant differences in the NAG indices either between fullterm and preterm babies or between appropriate for gestational age (AGA) and small for gestational age (SGA) neonates of the normal group. The NAG indices on the first day of life were significantly higher in the case of polycythemic newborns in comparison with the normal group ($p < 0.01$). On the 14th day, after the partial plasma exchange, the NAG indices returned to the normal range. The premature babies suffering from IRDS received an average 10.1 days oxygen supplementation. Their NAG indices were significantly ($p < 0.01$) higher on the 1st, 2nd, 4th days than those of the healthy prematures of the normal group and decreased considerably up to the 14th day. Finally the NAG indices reached the normal value on the 28th day. These results support the assumption that the urinary NAG index is a suitable indicator of the renal tubular damage during the newborn period.

INTRODUCTION

The nephrotoxic drugs widely applied in intensive neonatal treatment, the temporary hypoxic periods and the pathological oscillation of the oxygentension may cause tubular damage, which has been studied very intensively /6,14,18,21/. The degree of the damage can be estimated on the basis of the

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characteristic increase in either the total urinary protein content or some typical enzyme activities of the urine, e.g. N-acetyl-β-D-glucosaminidase (EC.3.2.1.30).

The different NAG isoenzymes /29,31/, which can also be found in high concentration in the proximal tubule cells of the kidney, are lysosomal hydrolases with a molecular weight of about 140 000 daltons.

Physiologically they play an important role in the catabolism of both glycoproteins and mucopolysaccharides. The NAG satisfies the Gonick's criteria for selecting enzymes potentially useful in the detection of different renal diseases /7/.

In connection with adult patients recently a lot of publications have been reported on the successful diagnostic applications of this enzyme /10,12,14/ e.g. lead poisoning /19/, Allopurinol treatment /20/, for the indication of rejection reaction after renal transplantation /13/, for the diagnosis of renal complication in diabetes mellitus /23,33/, for the diagnosis of renal changes during pregnancy /26/, and for the diagnosis of other renal diseases /11,31/. However, relatively little data can be found in the literature in relation to the application of this method during neonatal period and in childhood /8,22,27,28,30,31,32/. Only one research group reported on the normal value for healthy neonates on the basis of a few cases /15/. Therefore, our main goals were the investigation of the applicability of this method i.) for measuring the degree of the damage and ii.) for the diagnosis of hypoxic tubulopathy, caused by polycythemia and hypoxia, iii.) for the detection of the temporal development of the process.

PATIENTS AND METHODS

Between November 1st, 1988 and May 1st, 1989 101 normal and 20 polycythemic patients were investigated. The details of these patients are summarized in Table I. The normal neonates did not need oxygen supplementation, did not have urinary infection and were not given antibiotics.

TABLE I

Details of te normal and polycythemic patients

		Number of patients[a] male	female	Gestational age[b] weeks (mean ± SD)	Birth weight[b] gram
Full-term	AGA	30 (0)	20 (0)	38.9 ± 1.3	3353 ± 417
	SGA	12 (3)	10 (0)	38.6 ± 1.1	2210 ± 233
Preterm	AGA	9 (5)	17 (9)	35.4 ± 0.9	2280 ± 125
	SGA	2 (2)	1 (1)	35.0 ± 1.1	1740 ± 80

a: the number of polycythemic patients is indicated in parenthesis

b: there were no significant differences between healthy and polycythemic patients in gestational age and birth weight

The hematocrit (Htc) was determined from venous blood within 2 hours or between 12 - 24 hours after birth /5,17/. In the case of any following pathological alterations: tachypnoe, hypoglycaemia, hypocalcaemia, thrombocytopenia and if the Htc was greater than 65 per cent, partial exchange transfusion was performed without delay, with 5 % human albumin. If the above mentioned pathological alterations could not be detected, the partial plasma exchange was only made if the Htc was greater than 70 per cent.

The urine specimens were collected on the days 1st, 4th, 14th, for twelve-hour-periods, using urine collection bags. These 101 patients were divided into subgroups according to sex, gestational age, the degree of retardation if any, and polycythemy /Table I/.

During this period 50 preterms with IRDS were investigated in the Neonatal Intensive Care Unit, namely: 25 males, 25 females, gestational age: 31.8 $\pm$ 2.5 weeks (mean $\pm$ SD), birth weight: 1590 $\pm$ 410 gram, one minute Apgar score of: 7.5 $\pm$ 1.5, five minute Apgar score of: 8.3 $\pm$ 1.4. During the observation period half of the patients (15 males, 10 females) died. The neonates required oxygen supplementation for 10.1 $\pm$ 7.0 days on average. The average oxygen concentration (p_cO_2) and oxygen saturation (cap.$SatO_2$) of these patients on the first six days of life can be seen in Table II. Urine samples were obtained on the 1st, 2nd, 4th, 14th, 28th days and were immediately frozen (-20°C) and analysed within two weeks.

For determining the NAG activity the method of Horak et al /9/ was used with slight modifications. The specimens of urine were allowed to thaw at 4°C. After centrifugation (1000 g, 5 min). 1 ml aliquots of the samples were filtered on 8 x 1.5 cm fine mesh Sephadex G - 25 columns (Pharmacia AB, Uppsala, Sweden). The enzyme activity in the eluents was determined by the application of chromogenic p-nitrophenyl-β-D-glucosaminid substrate /16/. The reaction was carried out in 0.1 M sodium citrate buffer, pH 4.4 at 37°C and quenched with 0.2 M sodium borate buffer, pH 10.0. The amount of the liberated p-nitrophenolate was determined spectrophotometrically at 400 nm with a SPECORD M -40 device, by the use of calibration curves. In every case substrate blank solution was used. The enzyme activity was expressed in terms of the hydrolysed p-nitrophenyl-β-D-glucoside (μmol/min/l).

The urine creatinine concentration was determined by Jaffe' method /2/ with Centrifichem autoanalyser.

Statistical analysis was made on the basis of the Student's t-test /3/.

RESULTS

The NAG indices of the normal patients on the 1st, 4th, 14th days after birth according to sex, gestational age (fullterm - preterm), the degree of retardation (AGA - SGA) are shown in Fig. 1. The increase of the NAG index observed in the females

TABLE II

Average capillary oxygen tension (p_cO_2) and oxygen saturation (cap. $SatO_2$) in preterm infants with idiopathic respiratory distress syndrome on the first six days of life.

p_cO_2 (Hgmm) (mean ± SD)	53 ± 19	57 ± 22	62 ± 21	58 ± 22	54 ± 20	61 ± 24
cap.$SatO_2$ (%) (mean ± SD)	77 ± 16	82 ± 15	86 ± 11	80 ± 18	79 ± 17	82 ± 16
Days	1	2	3	4	5	6

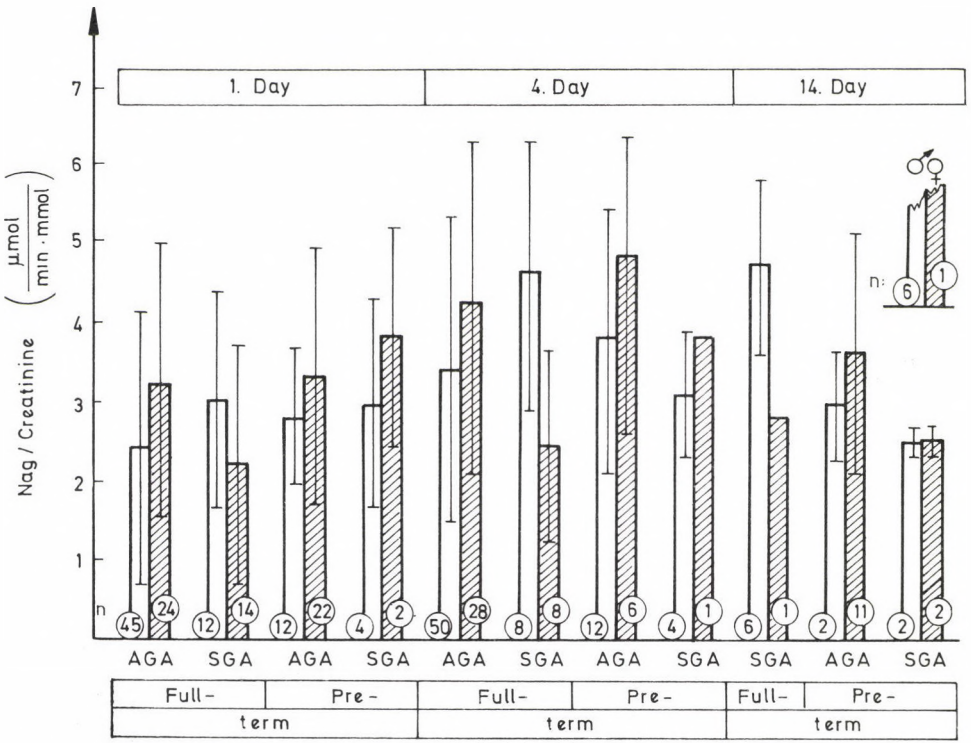

Fig. 1. Urinary NAG index in healthy full-term and preterm infants (mean ± SD).

was only significant in the fullterm AGA group in the first day of life ($p < 0.05$). Moreover, there were no significant differences between the fullterm and preterm, AGA and SGA groups compared on any day of observation.

The NAG indices of the polycythemic neonates can be seen in Fig. 2. It is noteworthy, that the NAG indices of this group were significantly higher than the normal values in all sub-groups on the day of life ($p < 0.01$). On the 4th day considerable decrease of the NAG indices could be observed except in the preterm AGA boys, but these values still exceeded the normal ones ($p < 0.05$). On the 14th day the NAG index went back to the normal range. Simultaneously, the differences in the NAG index between the males and females both of the preterm AGA and preterm SGA sub-groups were negligeable on any day during the investigation.

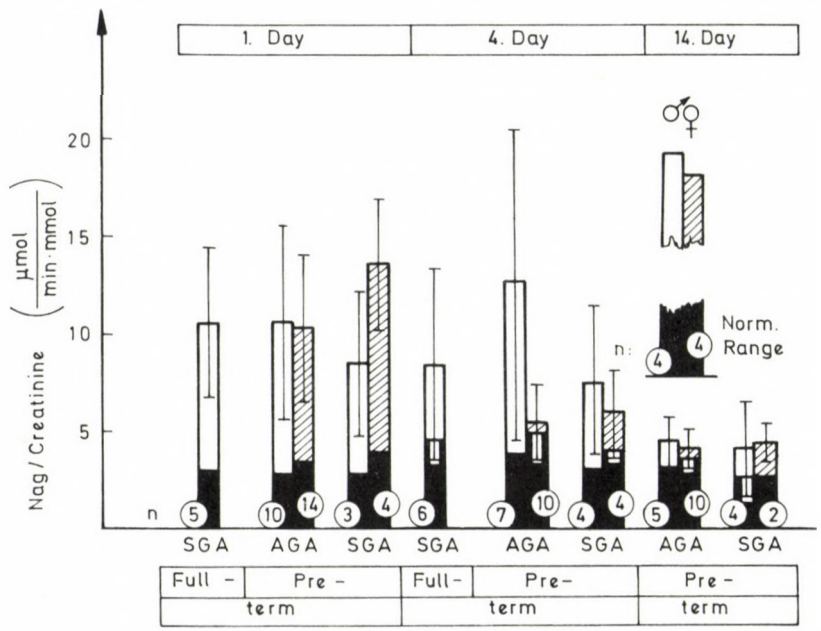

Fig. 2. Urinary NAG index in full-term and preterm infants with polycythemia (mean ± SD).

Fig. 3 indicates that the NAG indices of the preterms with IRDS who participated in intensive therapy were significantly higher on the 1st, 2,nd, 4th, days than the normal values ($p < 0.01$). The NAG index decreased up to the 14th day and became normal on the 28th day. On the 1st, 2nd, 4th, 14th days there was no significant difference between the NAG indices of males and females. On the other hand, on the 14th, and the 28th days the NAG indices of males exceeded the corresponding values of females which might be attributed to the prolonged reparation of males.

DISCUSSION

The urinary proteins and enzymes (alanin-aminopeptidase, β_2-microglobulin, lysozym) are extensively applied nowadays for the detection of the nephrotoxic effects of different drugs (e.g. aminoglycosides, indomethacin hypoxia, etc. The measuring of the β_2-microglobulin (β_2M) of the urine has become an especially widely used method in the medical routine /1,4,25/ and the possible diagnostic application of the urinary NAG determination also seems to be promising. Some publications have reported that the determination of the NAG index, which is a relatively simple procedure, gives useful and valuable information on the condition of the renal tubulus as can be obtained by the application of β_2M tests /8,24/. Moreover, Rajchgot and his co-wokers found that the increase of the NAG index in some cases is an earlier and more sensitive indicator of tubular damage than that of the β_2M level /22/. Although a lot of papers have already been published on the tubulopathic effects of aminoglycosides and other nephrotoxic drugs in childhood and adults /22,27,28,32/, the tubulopathy caused by hypoxia in neonatal period is a much less investigated field.

First of all, we determined the normal ranges of NAG indices for healthy neonates and preterms as a function of sex, gestational age and birth weight (Fig. 1). We assumed that in

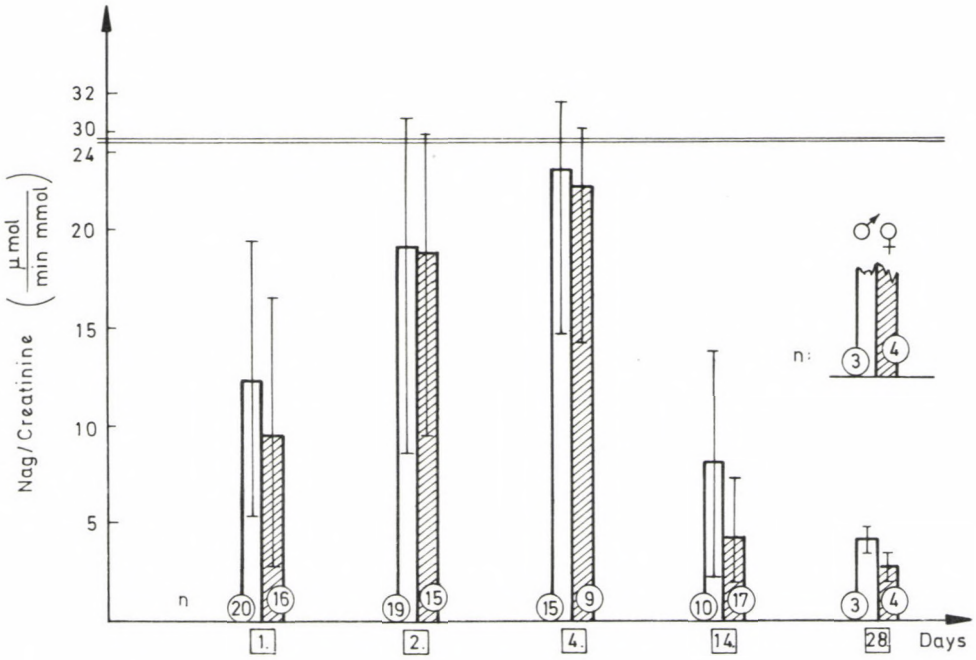

Fig. 3. Urinary NAG index in preterm infants with idiopathic respiratory distress syndrome (mean ± SD).

the case of retarded infants a relative placenta insufficiency led to the observed retardation which might also caused tubular dysfunction in the kidneys. But our investigation did not support this assumption because the NAG indices of the retarded infants did not differ significantly from that of the normal group either in the case of fullterm or preterm babies /Fig.1 /. Therefore, we could conclude that the different processes which led to retardation had no tubulopathic effect detectable with this method. Regarding the dependence of the NAG indices on sex, we found, that they were higher in females, which is in good accordance with previous observations /14/. But this difference between males and females was significant ($p < 0.05$) only in the full-term AGA group on the first day of life. In the case of polycythemic neonates we observed high NAG indices on the 1st day of life, which decreased to the normal value on the 4th day in females and on the 14th day in males, in consequence of partial plasma exchange (Fig. 2). As it can be seen, the NAG indices of the fullterm and preterm polycythemic groups hardly differed from each other (Fig. 2), that is the hypoxic tubular damages were essentiallly independent of the gestational age after the 35th week. The kidney functions of preterms with IRDS, ventillated for a long period of time with a higher concentration of oxygen, can be damaged by hypoxia, pathologic changes in the oxygen tension, as well as by the necessarily applied medicines. Our patients required oxygen supplementation for about 10 days. The continuous increase of the NAG indices on the 1st, 2nd, 4th days persuasively indicate the progress of tubulopathy. On the other hand, the decrease of the NAG index after the 4th day shows that the pathological processes were reversible in these cases, which is a good evidence of the very high regeneration capacity of the kidney (Fig. 3).

On the basis of our results we think that the urinary NAG index determination, a relatively simple and fast procedure, will become a suitable tool for the reliable indication of neonatal hypoxic tubulopathy and for the detection of this process in the near future.

Besides the diagnostic significance, the NAG index may also be applied in planning therapeutic steps for the influence of the renal functions.

ACKNOWLEDGEMENTS

The authors are indebted to Mrs. Anna Oláh - Varga, B.Sc., Károly Erdei and László Somorjai, M.D. for helpful suggestions.

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Acta Paediatrica Hungarica 31 (2), pp 215-221 (1991)

THE EFFECT OF SOCIO-ECONOMIC CONDITIONS ON THE TIME OF DIAGNOSIS AND COMPLIANCE DURING TREATMENT IN GROWTH HORMONE DEFICIENCY

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Received 6 December 1989

In 78 patients with idiopathic growth hormone (GH) deficiency the effect of the fathers' educational level on the age and the extent of growth retardation at diagnosis was studied. There was a tendency for an increase of the age and the degree of growth retardation with the decrease of the fathers' completed grades. The occurrence of height SD scores less than -4.5 was conversely related to the number of grades completed by the father ($chi^2=19.2$ $p<0.001$). Eighteen of the 70 patients treated with growth hormone discontinued treatment after 0.3 to 6 years. Compliance was closely related to the grades completed by the father ($chi^2=24.7$ $p<0.001$). Six out of 7 patients with a height SDS less than -4.5 at diagnosis and with a father of low level of education (less than 8 grades) became non compliant.

It is concluded that the degree of growth retardation at diagnosis and compliance at treatment in GH deficiency is related to the educational level of the father.

INTRODUCTION

With the treatment methods employed in the last 20 years the final height of many growth hormone deficient patients remains below the 3rd percentile. Among the factors which determine final height the degree of growth retardation at the beginning of treatment seems to be the most important /1, 2, 4, 7/. Consequently, an earlier diagnosis at less severe growth retardation would result in greater final height. Another factor which in our experience unfavorably affects final height in many patients has been bad compliance during treatment. The factors which determine the time of diagnosis and compliance during treatment have not been studied previously, but the importance of socio-economic status may be supposed.

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The present paper aims at determining to what extent the fathers' educational level affects the degree of growth retardation at diagnosis and compliance during treatment.

PATIENTS AND METHODS

The study concerning the time of diagnosis was carried out in 78 patients with idiopathic growth hormone deficiency. The criteria for the diagnosis of growth hormone deficiency have been published recently /3/. The degree of growth retardation was expressed in SD scores. For comparison local standards were used.

In the study of compliance 70 patients were included who had been treated between 1973 and 1988. Growth hormone was given 2-3 times a week and the patients returned for check-up every 3 months at our clinic to be examined by the same doctor. Medical care including growth hormon was free for all patients. A patient was regarded non compliant if he discontinued treatment completely or interrupted treatment for at least one year.

The data concerning the fathers' occupation could be obtained from the records in all cases, the number of grades completed was asked during check-ups or in a questionnaire sent out to the parents. In the case of 61 out of 62 fathers the occupation was in accord with the educational level so that in the 8 fathers whose educational level was not known it was deduced from the occupation. The information available about mothers' education was not sufficient for analysis. For the statistical analysis Students' t-test and the chi^2 test were used.

RESULTS

There was a tendency for an increase of the age at diagnosis and the degree of growth retardation with decreasing paternal educational level, the difference between the groups, however, was not significant (Table I). The occurrence of patients with severe growth retardation ($<$ -4.5 SDS) at diagnosis was significantly related to the number of grades completed by the father (Table II). Of the 70 patients treated in the given period 18 interrupted treatment for more than 1 year or discontinued it definitely. There were relatively more girls

TABLE I

The relationship of the educational level of the father to the age and extent of growth retardation at diagnosis

		Grades completed			
		< 8	8	11-12	>12
	n	8	31	29	10
Age at diagnosis, years. Mean ± SD		9.9±52	8.6±3.6	7.9±3.4	6.9±3.0
Height at diagnosis SDS Mean ± SD		-6.4±2.4	-4.2±0.9	-3.8±1.0	-3.7±0.7

TABLE II

The occurrence of severe growth retardation at diagnosis in relation to the fathers' educational level

	Grades completed			
Height SDS	< 8	8	11-12	>12
≥4.5	1	15	22	9
<4.5	7	16	7	1

chi^2 = 19.2 $p < 0.001$

than boys in the non compliant group 10:8 vs 18:36 in the compliant group (chi^2=2.5 non significant). The average distance of the clinic from the patients' home was 90 km in the non compliant and 130 km in the compliant patients. In the year preceding the interruption of treatment growth was unsatisfactory (< 4 cm/year) in one patient. There was a significant relationship between the fathers' educational level and compliance (Table III). Six of the seven patients whose height was below -4.5 SDS at diagnosis and whose father completed less than 8 grades became non compliant.

TABLE III

The occurrence of non compliance during treatment in relation to the fathers' educational level

	Grades completed			
	< 8	8	11-12	>12
Non-compliant	8	6	4	-
Compliant	1	15	26	10

chi^2 = 24.7 $p < 0.001$

DISCUSSION

The growth rate of children with growth hormone deficiency before treatment is variable so that the time necessary to accumulate the same degree of growth retardation is different from patient to patient. We have shown that the most important factor which determines the age at diagnosis is the extent of growth retardation /5/.

No relationship has been found between the degree of growth retardation at the time of diagnosis and the sex or the nutritional state of the patient, or the height of the parents. In agreement with Herber et al we have also observed that in the last years growth hormone deficiency has been detected at a less severe growth retardation than previously /5, 6/ suggesting an improved familiarity of the doctors with growth problems. In the present paper we demonstrated that socioeconomic factors are also of importance in early diagnosis, and, in consequence, in the final height of patients.

To our knowledge, the problem of compliance during growth hormone treatment has not been studied previously. Apart from diabetes mellitus, growth hormone deficiency is the only condition in childhood where chronic treatment consists of the regular administration of injections. In diabetes mellitus some of the consequences of irregular treatment become apparent in a short time, while in growth hormone deficiency the results of treatment or non treatment can only be appreciated after at least a year which may unfavorably affect compliance. It has been shown that due to the exaggerated expectations raised by the doctors /8/, some patients and parents are disappointed in the treatment results. In our own experience discontent with treatment seems to be less important since in most of these patients treatment tended to be irregular already in the first year. As shown by our data and our personal impression negligence of the parents associated with low socio-economic status is the most important determinant of non compliance during growth hormone treatment. Although in our material, as well as in that of others, growth hormone deficiency was more

frequent among boys than girls, there were more girls in the non compliant group which may reflect a difference in the attitude of parents to the importance of height in the two sexes.

If a patient failed to turn up for checkup for six months, a letter inquiring about the cause of interruption of treatment was sent to the parents. In some cases this resulted in the resumption of treatment which, however, usually lasted for a short time only. Our results suggest that only an active search for children with pathological growth retardation and new approaches to the improvement of compliance can increase final height in a group of growth hormone deficient patients.

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Acta Paediatrica Hungarica 32 (2), pp 223-231 (1991)

DECREASED SENSITIVITY OF CYTOSTATIC DRUGS IN GLUCOCORTICOID RECEPTOR-FREE ACUTE MYELOID LEUKAEMIA CELLS. CLINICAL AND EXPERIMENTAL OBSERVATIONS

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Received 4 Dec 1989

Preliminary clinical observations have suggested that low cellular glucocorticoid receptor (GR) levels might have been connected with multidrug resistance in children with acute myeloblastic leukaemia (AML). To test this possibility, we have developed glucocorticoid resistant subclones of two recently established human myeloid leukaemic cell lines. The cause of glucocorticoid resistance was GR negativity in these subclones. GR positive parent cell lines or GR negative subclones were incubated for 1 h in the presence of Adriamycin, Cytosine-arabinosid, Etoposide or Vincristine, respectively. After short-term (1 h) incubation in suspension cultures cells were washed and plated in clonogenic agar cultures. Each anticancer drug was more potent against both GR positive parent cell lines than against the GR negative subclones. The results of this study suggest that the absence of GRs is a useful marker of multidrug resistance in childhood AML.

INTRODUCTION

Drug resistance is a major reason for treatment failure in childhood acute myeloblastic leukaemia (AML) /8/. Mechanisms of resistance are not well understood /5/, although the overexpression of a plasma membrane glycoprotein, P-glycoprotein, has been observed in several multidrug resistant cell lines and clinical specimens including AML cells /1, 10/. On the other hand, Adriamycin resistance of the promyelocytic human leukaemia cell line HL-60 has been reported in the absence of detectable P-glycoprotein /11/. Recent clinical observations have suggested that glucocorticoid resistance might also have been connected with pleiotropic drug resistance

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in leukaemic children resulting in treatment failure /12/.

In the present study, glucocorticoid receptors (GR) of primary AML cells were investigated and GR level of the cells was related to remission induction. Moreover, in vitro sensitivity in two recently established human myeloid leukaemia cell lines, BRM and DD, and in their glucocorticoid resistant subclones, BRMd and DDd, was studied using 4 anticancer agents: Adriamycin (ADR), Cytosin-arabinoside (ARA-C), Etoposide (VP-16) and Vincristine (VCR).

MATERIALS AND METHODS

Chemicals. VP-16 and its solvent consisting of 150 mg benzyl-alcohol and 3250 mg polyethyleneglycol-300 in 5 ml destillated water was provided by Bristol Laboratories. RPMI-1640 fetal calf serum (FCS) and phosphate buffered saline pH 7.4 (PBS) were purchased from GIBCO. Lymphoprep was a product of Nycomed. (1,2,4 ^{3}H)-Dexamethason was made by Amersham. Agar was purchased by Difco. All other chemicals were purchased by Sigma. ADR and VCR were dissolved in ethanol-PBS at 1:1 v/v. ARA-C was dissolved in RPMI-1640. Solvents were present in control flasks at equivalent levels.

Patients. 10 children with AML participated in the study. Two patients were examined both at the onset of the disease and in relapse. Diagnosis was established according to FAB criteria /2/. All patients were treated according to the protocols of the Hungarian Leukaemia Study Group for Children.

Cell lines and culture conditions

BRM (CD13+, CD14+, CD33+, VIM2+, HLA-DR-, TdT-, myeloperoxidase-) and DD (CD24+, VIM2+, HLA-DR-, TdT, myeloperoxidase-) are recently established myeloid leukaemia cell lines. DD cells were provided kindly by Dr. G. Szegedi (Department of Internal Medicine III of the Medical University of Debrecen). Two variants, BRMd and DDd, resistant to growth inhibitory action of dexamethasone were isolated by culturing BRM cells for 62 subcultures and DD cells for 54 subcultures (3- to 4-day intervals), respectively, in the presence of increasing concentrations of dexamethasone /6/. Both parent cell lines and glucocorticoid-resistant variants were grown in plastic Petri dishes (Nunc) at 37 ^{o}C in a humidified atmosphere of 5% CO_2 in air in RPMI-1640 supplemented with 2mM L-glutamine, 100 U/ml Penicillin, 100 ug/ml Streptomycin and 10 % FCS (complete culture medium; CCM). Cells in exponential growth phase were used.

Dexamethasone binding assay

Glucocorticoid binding of whole cells was carried out with (1,2,4-^{3}H)-Dexamethasone as described previously /7/. Briefly, various mixtures of (^{3}H)-Dexamethasone and cold dexamethasone were added to 0.1 ml cell suspensions at 37 ^{o}C for 30 min.

After terminating the reactions by rapid cooling and washing in ice cold PBS, cells were resuspended in 5 ml of scintillation fluid. Radioactivity was measured in a Nuclear ISOCAP 300 radiospectrofluorimeter. All assays were outlined in triplicates. Specific dexamethasone binding was defined as the difference in radioligand binding in the absence and presence of unlabeled hormone. Binding sites per cell (GR level) were calculated according to Scatchard /13/ or to the abbreviated single point assay /4/ if the number of available cells was limited. Dissociation constants (K_D) were determined from the Scatchard graphs.

In vitro drug treatment. BRM, DD, BRMd and DDd cells at a density of $5x10^5$ cells/ml in CCM were exposed to various concentrations of anticancer agents for 1 h at 37 ^oC in 5 % CO_2.

Colony-forming assay. After short-term (1 h) incubation, cells were washed twice in RPMI-1640, counted and subsequently plated at $5x10^2$ cells/ml in 0.3 % agar medium containing RPMI-1640 supplemented with L-glutamine, antibiotics and 20 % FCS. Cultures were incubated for 7 days at 37 ^oC in a humidified atmosphere of 5 % CO_2 in air. Colonies containing more than 15 cells were counted using an inverted microscope (Leitz). Cytotoxicity was determined by comparing colony counts from drug-treated cells with counts from cells incubated only with solvent (controls) in per cent of controls.

Statistics. Agar cultures were performed in triplicates. Mean $\pm$ S.D. of two to three repeated experiments were presented. Group data were compared by Student's t-test after checking the normal distribution of the data by Geary's test and comparing S.D.-s by the t-test.

RESULTS

GRs and treatment results in AML patients. GRs were detected in each primary AML cases (Table I). Patients had GR levels ranging between 114 to 10763 (mean$\pm$S.D.:4623$\pm$3566), while values of control subjects scattered between a remarkably narrower range: 1027 to 9311 (mean$\pm$S.D.:2523$\pm$1646), as determined previously /7/. K_D values of patients were in the nanomolar range as in control persons. Two patients were investigated in relapse (cases N^o 2/b and 5/b in Table I). However, both relapsed patients exhibited very low GR levels. Less than 1000 GRs/cell were observed in three cases (cases N^o 2/b, 5/b and 10 in Table I). These cases did not respond to antileukaemic treatment protocol, while remission induction was successful in 8 out of 9 cases exhibiting more than 1000 GRs per cell.

TABLE I

Glucocorticoid receptors (GR) in acute myeloid leukaemic children

	GR/cell	K_D (nM)		Remission induction	Outcome of the disease
1.	10763	5.7	M5b	successful	2nd CCHR
2/a.	6772	-	M2	successful	relapsed
2/b.	250	-		unsuccessful	died
3.	6474	-	M4	successful	died
4.	6330	-	M6	unsuccessful	died
5/a.	5833	-	M1	successful	relapsed
5/b.	0	-		unsuccessful	died
6.	3416	-	M1	successful	died
7.	2261	-	M1	successful	died
8.	1176	10.3	M1	successful	died
9.	1000	-	M1	successful	died
10.	114	-	M1	unsuccessful	died

CCHR denotes complete clinical and haematologic remission

In vitro anticancer drug sensitivity of GR positive lines and GR negative subclones

BRM and DD cells were characterized by a high number of GRs, 15476 and 7270 GR/cell respectively. K_D values were 5.4 nM in case of BRM cells and 13.4 nM in case of DD cells. BRMd and DDd cells which were resistant to growth inhibitory action of dexamethasone, did not have specific dexamethasone binding sites, i.e. GRs.

The effect of the anticancer drugs on colony formation of glucocorticoid-sensitive and -resistant cell lines were summarized in Fig. 1 and 2. Each drug caused a concentration dependent inhibition of colony formation of the investigated cell lines. ADR and VP-16 were proven to be the most effective agents used in pharmacologic concentrations /3, 14/. VCR was moderately inhibitory. ARA-C was the least effective in these cell lines. Identical concentration of each four drugs resulted in significantly greater decrease in colony formation of BRM and DD cells than of BRMd and DDd cells.

DISCUSSION

Low GR level is a proven unfavourable prognostic factor in childhood acute lymphoid leukaemia (ALL) /9/. In our previous study we have chosen the arbitrary value of 1000 GR/cell in childhood ALL /7/. All children having less than 1000 GR/cell exhibited a significantly worse outcome of the disease than patients with GR levels between 1000 to 10000 /7/. The observations made by AML children suggest that less than 1000 GR/cell may be a useful marker of poor disease outcome in childhood AML as well. Because of the limited number of patients we did not perform statistical evaluation. In order to study the possible connection between low cellular GR level and multidrug resistance in AML, as suggested by the clinical observations, an in vitro model was developed using glucocorticoid sensitive, GR positive human myeloid leukaemia cell lines and their glucocorticoid resistant, GR negative

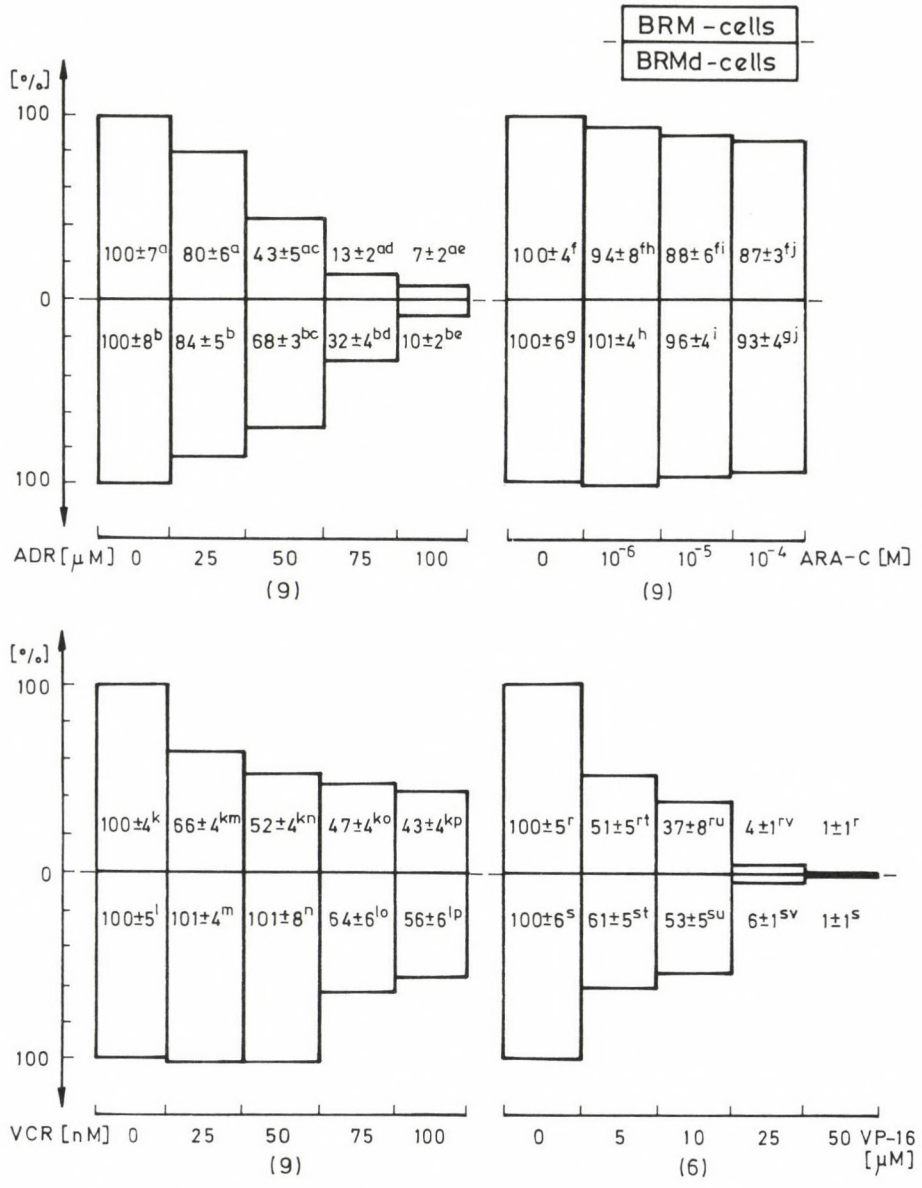

Fig. 1. Effect of antileukaemic drugs on colony formation of BRM and BRMd cells.
Mean ± S.D. values of relative plating efficiencies are given in per cent.
$^{a-v}$indicate statistically significant differences between the corresponding groups

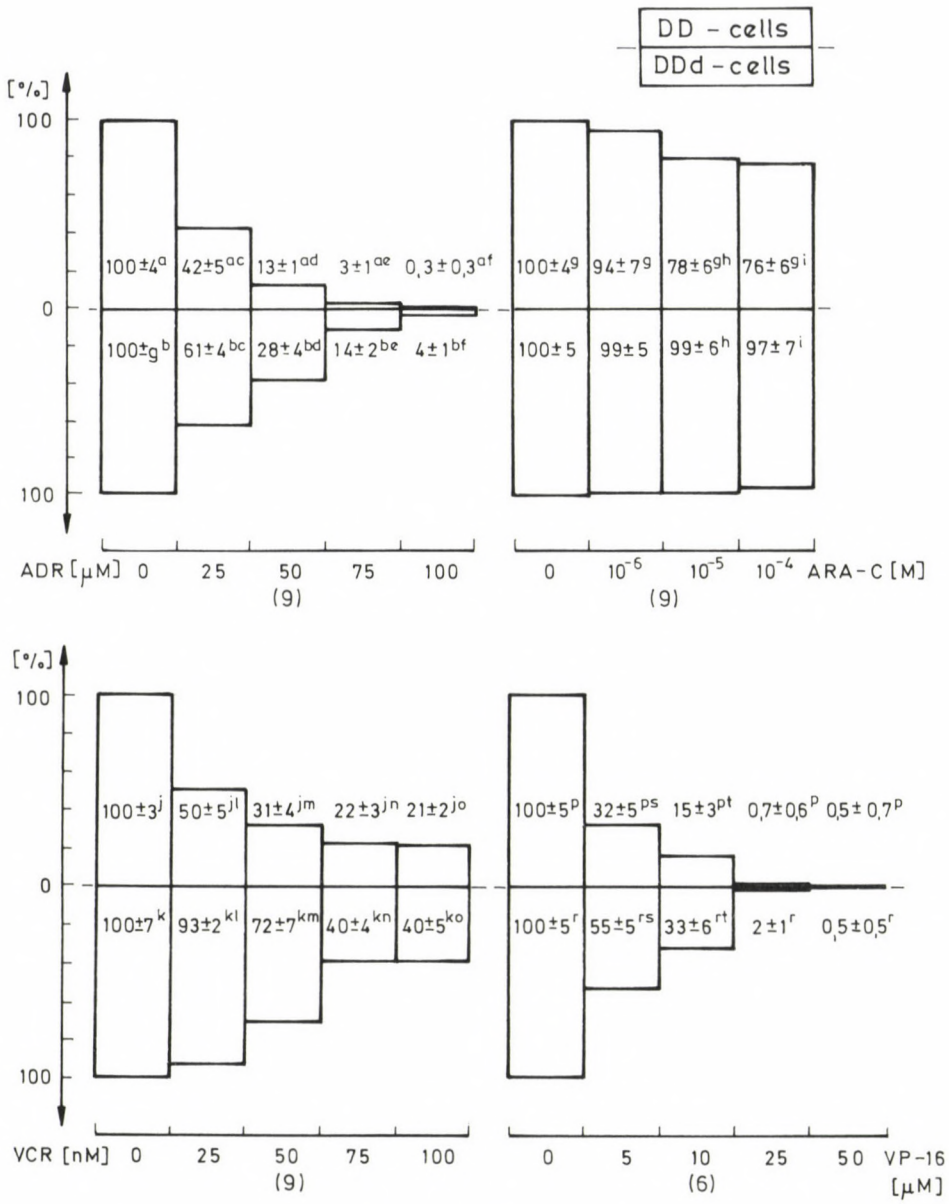

Fig. 2. Effect of antileukaemic drugs on colony formation of DD and DDd cells.
Mean ± S.D. values of relative plating efficiencies are given in per cent.
$^{a-t}$indicate statistically significant differences between the corresponding groups

subclones. In vitro clonogenic assay was performed after short-term incubation with ADR, ARA-C, VP-16 and VCR. Each investigated anticancer drug was significantly less toxic against the GR negative subclones than against the GR positive parent cell lines.

Our results suggest that glucocorticoid resistance caused by the absence of or decrease in the cellular GR content of AML cells may be a useful indicator of pleiotropic drug resistance.

ACKNOWLEDGMENT

C.K. was supported partially by the University of Ulm, F.R.G.

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Acta Paediatrica Hungarica 31 (2), pp 233-239 (1991)

EUTHYROID SICK SYNDROME IN CHILDREN WITH ACUTE VIRAL HEPATITIS A

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Received 3 January 1990

According to the clinical findings, the activity of serum asparate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2) and the level of total bilirubin, 45 children with acute viral hepatitis A were divided into two groups: with mild and moderately severe degree of disease. By determining the products of the peripheral thyroxine metabolism - T3 and rT3, as well as the other thyroid parameters (T4, FT4, TSH and TBG) we have found significantly lower T3 level and significantly higher T4 and TBG levels in both groups of patients in comparison with control group. At the same time, the level of biologically less active rT3 was increased in patients with moderately severe form of disease, while no differences were found in the values of TSH between the ill and control patients. TRH induced TSH release was normal in all patients. The results of this study point to the development of euthyroid sick syndrome or low T3 syndrome in children with viral hepatitis A.

INTRODUCTION

Liver plays the major role in extrathyroid T4 deiodination where highly active T3 and less active rT3 are produced /1,2,10/. Deiodination is performed by the action of two enzymes: 5'-monodeiodinase and 5-monodeiodinase, respectively. Their subcellular localization is currently under investigation /6/, but separate cell particles i.e. microsomal fraction are assumed to be involved /8/.

Peripheral T4 deiodination may be altered during the acute and chronic liver diseases, especially in cirrhosis when the conversion of T4 to rT3 is increased, while to T3 decreased

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/3,4,7,11/. Conversion disturbances lead to the changes of thyroid hormones serum level designated as "euthyroid sick syndrome" or a "low T3 syndrome" /12/, characterized by the following serum hormone concentrations: low T3, normal or increased rT3, variable T4, normal or increased FT4 and normal TSH /5/, as well as the inability of TRH to induce excessive TSH increase typical for hypothyroidism /12,14/. Because there are no published studies, the possible connection between viral hepatitis and euthyroid sick syndrome might be only inferred. We therefore assumed that such syndrome might be present during viral hepatitis A in children and that, like in hepatic cirrhosis, may be based on the decreased conversion of T4 to T3. The results of our study of basal T3, T4, FT4, rT3, TSH and TBG and TRH induced TSH serum levels aimed at checking this assumption were found to be confirmatory.

PATIENTS AND METHODS

According to epidemic history, clinical and laboratory findings, 45 children of both sexes (20 females and 25 males; 18 prepubertals and 27 pubertals) with viral hepatitis A, aged 4.5 to 15 years (mean 10.5 $\pm$ 2.8) admitted at the Department of Infectious Diseases were divided into two groups: one with moderate (n = 27) and the other with moderately severe (n = 18) liver disturbances. Both groups were of comparable mean body weights (kg) (34.6 $\pm$ 8.8:30.3 $\pm$ 9.0) and body heights (cm) (136.2 $\pm$ 13.0:131.0 $\pm$ 14.2). The control group consisted of 30 healthy children of both sexes (16 females and 14 males; 13 prepubertals and 17 pubertals) aged 6 to 14 years (mean 10.1 $\pm$ 2.7), with mean body weights (kg) (33.3 $\pm$ 10.7) and body heights (cm) (135.0 $\pm$ 15.0).

The activities of the enzymes in the groups with moderate and moderately severe disturbances were the following: aspartate amino transferase (nkat/L) 1527 to 9255 : 10520 to 36560 (Normal value to 567), alanine aminotransferase (nkat/L) 2025 to 15940:8484 to 33090 (Normal value to 750), and the level of total bilirubin (umol/L) 116 to 180:116.8 to 240 (Normal value 6.8 to 20.4).

For the determination of tyroid functional parameters in all three groups after an overnight fasting, cannulation of an antecubital vein blood was taken, before the initiation of treatment. Besides this sample, the additional 3 ml of vein blood was withdrown at 30 and 60 min intervals after i.v. administration of synthetic TRH (Roche) in a dose of 200 ug/m^2 /9/. After the centrifugation, serum was pipetted and

stored at -20° C until the hormone level determination. For the determination of total T4 and T3, RIA kit (Institute of Nuclear Sciences "Boris Kidrič", Vinča) was used. Free T4 was determined by RIA-coat FT4 (Mallincrodt Diagnostica, Dietzenbach), total rT3 level by rT3 kit (Biodata, Roma), TBG by RIA-hTBG complet (INEP, Zemun) and level by RIA-hTSH (INEP, Zemun).

All data were represented as the mean 1 ± SD. The statistical analysis of the results was done by Student's t-test.

RESULTS

Table shows that the pattern level of serum hormone concentrations in both groups of studied patients in relation to the control one was the same. Namely, the significant decrease of T3 and increase of T4 and TBG were found in both groups, whereas rT3 concentrations were significantly higher in the group with more severe stage of the disease.

A better insight into the results may be obtained from the Figure in which individual values of the studied parameters are presented. It is evident that the levels of T4, rT3 and TBG rise progressed with the severity of the disease, while T3 concentrations followed the opposite direction. TRH induced TSH release was normal in all patients.

DISCUSSION

The results of our study in children with viral hepatitis A have shown that depending on the severity of the disease, the level of the thyroid serum hormones changes in a way that the values of T3 decrease and T4 and TBG increase in relation to the control group. Simultaneously, the level of less biologically active rT3 rise in patients with severe form of liver disturbances. No changes have been found in TSH and FT4 levels.

High serum concentrations of total T4 and TBG in our patients are in agreement with the findings of Schussler et al.

TABLE

Serum concentrations of thyroid parameters in children with viral hepatitis A and in controls

Patients	Thyroid parameters (X ± S.D.)					
	T3 (nmol/L)	T4 (nmol/L)	FT4 (pmol/L)	rT3 (nmol/L)	TSH (mIU/L)	TBG (mg/L)
Healthy (control n = 30)	2.2 ± 0.5	108.0 ± 29.0	20.1 ± 4.9	0.3 ± 0.1	2.4 ± 1.5	24.2 ± 3.1
Mild hepatitis (n = 27)	1.7 ± 0.5[X]	137.0 ± 22.7[X]	22.2 ± 4.2	0.3 ± 0.2	2.4 ± 1.7	57.2 ± 21.2[X]
Moderately severe hepatitis (n=18)	1.7 ± 0.5[X]	171.9 ± 23.5[X]	21.8 ± 4.2	0.5 ± 0.1[X]	1.6 ± 1.4	59.2 ± 16.8[X]

[X]Statistically significant difference in relation to the control with the P values from 0.001 to 0.0005.

/13/, as may be stated for T3 as well, but which in our study have shown large dissipation around the mean value in comparison with control group (see Figure).

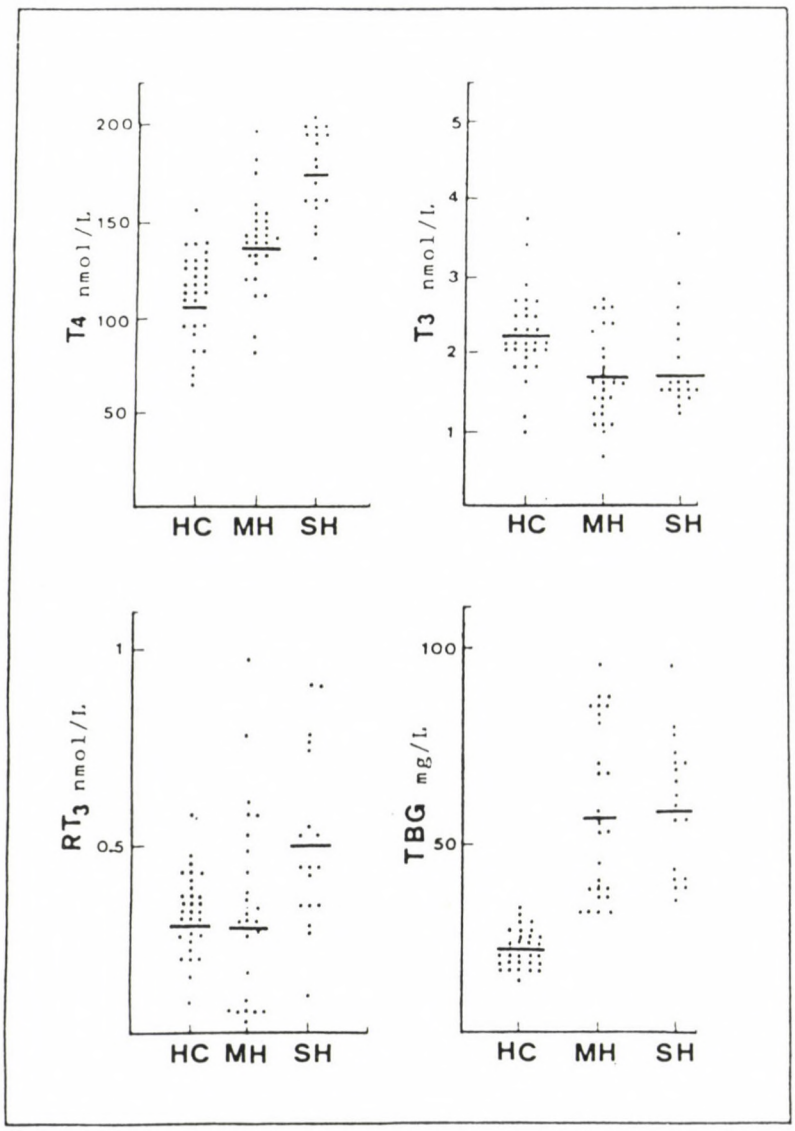

Figure: Individual values of T4, T3, rT3, and TBG in healthy (HC); children with mild hepatitis (MH) and moderately severe hepatitis (SH)

An interesting finding in our study is the normal serum value of rT3 in patients with mild stage of the disease, as well as its increase in patients with moderately severe form. The increase of this parameter was also observed by the other authors in patients with liver cirrhosis /7,11/ and the rT3 increase may be associated with more severe liver disturbances.

According to Schussler et al /13/ the rise of total T4 in both groups of diseases, i.e. in liver cirrhosis and chronic liver disease, is due to TBG increase which is considered as non-specific marker of the inflammatory processes. This is confirmed by our results with FT4, which in both groups of patients were similar to the values of control group.

Finally, the most important finding in our study was low T3 level in both groups of patients, pointing thus to the decreased monodeiodination of other thyroxine ring, to which the simultaneous increase of T4 level may also contribute. Namely, it is known that the enzyme 5-monodeiodinase shows the high affinity to rT3. So in addition to this, the altered enzyme synthesis due to hepatocyte damage leads to the both: lowered T3 production and impede rT3 degradation.

In conclusion, the results of this study show that during the viral hepatitis A in children, euthyroid sick syndrome develops, which is confirmed by the changing of the serum thyroide hormone levels in the presence of the normal TRH test values.

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Acta Paediatrica Hungarica 31 (2), pp 241-253 (1991)

PULMONARY HYDATIDOSIS IN CHILDHOOD. REVIEW OF 21 CASES

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Received 13 November 1989

Spain is one of the countries with a very high incidence of hydatidosis in the childhood. It represents 16.8 % of all cases intervened for thoracic surgery for hydatidosis cysts in our department during the last ten years with a total of 21 children (inferior to 14 years) operated due to hydatidosis. Cough and pain were the symptoms more frequently encountered. The ratio unruptured/ruptured cysts was 3/1, higher than in the adults, with an average of 2.62 cysts per patient. Specific immunoglobulin E and histamine liberation test were the most useful tests in the laboratory. The usual surgical technique was a cystopericystectomy with total extirpation of the parasite of its rests. No recurrence was found in the follow-up of our patients.

INTRODUCTION

Spain is one of the countries with the highest incidence of hydatidosis in the world /1/. This disease results endemic to the Mediterranean region (particularly in Algiers and Morocco), South America , Australia and New Zaeland /2/.

In its adult stage, this parasite lives in the intestine of dogs, while man represents an intermediate host who contracts the disease from water or food or from direct contact with dogs. The embryos may later overcome the hepatic obstacle and may become lodged in the lung.

The present study reviews the patients who underwent surgery for pulmonary hydatidosis in their childhood during the last ten years in our department.

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MATERIALS AND METHODS

We review the casuistic of the Thoracic Surgery Department of the Hospital Ramon and Cajal in Madrid, finding 21 children, who were surgically intervened for pulmonary hydatidosis in the last ten years. This group represents 16.8 % of the total of 125 patients operated for this disease in our Thoracic Surgery Department.

The mean age was 10.9 years (S.D. 2.76). The highest number of cases were between 12 and 14 years (42.9 %) with a total of 15 boys (71.4 %) and 6 girls (28.6 %).

The two symptoms more frequently registered were cough and pain followed by fever and hemoptysis. Four children were found asymptomatic, being discovered only after an X-ray was taken for another problem (Table I). In 7 cases (33.3 %) there was a prior contact with dogs.

TABLE I

Symptomatology

	Cases	%
Cough	10	47.61
Pain	9	42.85
Fever	6	28.57
Hemoptysis	4	19.04
Asymptomatic	4	19.04
Dyspnoea	3	14.28
Vomica	1	4.76

From the radiological study based on conventional methods and CT, 15 cases presented unruptured cysts (71.4 %) and 6 ruptured cysts (28.6 %) (Fig. 1, 2, 3). 13 cases were constituted by single cysts (61.9 %) and 8 by multiple cysts (38.1 %), with an average of 2.6 cysts per patient (4 with unilateral multiple cysts and 4 bilateral), (Table II), with the typical images of ruptured and unruptured cysts (Fig. 4, 5, 6).

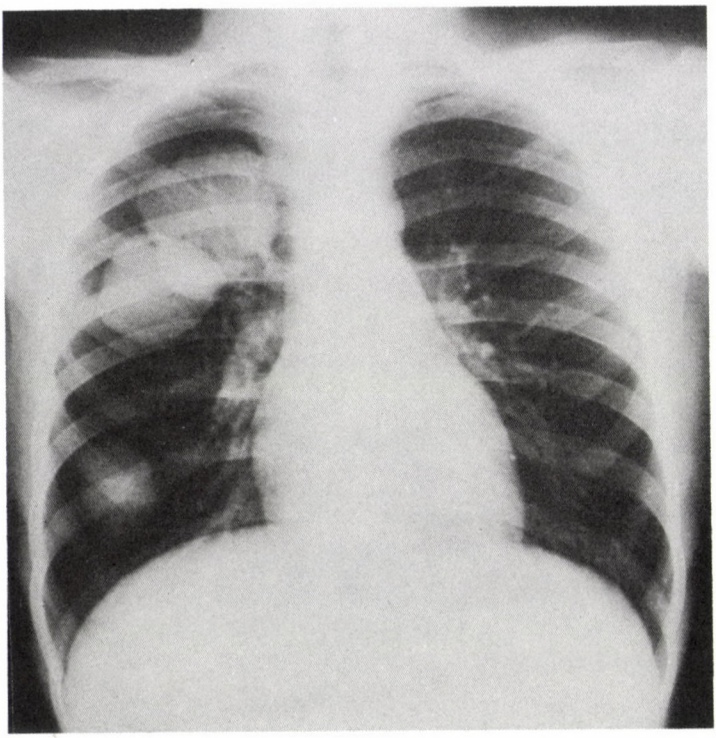

Fig. 1. P-A X-Ray: image of three cysts in the right lung

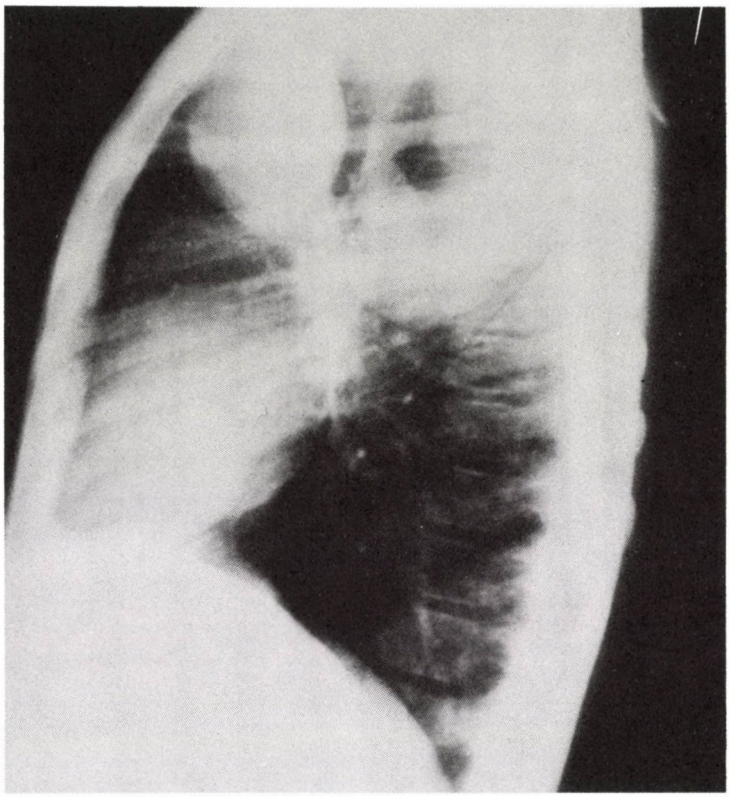

Fig. 2. Lateral view of the same case of Figure 1

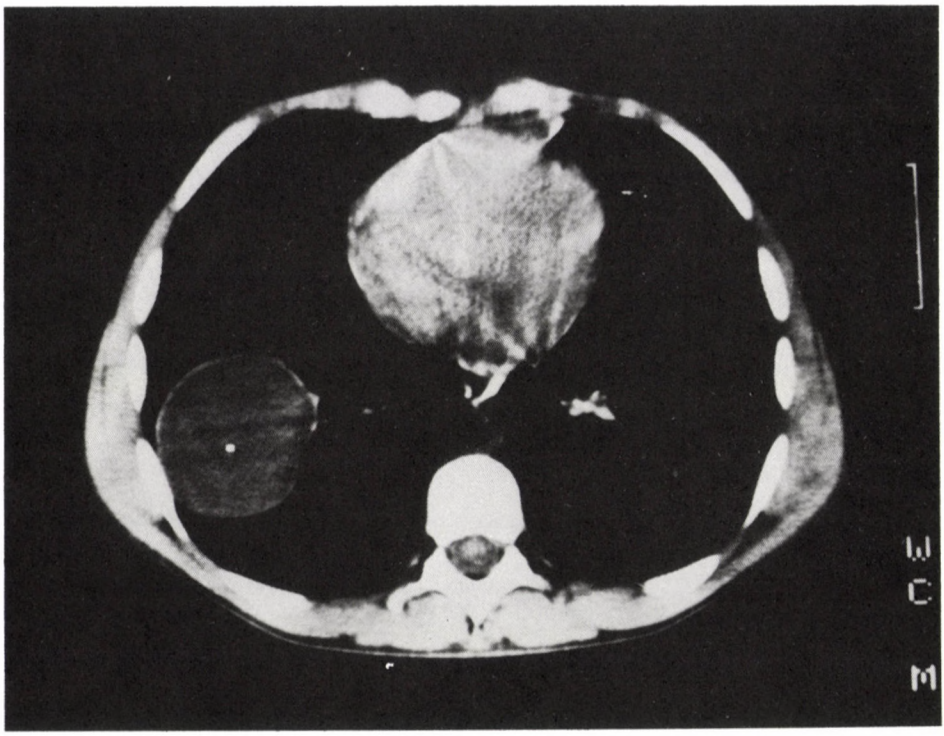

Fig. 3. Thorax CT: unruptured cyst in the right lower lobe.

TABLE II

Radiology

	Cases	%
1. Cyst type:		
Unruptured	15	71.4
Ruptured	6	28.6
2. Number of cysts:		
Single	13	61.9
Multiple	8	38.1

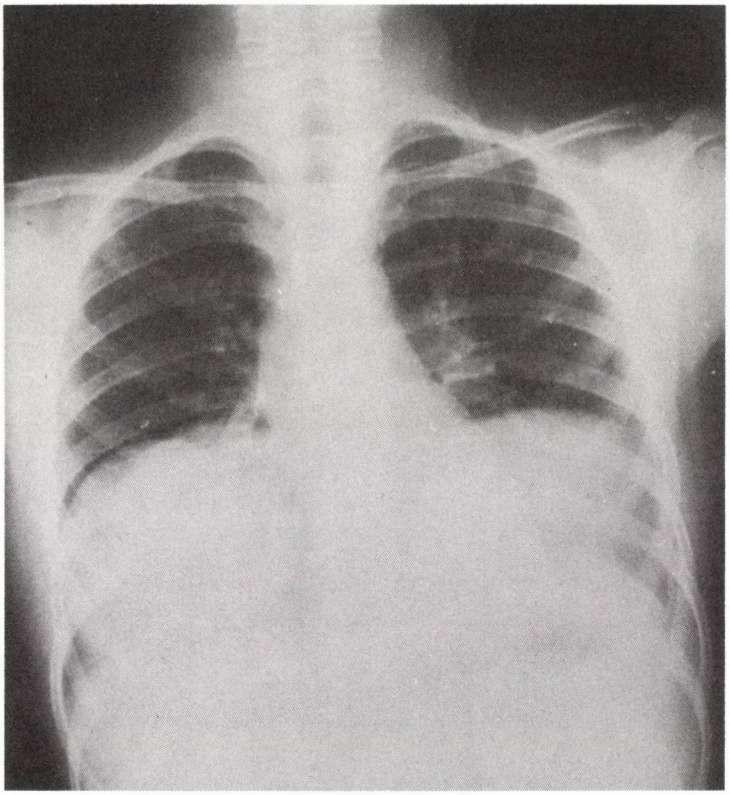

Fig. 4. P-A X-Ray: bilateral unruptured cyst.

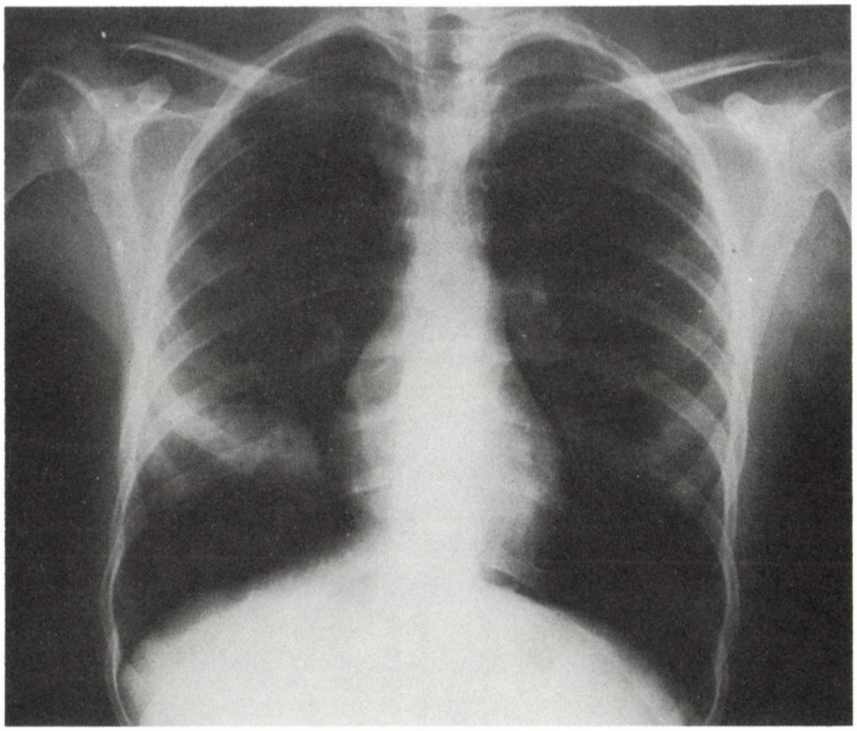

Fig. 5. P-A X-Ray: ruptured cyst in the right lower lobe

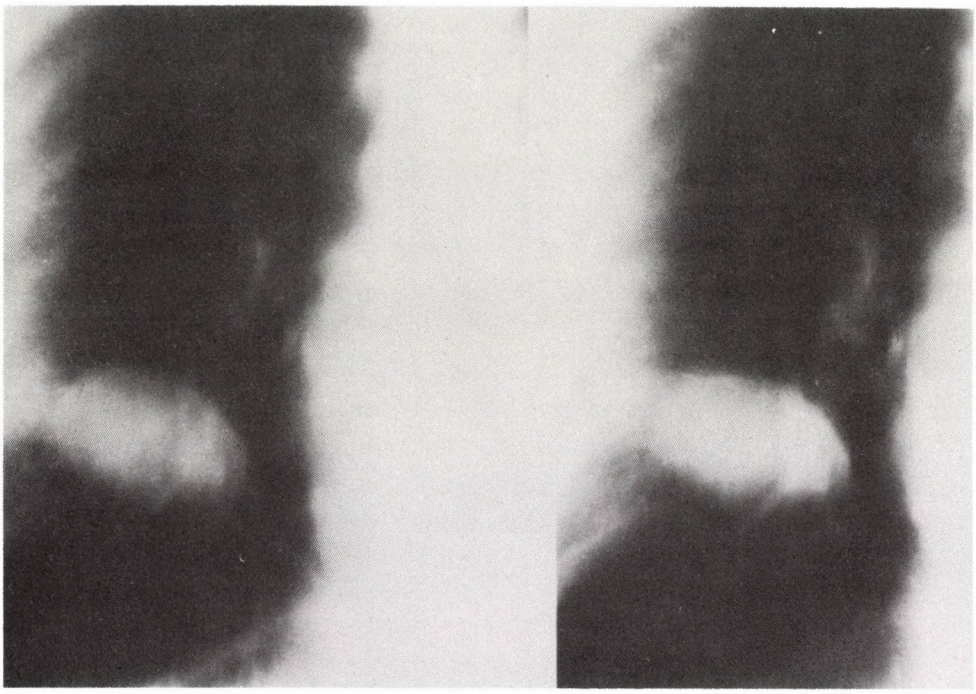

Fig. 6. Tomography (5, 5-6 cms.): same case of Figure 5.

Both inferior lobes were most often affected, with the left inferior lobe in high proportion. Reference should be made additionally to the presence of a child with 5 cysts in the mediastinum, who had been operated previously for a ruptured cyst in a different hospital (Table III).

TABLE III

Cyst location

	Cysts	%
Upper right lobe	5	14.3
Middle lobe	2	5.7
Lower right lobe	9	25.7
Upper left lobe	2	5.7
Lower left lobe	12	34.3
Mediastinum	5	14.3

The most sensitive tests were in the ruptured cysts (p inferior to 0.005) in comparison with the unruptured cysts, showing the high specificity obtained with specific IgE and histamine liberation test. In unruptured cysts indirect hemagglutination was positive in 10/15 cases (66.6 %), indirect immunofluorescence in 9/15 cases (60 %), immunoglobulin E (specific) in 12/15 (80 %) and histamine liberation in 13/15 cases (86.7 %). In ruptured cysts, indirect hemagglutination was positive in 4/6 cases (66.6 %), indirect immunofluorescence in 3/6 (50 %), immunoglobulin E (specific) in 4/6 (66.6 %) and histamine liberation in 5/6 cases (83.3 %) (Table IV).

TABLE IV

Laboratory tests

	Unruptured		Ruptured	
		%		%
Indirect hemagglutination	10/15	66.6	4/6	66.6
Indirect immunofluorescence	9/15	60	3/6	50
Immunoglobulin E	12/15	80	4/6	66.6
Histamine liberation	13/15	86	5/6	83.3

RESULTS

The approach most often used was the posterolateral thoracotomy in 12 cases (57.1 %), followed by the axillary thoracotomy in 5 (23.8 %). In 4 patients with a bilateral hydatidosis we performed a sequential bilateral thoracotomy with a mean interval between both surgeries of 22.4 days. In these cases of second surgeries this approach was preferred to the median sternotomy.

The surgical technique most frequently used was the subtotal cystopericystectomy with total extirpation of the cysts or its rests in the ruptured cysts followed by close of the bronchial leaks, washing the cavity with hydrogen peroxide 10 % during 3 minutes and subtotal extirpation of the pericyst leaving the hilar pole. In 4 cases it was necessary to perform a lung resection (19.1 %), 2 due to the size of the cysts and 2 due to the status of the distal parenchyma (Table V). In the cases of two surgeries, a total of 3 cystopericystectomies and one wedge resection were performed on the contralateral lung. The operative mortality was nil, while the postoperative morbidity was low. No recurrence was registered during the follow-up of our patients (Table VI).

TABLE V

Surgical technique

	Cases	%	Bilateral surgeries	%
Cystopericystectomies	17	81.0	3	14.2
Wedge resections	3	14.3	1	4.8
Lobectomy	1	4.8		

TABLE VI

Complications

	Cases	%
Atelectasia	2	9.5
Hydropneumothorax	2	9.5
Wound infection	1	4.8
Hemothorax	1	4.8

DISCUSSION

The clinical presentation of hydatidosis in children has some different aspects with regard to the adults. This disease rarely occurs before two years of age, and thus in our series the youngest case was a six-year-old boy.

Diagnosis is based in the X-ray and laboratory tests, while the clinic and the antecedents of contact with dogs prove also very useful. The presence of hydatidic cyst expectoration is lower than in the adults, as the ratio between unruptured and ruptured cysts was 3/1, inverse to the ratio found in the adults, due to an earlier diagnosis of thoracic hydatidosis in the childhood /3, 4/.

The lobe distribution between both lungs is similar to the adults, except for the predominance of the left lower lobe to the right lower lobe /5/.

The laboratory tests were more sensitive for the ruptured cysts. Specificity obtained in our experience with the indirect hemagglutination, immunoglobulin E specific or the histamine liberation tests should be pointed out /6/.

Surgery continues to represent nowadays the treatment of choice in the management of thoracic hydatidosis, despite the benefits apported by the chemotherapy in the last years. It is important to perform the surgical intervention immediately after the diagnosis has been made to avoid complications /7/. The results achieved were excellent, even though we tend to be more conservative than in the adults /8/.

The surgical techniques available for thoracic hydatidosis in the lung can be divided initially to: those involving conservation and those involving removal of the parenchyma /9/. Of the procedures conserving the lung, the most commonly used is the cystopericystectomy, with extirpation of the cyst or its rests, followed by partial resection of the pericyst, since total resection of the pericyst in its hilar pole is of high-risk /10/.

After the extirpation of the parasite, followed by closure of the bronchial air leaks, it is very important to perform a

lavage of the residual cavity during 3 minutes with hydrogen peroxide 10 % solution. This represents the perfect scolicid solution for children, as the complications that can arise from formol or hypertonic solution are well known nowadays and in the childhood these complications may be extremely grave /11/.

It must not be forgotten that for children, like in adults, complementary or preventive treatment with mebendazole may give good results in some cases to avoid recurrences /12/. Five of our cases were treated with adjuvant chemotherapy but no definitive conclusions have arrived in our study.

Due to the parasitic character of this disease, we always investigate the family and carry out a serological and radiological study with an X-ray and abdominal echography on family members who live with the patients. In this idea we have found an incidence of hydatidosis in asymptomatic members of 18.1 % /13/. It is important also to investigate in every patient the concomittance of a liver hydatidosis and we have found in our series 5 cases with hydatidosis cyst in liver who were operated by laparotomy posteriorly.

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Acta Paediatrica Hungarica 31 (2), pp 255-261 (1991)

CARNITINE CONTENT OF RED BLOOD CELLS OF HUMAN SUBJECTS TREATED WITH PIVAMPICILLIN AND CARNITINE

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Received 15 November 1989

Total- and free carnitine content of washed pooled red blood cells collected from five children prior to and on the last day of combined pivampicillin and equal molar carnitine treatment were measured. On the last day of treatment (day 7) the level of total carnitine decreased from 47.5 ± 3.39 to 37.5 ± 2.48 nmol/ml, mean ± SEM ($p < 0.05$) with a concomitant decrease of free carnitine (from 19.2 ± 0.97 to 15.5 ± 0.99 nmol/ml, $p < 0.05$) as compared with the pretreatment control day (day 0). The calculated amount of acid soluble carnitine esters also fell (from 28.2 ± 3.38 to 21.9 ± 1.78 nmol/ml). The same effects were found when the carnitine levels were referred to haemoglobin or water content of samples.
These results demonstrate that in pivampicillin treatment the carnitine pool of erythrocytes also alters. In agreement with previous findings the data presented here suggest, that the administered carnitine was not sufficient to meet the enhanced needs of the organism caused by the pivalate load and that the organism utilized some of its stores for pivaloylcarnitine production. The decreased carnitine ester level of erythrocytes suggest, that the red blood cells do not participate in significant extent in pivaloylcarnitine transport or production.

INTRODUCTION

Pivampicillin (pivaloxymethyl ester of ampicillin) is widely used antibiotics in some countries. It was developed to reach better intestinal absorption of the drug /10/. The absorbed pivampicillin is hydrolysed to the active substrate (free ampicillin) and a part of the liberated pivalic acid conjugates

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with carnitine. The pivaloylcarnitine is eliminated probably predominantly via kidneys /5,11/. The urinary loss of carnitine as pivaloylcarnitine may ultimately produce carnitine deficiency/5/.

Although carnitine deficiency can be well tolerated, it is considered a potentially dangerous state because an additional metabolic stress like fasting, fever or acute illness may lead to metabolical changes with clinical symptoms, even life-threatening crises have been reported /3,9/.

In several types of carnitine deficiency administration of exogenous carnitine is recommended /1,2,7,9/. When pivampicillin was administered with equal molar carnitine in human subjects the magnitude of changes in urinary excreted carnitine esters showed that exogenous carnitine was a very good substrate for pivaloylcarnitine production /6/. However, the dose of carnitine was probably not sufficient to meet the enhanced needs implied by the plasma and urine carnitine values. The present work was undertaken to study the fate of erythrocyte's carnitine pool (as a different compartment) under the same conditions.

MATERIALS AND METHODS

Patients. Pooled erythrocyte samples were analyzed. The samples collected originally from five children (females, mean weight 36.4 kg, range 26.5 - 47.5 kg; mean age 9.4 years, range 8 - 12 years) who were participants of another study /6/. The clinical indications for the antibiotic treatment were ampicillin sensitive bacteriuria and upper respiratory tract infection. The daily dose of pivampicillin (Pondocillin, Leo Pharmaceuticals, Denmark) was 1.000 mg (n = 3) and 1.500 mg (n = 2) divided in 500 mg doses two- or three times daily. The dose of administered carnitine was equimolar to the pivampicillin: to each 500 mg tablet (containing 1.08 mmoles pivalic acid) 173.9 mg L-carnitine oral solution (100 mg/ml, Sigma-Tau Pharmaceuticals, Italy). Informed consent was obtained from the parents of participants.

Procedures. Prior to (day 0) and on the last day (day 7) of the treatment blood was taken between 7:30 and 8:30 AM after an overnight fasting into heparinized tubes. The samples were immediately centrifuged (600 g 5 min). After the removal of

plasma the sediment was washed twice in 0.9 % NaCl and centrifuged as previously. The sedimented red blood cells were stored at -20°C until analysis.

Chemical methods. Carnitine content of red blood cells (defined here as the carnitine within the cells and/or bound to cells) was measured by the DTNB method as described /8/ after partial purification of the samples. 500 µl erythrocyte mass was diluted with 100 µl distilled water and the protein was precipitated on ice with 100 µl concentrated perchloric acid. After centrifugation the pellet was washed with 500 µl 0.5 N perchloric acid. The combined supernatant was neutralized with 10 N KOH to pH 7.5 after addition of 100 µl 0.5 M phosphate buffer (pH 7.5). The perchlorate sediment was washed with 300 µl ethanol (40 %) and after centrifugation the supernatant was added to the above neutralized solution. The volume of the solution was measured and 20 µl DTNB (5 mM) was added to trap the free -SH groups. 250 µl mixture was applied to a small column (0.5 x 4.0 cm) containing Dowex 1X8 Cl- (200-400 mesh) resin and was washed with 2 x 250 µl water (the resin binds the free and sulphydril bound DTNB). The effluent was used for carnitine determination.

For total carnitine determination 500 µl red blood cell mass was treated with 100 µl 5 N KOH (60 min, 55 oC). After this hydrolization of the esterified carnitines the same procedure was done as described above.

The haemoglobin was measured by routinely used KCN reaction. For calculation of water content of samples 200 µl erythrocyte mass was stored in desiccator until reaching a constant weight. Thus, the water content measured in the present work is not equal to the cellular water because small amount of extracellular water was also involved.

Statistics. The Student's t test for paired samples was used with the help of an IBM compatible computer package.

RESULTS

The carnitine content of the pooled ruptured red blood cell mass is shown in Table I. Both fractions (total and free, amount of carnitine esters was calculated by substraction: total minus free) of carnitines decreased on the last day of treatment (all patients responded) as compared with the initial pretreatment value (b versus a in the Table I). No differences were found in the haemoglobin and water content of the samples comparing the day 0 and day 7 values (not shown) indicating that the yield of the erythrocytes was the same in the samples obtained prior to and on the last day of treatment (no effect of the drugs, washing and pooling procedures on these

parameters). Thus, when the carnitine levels were expressed as µmol/mmol haemoglobin on nmol/ml water the same feature was found like when the carnitine values were expressed as nmol/ml erythrocyte mass.

TABLE I

Carnitine content of red blood cells of patients taking pivampicillin and carnitine (a: day 0; b: day 7).

		total	acyl	free
nmol/ml red blood cell	a	47.5 ± 3.39	28.2 ± 3.38	19.2 ± 0.97
	b	37.5 ± 2.48*	21.9 ± 1.78*	15.5 ± 0.99*
µmol/mmol haemoglobin	a	2.44 ± 0.20	1.45 ± 0.19	0.99 ± 0.06
	b	1.94 ± 0.14*	1.14 ± 0.09*	0.80 ± 0.06*
nmol/ml water content	a	75.2 ± 3.96	44.5 ± 4.41	30.7 ± 1.73
	b	60.6 ± 3.29*	35.4 ± 2.40*	25.1 ± 1.45*

*$p < 0.05$ or less

DISCUSSION

The data show proportionally decreased total, free and esterified carnitine content of red blood cells collected from patients on the last day of combined pivampicillin and equimolar carnitine treatment compared with the initial carnitine status.

As it was shown previously, pivampicillin treatment causes exceptional formation of pivaloylcarnitine in humans evidenced

by marked excretion of this xenobiotic acyl ester of carnitine in the urine /5/. The pivampicillin treatment was associated with changes of plasma carnitine levels: the plasma total carnitine decreased due primarily to a decrease of the free carnitine, whereas an expansion in the short-chain acylcarnitines was seen /5/. These effects of the drugs were recently confirmed by others /4/, who noticed that the resulting carnitine deficiency should be considered as a risk factor regarding that in many forms of carnitine deficiency serious complications have been observed. Indeed, the present knowledge is that carnitine deficiency (insufficiency) a multiple deterioration of intracellular processes may develop leading to a spectrum of pathological changes /3,9/.

Therefore, in a previous work the fate of supplemental carnitine was studied in pivampicillin treatment /6/. At the end of the study the ratio of acylcarnitine/free carnitine in plasma increased due to a decrease of circulating free carnitine and an increase of carnitine esters /6/. The increase of carnitine esters was probably caused by the presence of pivaloylcarnitine in the plasma as suggested by gas-chromatographic analysis of an exctract of pooled, combined and concentrated plasma samples /6/. Although the ester profile of red blood cell acylcarnitines was not determined in the present study, the decrease of carnitine esters in the erythrocytes (Table I) on the last day of treatment strongly suggests that this compartment does not participate significantly or at all in the pivaloylcarnitine transport and production. By contrast, the decrease of total, free and acylcarnitine on the last day of treatment shows that the carnitine pool of red blood cells contributed to the enhanced needs caused by the increased pivaloylcarnitine generation.

Based on the present data it is raised, on the other hand, that measurement of carnitine levels of red blood cells may be a useful additional parameter in the evaluation of carnitine status of human subjects. It should be considered particularly during surveys on efficiency of carnitine supplementation.

ACKNOWLEDGEMENT

The authors are grateful to Sigma-Tau Pharmaceuticals, Rome, Italy for providing carnitine and for Leo Pharmaceuticals, Ballerup, Denmark for pivampicillin. We thank Ilona Szántó for her skilled technical assistance.

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Acta Paediatrica Hungarica 31 (2), pp 263-274 (1991)

PRENATAL DIAGNOSIS OF CYSTIC FIBROSIS BY MICROVILLAR MEMBRANE ENZYME ANALYSIS IN AMNIOTIC FLUID

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Received 4 December 1989

Prenatal diagnosis was performed in 92 pregnancies high-risk for cystic fibrosis during six years. Amniotic fluid samples obtained by amniocentesis were examined with regard to their microvillar membrane enzyme activity. Though trehalase, alkaline phosphatase isoenzymes and L-gamma-glutamyltransferase in the amniotic fluid are not specific markers of cystic fibrosis, their activity is significantly lower than in normal pregnancies. By measuring the three enzymes simultaneously, sensitivity, specificity and reliability of the method were found to be over 92 %. It is concluded that mid-trimester amniotic fluid diagnosis is indispensable for some heterozygotic couples for cystic fibrosis even in the possession of DNA (desoxyribonucleic acid) methods.

INTRODUCTION

Since 1983, the use of amniotic fluid microvillar membrane enzyme analysis for the prenatal diagnosis of cystic fibrosis (CF) has been suggested by several laboratories. The most important enzymes are: peptidases, e.g., L-gamma-glutamyltransferase (GGT), disaccharidases (DS), (e.g., trehalase, lactase) and phosphatases, e.g., alkaline phosphatase (ALP). Since the glandular cells of affected organs (sweat glands, pancreas, intestines, bronchi) are rich in microvilli, it is not surprising that in the case of fetal CF, microvillar enzyme activity in the amniotic fluid is lower than

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in normal pregnancies /2, 5, 10, 14/.

We described our results with this method first in 1984, then in the following year /13, 14/. During the past 6 years we have had a sufficient number of cases to evaluate retrospectively and determine the place and value of amniotic fluid enzyme analysis, besides molecular genetic methods, in the prenatal diagnosis of CF.

MATERIALS AND METHODS

Between 1 January, 1983 and 31 December, 1988, amniocentesis was performed in the 16-21 gestational weeks in 92 pregnancies because of high risk for CF (25 %) or pathological ultrasound findings (dilated intestinal lumen and/or meconium plug) at the Genetic Counselling Unit of the Department of Obstetrics and Gynecology, Debrecen.
The enzyme analysis of amniotic fluid samples taken from these 92 and from 175 healthy pregnancies of the same gestational age was done by the following methods:

1. Determination of the activity of two disaccharidases (trehalase and lactase) (see 14).
2. Kinetic determination of alkaline phosphatase (ALP) activity. The reagent contained: 0.60 ml buffered substrate (1.02 mol/l dietanolamin-HCL, pH 9.8, 10.27 mmol/l p-nitrophenylphosphate-chlorid, 0-5 mmol/l $MgCl_2$ and 60 ul amniotic fluid supernatant. Enzyme activity was determined from p-nitrophenol released after a 3-minute incubation period by photometry at 405 nm.
2. Measurement of enzyme activity to determine ALP isoenzyme activity in the presence of 5.0 mmol/l L-phenylalanine (a placental and intestinal isoenzyme inhibitor) was performed as described above.

$$\text{Inhibition ratio (\%)} = 100 - \frac{\text{activity in the presence of inhibitor}}{\text{ALP activity}} \times 100$$

4. L-gamma-glutamyltransferase activity (GGT) was measured by Merckotest 14302 gGT (FRG) kinetic kit. The reaction mixture contained: 1.0 ml buffered substrate (110 mmol/l of TRIS, pH 8.25, 110 mmol/l of glycilglycin, 4.4 mmol/l of L-gamma-glutamyl-3-carboxy-4-nitroanilid and 20 ul of amniotic fluid supernatant. After a 1-minute lag phase, increase in absorption was recorded every minute during a 3-4 minute duration. Enzyme activity was expressed in volume activity (U/l).

To discriminate "affected" (P1) from "healthy" groups (P2), we have found a value designated by Xcrit, which falls between

the average of the two groups. If the enzyme value was greater than the Xcrit, the patient was judged healthy, if it was lower, he/she was judged affected. This Xcrit value was chosen according to the principle of "maximum likelihood".

Let the P1 population be of normal distribution with m_1 expected value and sl standard deviation, and similarly the P_2 population with the parameters m_2 and s_2, where $m_2 > m_1$. In this case, the likelihood function is:

$$L_1(x) = \frac{1}{\sqrt{2\pi} \cdot s_1} \cdot e^{-\frac{(x - m1)^2}{2s_1}}$$

for the P1 population and

$$L_2(x) = \frac{1}{\sqrt{2\pi} \cdot s_2} \cdot e^{-\frac{(x - m_2)^2}{2s_2}}$$

for the P2 population.
A patient having a given x enzyme value is considered healthy if

$$L_2(x) > L_1(x).$$

After the necessary algebraic transformations we have the following quadratic unequality:

$$x^2 \left(\frac{1}{s_1^2} - \frac{1}{s_2^2}\right) - 2x \left(\frac{m_1}{s_1^2} - \frac{m_2}{s_2^2}\right) + \frac{m_1^2}{s_1^2} - \frac{m_2^2}{s_2^2} - 2 \cdot \log \frac{s_2}{s_1} > 0$$

If $s_1 = s_2$, then Xcrit = $(m_1 + m_2)/2$, if $s_2 > s_1$, then Xcrit will have 2 values, one of which is smaller than m_1, and if $s_1 > s_2$, then one of the Xcrit values is greater than m_2, but in the two latter cases one of the solutions is smaller than m_2. If the Xcrit value falls between m_1 and m_2, this value is accepted, and if it is below m_1 or above m_2, distinction is not possible from a clinical point of view (it occurs when m_1 and m_2 are very close to each other, which is significantly different in our material).

The means and standard deviation were used in computing the data.

RESULTS

For data evaluation, the amniotic fluid samples were classified into three groups. The first group included samples of fetuses with CF. The second group consisted of healthy infants of heterozygotic parents for CF. The theoretical

probability of these being heterozygotic was 2/3. The third group was the control group, where amniotic fluid samples were taken from pregnancies unrelated to high risk for CF. The number of cases was not identical in each group, ALP and GGT examinations were introduced later.

The median values of enzyme activity in the amniotic fluid samples of healthy infants in the 16-18 gestational weeks are:

Enzyme	16th week	18th week	Unit
Trehalase	1.22	0.71	U/g protein
ALP	16.81	20.87	U/l
Inhibition (%)	76.64	71.14	%
GGT	269.21	171.51	U/l

There is a significant difference between the affected and healthy groups for each of the enzymes ($p < 0.0001$). It means that the selected enzymes are very good markers, even if not all are of specifically intestinal origin.

The values of enzyme activity - considering the means of the control group as median - were counted in MoM (multiple of the median) units (Table I). Table I shows that in relation with the control group, enzyme activity values in the affected group are around 0.5 MoM, while activity values of the 2/3 CF-heterozygotic group are practically identical to those of the normal cases.

In distinguishing normal and affected populations we were looking for a value, which - according to the principle of "maximum probability", and the normal distribution curve would be the most suitable for the discrimination between the two groups and which can be used for classifying the enzyme values into affected and healthy groups /14/.
Our Xcrit results were:

Trehalase = 0.832 MoM
ALP = 0.817 MoM
Inhibition (%) = 0.808 MoM
GGT = 0.621 MoM

TABLE I

Microvillar membrane enzyme activity in amniotic fluid samples in 15 - 20 weeks of gestation
(Values in MoM, 95 % confidence limits in brackets)

Enzyme	CF-affected	2/3-heterozygotic	Control
Trehalase	n = 42 0.445±0.168 (0.278-0.613)	n = 52 0.928±0.163 (0.765-1.091)	n = 175 1.0±0.102 (0.892-1.102)
Lactase	n = 25 0.581±0.610 (0.0-1.191)	n = 16 0.817±0.262 (0.555-1.080)	n = 44 1.0±0.208 (0.794-1.206)
ALP	n = 25 0.525±0.162 (0.363-0.687)	n = 29 1.051±0.247 (0.803-1.299	n = 132 1.00±0.089 (0.911-1.089)
Residual activity (%)	n = 25 0.668±0.110 (0.558-0.778)	n = 29 1.022±0.049 (0.973-1.071)	n = 128 1.0±0.030 (0.970-1.030)
GGT	n = 25 0.378±0.101 (0.278-0.479)	n = 29 1.059±0.186 (0.878-1.245)	n = 132 1.0±0.079 (0.921-1.079)

(Since lactase activity is very low in the second trimester, and measurement of a reduced value is not reliable, therefore these analyses were dropped).

If a single enzyme activity was greater than or equal to the Xcrit value, it was classified into the "healthy", if it was lower into the "affected" group.

If we include individual enzyme values retrospectively into the "healthy" and "affected" groups, we get the detection rate for each enzyme activity (Table II).

Since it is believed that with the combination of several parameters the health status of the fetus can be evaluated with a greater accuracy, all the parameters, including the Xcrit value have been taken into consideration both in the group of

TABLE II

a) Detection rate for individual enzyme activities

Enzyme /Xcrit value/	Affected fetus found/total ("sensitivity")	Healthy fetus found/total ("specificity")
Trehalase /0.832/	23/25 (92.0 %)	67/125 (53.6 %)
ALP /0.817/	22/25 (88.0 %)	77/125 (61.6 %)
Inhibition (%) /0.808/	18/25 (72.0 %)	113/125 (90.4 %)
GGT /0.621/	24/25 (96.0 %)	97/125 (77.6 %)

b) Reliability of individual enzyme activities

Enzyme	total found/total cases
Trehalase	90/150 (60.0 %)
ALP	99/150 (66.0 %)
Inhibition (%)	131/150 (87.3 %)
GGT	121/150 (30.7 %)

high risk pregnancies for CF and that of the control group (Table III).

From the data in Table III we can conclude:

1) In 92 % of affected fetuses (23 cases) at least three or four enzymes (parameters) are below the Xcrit value.
2) In 92 % of healthy fetuses (115 cases) at least two or more parameters are above the Xcrit value.
3) In 95.55 % of the 2/3 CF-heterozygotic group (28 cases) at least two or more parameters are above the Xcrit value.

According to these criteria, the cases can be classified into four groups:

True positive cases: 23
True negative cases: 143
False positive cases: 11
False negative cases: 2

TABLE III

Patterns of microvillar membrane enzymes in affected, healthy and 2/3 CF-heterozygote groups

Enzymes				Affected	Healthy	2/3 CF-
Tre	ALP	Inhib	GGT	fetus	fetus	heterozygotic
-	-	-	-	13	5	1
-	-	-	+	1	1	0
-	-	+	-	6	4	0
-	+	-	-	1	0	0
+	-	-	-	2	0	0
-	-	+	+	0	13	4
-	+	+	-	1	12	4
+	+	-	-	1	0	0
+	-	+	-	0	5	1
-	+	-	+	0	2	2
+	-	-	+	0	4	0
+	+	+	-	0	2	0
+	+	-	+	0	0	1
-	+	+	+	0	21	5
+	-	+	+	0	16	6
+	+	+	+	0	40	5
Total				25	125	29

(-) = measured value < Xcrit

(+) = measured value > Xcrit

On the basis of the above results, we have concluded that in the case of a combination of the four amniotic fluid parameters, the reliability of the method is 92.73 %, its sensitivity is 92.00 %, and its specificity is 92.86 %.

DISCUSSION

Disaccharidases develop in the gastrointestinal tract of the intrauterine fetus in the 11-23 gestational weeks, primarily in the small intestines. Trehalase production increases significantly in the 10-23 weeks, lactase is synthetized mainly in the last months of pregnancy /6/. The membrane-bound disaccharidases originate from the brush-border cells along the microvilli of the small intestines. The villi appear in the 8-10 weeks of fetal life and undergo a maturing process during gestation. The various disaccharidases located on them enter the amniotic cavity through fetal defecation and show a specific profile.

From the 10th gestational week, their value increases, and they reach their maximum in the 14-17 weeks, which is followed by a sudden decrease, and from the 21st week, their level is very low.

The sudden decrease of disaccharidases is explained by the innervation of the anus spinchter after the 20-21 weeks when fetal defecation ends or by the sudden change in the permeability of the intestinal mucosa, inhibiting enzyme outflow /9/.

In consequence, after the 20-21. weeks, because of the very low enzyme levels, the disaccharidases are not suitable for diagnosis.

We have called attention to the importance of trehalase enzyme analysis in the prenatal diagnosis of CF /14, 15/

The suitability of intestinal alkaline phosphatase isoenzyme for the prenatal diagnosis of CF was first shown by Brock et al /2, 3/. This enzyme prevails in the intestinal form in the amniotic fluid in the second trimester. It is of fetal origin and it enters the amniotic fluid during early defecation

supposedly from the desquamed mucosa cells. Overall ALP activity of the amniotic fluid has a tendency to decrease in the 15-20 weeks of pregnancy, but the distribution of forms inhibited by phenylalanine (L-Phe), intestinal and placental inhibitors or by homoarginin (bones, liver, kidney) is constant. In the case of fetal CF, the enzyme activity of the L-Phe inhibited form decreases significantly.
GGT occurs in high concentration in tissue microvilli. They have a role in the absorbing and secreting processes taking place on the epithelial cells of the brush-border membrane. In CF, GGT activity decreases significantly, which can be explained by developmental disturbances, and secondary atrophization of microvilli, or by the presence of an enzyme inhibitor secreted into the amniotic fluid /1, 5/.

Although several studies have confirmed the suitability of the enzymes in question for CF diagnosis, in the absence of standardized methods, the results cannot be compared. Kleijer et al /7/ considered 10 percentile as the lowest limit. According to them, trehalase and lactase enzymes are less informative. By combining GGT and ALP, Aitken et al /1/ found that the sensitivity of fetal CF detection was 84 % (if the lowest limit was 5 percentile with GGT), or 90 % (if in the presence of 2.5 mmol of Phe, residual ALP activity 80 %), thus the predictability of the affected fetus was 28:1 (96.5 %) /1/ Peretz et al /12/ considered 0.5 MoM and 52 % residual activity as cut off values. On the basis of their evaluation, predictive values of different enzymes for affected states are: ALP 69.4 %, inhibition: 93.2 %, GGT: 68.9 %.

In a multicentric study, by retrospective analysis of 258 cases, Brock concluded that if out of the amniotic fluid enzymes two or three had a lower value than 0.5 MoM in the relevant gestational week, fetal CF had a very good diagnostic predictability in the 17-20 weeks of pregnancy (false positive ratio 2.3 %, false negative ratio 4.4 %) /4/

Since these examinations were performed in different gestational weeks, MoM values had to be used for comparing these cases. The values of healthy normal controls measured in

a given week were related to those of high risk cases, and the groups were discriminated on the basis of the Xcrit values.

Our results show that trehalase, ALP, ALP-isoenzyme and GGT enzyme activities in the amniotic fluid measured in the 16-21 gestational weeks, in spite of their non specificity for CF, have not only a very good predictive value for fetal CF, but they also exclude the possibility of the disease.

Tissue specificity seems to be an important criterion in diagnoses based on amniotic fluid enzyme activity. Intestinal ALP isoenzyme proved to be much more reliable and specific than the less specific trehalase or GGT. Our results confirm this assumption. Though the differences between certain parameters of the group are highly significant, by combining enzyme values, fetal health status can be predicted much better and by this method, the number of false negative and false positive cases can also be reduced . By measuring overall enzyme activities, the reliability of the method is over 92 %. In this case, sensitivity and specificity also come to 92 %.

During the past few years, the use of first trimester DNA analysis for prenatal diagnosis of CF has brought about revolutionary changes. The predictability of CF from chorion villus is as high as 100 %. It has led us to believe that second trimester amniotic fluid enzyme assay with 92 % reliability should be used for the prenatal diagnosis of CF in the following cases:

(1) if the CF-heterozygotic couple, or the affected child are informative for none of the available RFLP (restriction fragment length polymorphism) markers:

(2) if the CF-heterozygotic couple has no living affected child, or the DNS of the stillborn affected child is not available:

(3) if, due to advanced gestational age, there is no possibility of genotypifying family members:

(4) if in the 18-20 gestational weeks, routine ultrasound examination is suggestive of meconium ileus in an otherwise not high-risk pregnancy on the basis of its medical history.

Since, even in 1988, more than 50 % of the CF-heterozygotic couples belonged to one of these four groups at our Genetic Counselling Center, mid-trimester amniotic fluid enzyme diagnosis is considered to be a valuable diagnostic method and its use is advisable in the prenatal diagnosis of CF.

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Acta Paediatrica Hungarica 31 (2), pp 275-278 (1991)

BOOK REVIEWS

Jennie Kline, Zena Stein, Mervyn Susser: Conception to Birth. Epidemiology of Prenatal Development issued as Vol. 14 in the series Monographs in Epidemiology and Biostatistics ed. by Brian MacMahon, Oxford University Press, New York, Oxford 1989. p 433
Received for reviewing: May 1990.

This study on the conception to birth is the 14th volume of the series "Epidemiology and Biostatistics" which offers a good review of the given field. The book contains 5 chapters structured as follows:

- I. Disorders of Development: Weighing the Evidence for Environmental Causes. This chapter is divided into 3 sub-chapters, dealing with a number of established criteria of causal factors.
- II. Conception and Early Gestation. Within this wide subject, three consecutive chapters are devoted to the probabilities of conception and of loss, as well as to their actual frequencies. Another three chapters treat miscarriages, both of aberrant and of environmental origin.
- III. Later Gestation. The 7 sub-chapters of this section deal with the still necessary distinction of preterm delivery, retarded growth and immaturity, as well as their etiological and familial influencing factors, maternity and birth in under developed countries are also discussed.
- IV. Age and Parity. The 3 sub-chapters of this section report on two main topics: maternal age and gravidity (fecundity, fertility, gestation, trisomy, number of pregnancies).
- V. The Physical Environment, Reproduction and Surveillance. Here some environmental aspects of reproduction are discussed together with the systematic analysis of surveillance.

The well-compiled and interesting book is closed with the most up-to-date informative bibliography and author and subject indices. The study is unique in its field and offers an exciting reading for obstetricians, human genetists and paediatricians. This work is a fundamental book, thus it can be recommended to every medical libraries.

M. Miltényi, MD

K. F. Schlegel and M. Aalam: Massage, orthopaedic techniques, occupational therapy. Hippokrates, Stuttgart, 1990. 222 pages, 138 figures, 6 tables.

The book is the third volume of the series Physikalische Medizin. Volume 2 (Curative gymnastics and kinesitherapy) was edited in 1989, volume 4 (Electro- and phototherapy) in 1988. Volume 1, dealing with basic principles, thermo- and hydrotherapy, balneology and medical climatology, is planned for this year.

The three main topics indicated in the title are described by twelve authors.

Part 1 deals with massage. Definition, indications and contraindications, methods, references are offered. Special attention is paid to classical massage, segment or connective tissue massage, manual lymph massage, various massage devices, subaqueous water jet massage.

In the following chapters massage therapy of various disease groups is described. The most important group is of course locomotor system disorders. Detailed description of the diseases of the spine, joints, tendons and muscles is followed by a separate chapter on states after joint replacement.

Cardiovascular and gastrointestinal diseases are briefly dealt with.

There is a short overview of absolute and relative contraindications of massage therapy.

The most extensive part is on orthopaedic techniques. More than 100 pages are dedicated to orthopaedic devices, ortheses, prostheses, various types of wheelchairs, orthopaedic shoes, foot-easers, etc. Structure, mechanics, application, instruction to the patients are discussed for each device.

All provisory and long-term devices are made of up-to-date material, by modern techniques, this part gives excellent insight to the most recent technical achievements in this field.

Since a large proportion of the population is affected by some orthopaedic disorder, economic viewpoints also emerge. One aspect is the patient's working capacity, the other is cost of the orthopaedic device.

Work or occupational therapy, ergotherapy is the issue of the third part of the book. Although this kind of therapy was already known in the antique world, appropriate organisatory structures, formation and training of specialists in this field have developed during the last few decades. The complex treatment methods aim at rehabilitation, rendering people affected by various mental, locomotor or other handicaps self-supporting and - in the ideal case - capable of working; if the original profession cannot be continued, searching for appropriate working activity is also task of the rehabilitation process. Here, many excellent examples are given on methods and devices making crippled persons able to lead a self-supported life.

This tripartite book can be warmly recommended to experts in orthopaedics, rheumatology, traumatology and rehabilitation.

T. Vízkelety, MD

Henry Ekert: Childhood Cancer: Understanding and Coping. Gordon and Breach Science Publishers, 1989, 185 pages, 16 figures.

The therapeutic achievements attained in the field of paediatric oncology during the last decade have deserved attention not only of the specialty but also of the public. Quite conceivably, parents and other family members of children afflicted by malignant disease are most interested in the issue. The principal aim of the author, leader of the haematooncological department of the Royal Children's Hospital in Melbourne, was to meet these parental needs. In this respect, the book fills up a large gap of international scale.

The book consists of two main parts. The first comprises nine chapters on paediatric malignancy in general; the main issues are incidence, aetiology, therapeutic approaches, bone-marrow transplantation and physiological guidance included, there is mention of alternative therapeutic methods and procedures used outside hospitals. The style is concise, most but not all essential questions are dealt with.

Part two describes various malignant diseases in detail, in 13 chapters. Very rare cancer types have not been included. Diagnosis, classification, prognosis, surgical, radiation and drug therapy of leukaemia, lymphoma and most important solid tumours are described. Possible complications and survival chances are also discussed. The style is again concise and easy to understand.

This detailed description is followed by a very useful glossary listing special terms and their explanations. There is also a list of the most frequently used cytostatic drugs, in which their names, side-effects and indications are given.

This book is a useful overview for everybody interested in malignant diseases occurring in children. Its explanations and descriptions are clear, simple and easy to understand. The book is primarily aimed at the layman who wants to know more about the issue. It does not add much to the knowledge of experts in the field.

Miklós Bartók, MD

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Abbreviations should be spelled out when first used in the text. *Drugs* should be referred to by their WHO code designation (Recommended International Nonproprietary Name); the use of proprietary names is unacceptable. The *International System of Units* (SI) should be used for all measurements except blood pressure.

References should be numbered in alphabetical order and only the numbers should appear in the text (in parentheses). The list of references should contain the name and initials of all authors (the use of et al instead of authors' names is not accepted). For journal articles the title of the paper, title of the journal abbreviated according to the style used in Index Medicus, volume number, first page number and year of publication should be given; for books, the title followed by the publishers and place and year of publication.

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Index: 26.021

Acta Paediatrica Hungarica

VOLUME 31, NUMBER 3, 1991

Akadémiai Kiadó, Budapest

ACTA PAEDIATR HUNG HU ISSN 0231-441X

ACTA PAEDIATRICA

HUNGARICA

A Quarterly of the Hungarian Academy of Sciences

Editor

M. MILTÉNYI

Acta Paediatrica publishes original reports of studies in paediatrics and borderline fields.

Manuscripts and editorial correspondence should be addressed to

ACTA PAEDIATRICA HUNGARICA

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H-1083 Budapest, Hungary

Subscription information

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P.O. Box 149,
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or its representatives

Acta Paediatrica Hungarica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
Prielle Kornélia u. 19–35.
H-1117 Budapest

Acta Paediatrica Hungarica is abstracted/indexed in Biological Abstract, Chemical Abstracts, Excerpta Medica, Index Medicus

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WHISTLING FACE SYNDROME
A case report and literature review

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Received 26 April 1990

The cranio-carpo-tarsal or "whistling face" syndrome was first described by Freeman and Sheldon in 1938. More than 60 cases with great variability of expression are known till now and autosomal dominant as well as recessive inheritance and sporadic cases suggest a genetic heterogeneity.

We review 60 well-documented cases of the literature and present a patient with a severe form, who died of bronchopneumonia at the age of 9 months. The facial stigmata of his mother and the ulnar deviations of his maternal grandfather support the autosomal inheritance of the syndrome.

INTRODUCTION

The cranio-carpo-tarsal syndrome or whistling face syndrome (WFS) first described by Freeman and Sheldon in 1938 is a disorder with great variability involving the face and the musculoskeletal system. We present a patient with a very severe form of this syndrome and review 60 cases of the literature.

CASE REPORT

A. S., a boy, was born after an uneventful pregnancy as the first child of non-consanguineous parents. His otherwise healthy mother has a small mouth, a high arched palate, and little mimicry. A photograph of the deceased maternal grandfather shows severe ulnar deviation of all fingers, which had been interpreted as sequelae of a "rheumatic disease" (Fig. 1).

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The child was delivered by cesarian section because of an abnormal cardiotocogramm. Birth weight was 2680 g, length 49 cm, head circumference 31.5 cm. The Apgar scores after 1, 5 and 10 minutes were 8,9, and 9, (respectively). The child was intubated and ventilated because of "poor respiration".

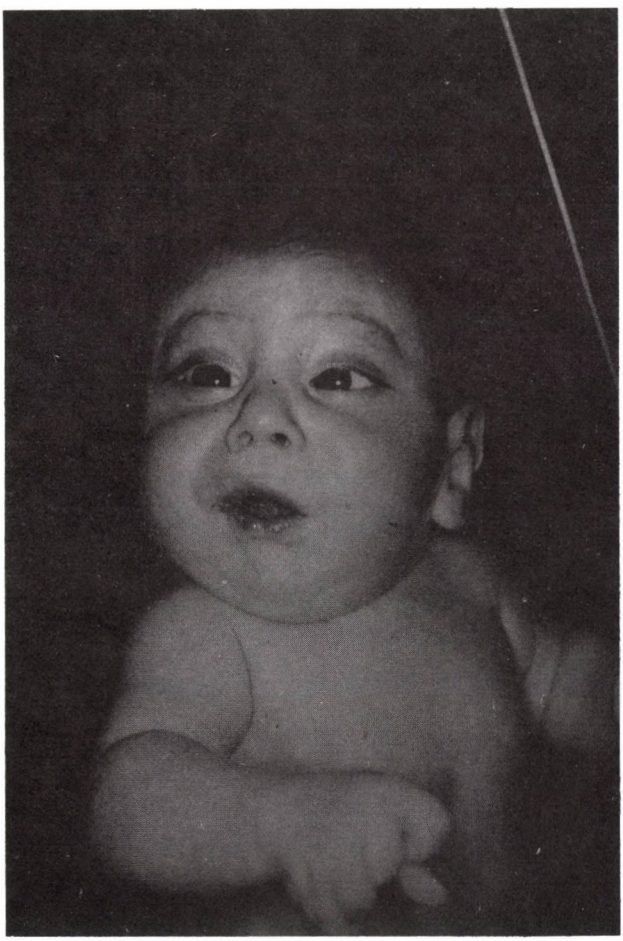

Fig. 1.

On examination at birth there were lack of mimicry, small and open mouth "pointed as for whistling", microcephaly with prominent supraorbital ridges and flat forehead, small anterior fontanel (<1) cm), ptosis of the eyelids, infrequent eye blink with missing blink reflex, and one row of long, straight and coarse eyelashes. His nose was small with plug-like nostrils, the philtrum long, the cheeks full but flabby, the mandible small, the large ears low set, and the neck short. The fingers were held in ulnar deviation and partial external rotation. The thumb and the fifth finger were flexed; the fingers could neither actively be bent nor flexed, which led to a pseudo-simian-crease. There were striking flexion contractures of the toes, lack of spontaneous movements and the stiffness of the skeletal muscles. The legs were adducted and internally rotated and the feet were in plantar flexion. The tendon reflexes were normal (Fig. 2).

Fig. 2.

Radiological examination revealed a steep fossa cerebri anterior (Fig. 3) but no other abnormalities. Ultrasound of the brain was normal, a cCT scan showed a moderately enlarged anterior part of the interhemispherical fissure and the pericerebellar space. Complete blood count, electrolytes, copper, and urine analysis were normal, no storage phenomena could be detected. Ophthalmoscopy revealed normal fundi and normal iris vasculature.

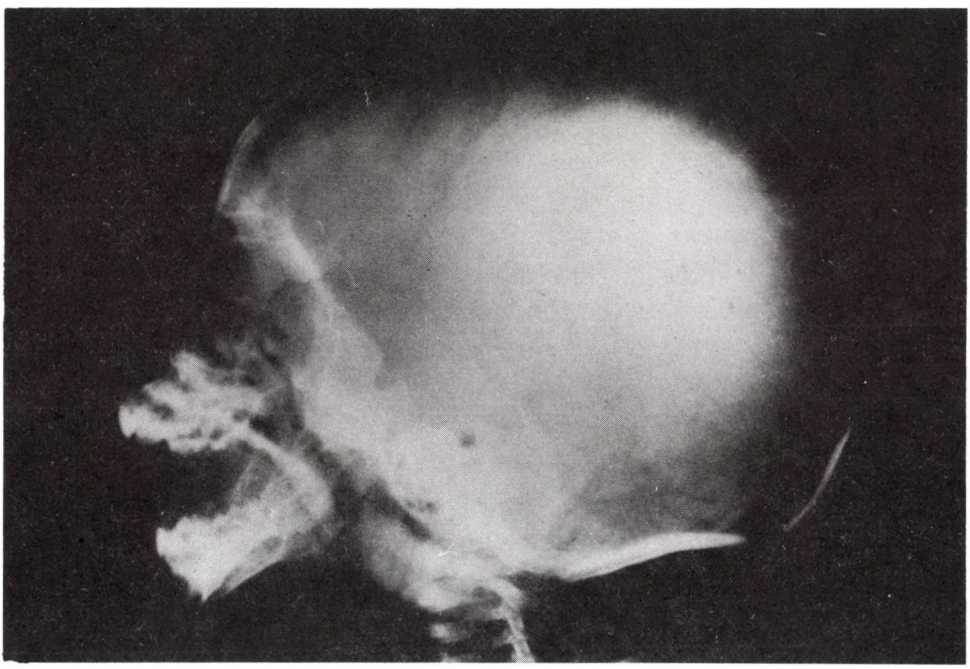

Fig. 3.

Course: due to respiratory and swallowing difficulties the child remained intubated and received parenteral nutrition for 11 days. He had to be fed by tube for the first weeks of life, thereafter bottle feeding was difficult and took about 60 minutes for each meal. At three months, generalized tonic-clonic seizures started, which responded to phenobarbital and valproic acid. His EEG was normal.

During the following three months the child remained stiff, but his motor activities improved gradually. The hips were externally rotated and the extensor posture of the feet disappeared. The baby was able to suck and swallow, but did not develop any mimicry. In prone position he could barely rotate or raise the head and his eyes did not follow objects or faces; the bulbi showed rotatory movements and sometimes diverging/converging strabism but no opsoclonus. He developed an asymmetric (convex to the left) scoliosis of the thorax with a corresponding rib bump and kyphosis of the thoracolumbar

area. Growth retardation, failure to thrive, and microcephalus became more striking.

Before further studies could be performed the patient died in another hospital at the age of nine months. Autopsy revealed severe bronchopneumonia as cause of death. The above described deformities of the spine were confirmed. The brain showed no macroscopic or microscopic abnormalities. The brain weight was normal, muscle tissue was not studied.

DISCUSSION

The British orthopaedic surgeon E.A. Freeman and the paediatrician J.H. Sheldon described the cranio-carpo-tarsal syndrome in 1938 /11/. In 1963 Burian introduced the term "whistling face" /3/.

More than 60 case reports have been published so far. The descriptions show great variability of expression. Wettstein et al /31/ described seven patients within three generations of one family: one of the adults had but the facial characteristics of the WFS. Others describe severely affected newborns with problems of sucking and swallowing and lack of mimicry. These babies later developed deformities of the extremities and spine and sometimes (especially during general anaesthetics) life-threatening bronchopulmonary complications /11,17,19,23,31, our patient/. A "lethal factor" for male patients has been discussed by Wettstein and Antley /2,31/.

Table I classifies the major and minor diagnostic signs of the WFS based on 60 well-documented cases of the literature and our case. The minimal diagnostic criteria for the WFS include the typical appearance of the face plus at least one of the major diagnostic abnormalities of the extremities or the spine. The spine deformities, ptosis of the eyelids, flabby cheeks and H-like dimple of the chin, and particularly the respiratory and feeding difficulties are probably sequelae of a myopathy.

The report of Burian /4/ is the first to emphasize features of a myopathy: flabby musculature of the abdominal wall and the cheeks, which "bulge like a membrane on blowing", a myopathic electromyogram and in muscle biopsy atrophic and vacuolated fibres. Sauk /25/ described a myopathic EMG and connective

TABLE I

Reported major and minor diagnostic signs of the WFS out of 60 well-documented cases in the literuture plus our patient.

Whistling Face Syndrome

Major diagnostic signs	Minor diagnostic signs
Face	
lack of mimicry	feeding difficulties (12/23)
microstoma	high arched palate
"whistling face" (61/61)	long philtrum
	hypoplastic alae nasi
	full flabby cheeks
	H-like dimple of the chin
	supraorbital ridge
	eyelid ptosis
	steep floor of ant. fossa (11/232)
Upper extremity	
ulnar deviation of the fingers (42/46)	contractures of the shoulder
flexion contractures of the fingers (36/41)	impaired rotation of the forearm
	dorsal swelling of the hand
	hypoplasia of the thenar
	flexion contracture of the thumb
Lower extremity	
club foot (24/41)	contracture of the hip and/or
contracture of the toes	contracture of the knee (11/15)
	hypoplasia of m. gastrocnemius
General	
(kypho-) scoliosis (26/31)	growth failure (16/26)
	thin oar-like ribs
	microcephalus (7/16)
	mental retardation (8/26)
	seizures (3/16)

tissue substitution in the muscles of his patient in 1974 and discussed a primary myopathy and/or muscle hypoplasia as the cause of his patients hand- and foot- deformities.

The percentage of lethal pneumonia and unexplained deaths in infancy may have been caused by involvement of the intercostal muscles, as mentioned first by Frazer /10/.

MacLeod noticed "fixed ribs in horizontal position throughout the respiratory cycle" in his patient /17/, who subsequently developed bronchopneumonia. Five (4 males, 1 female) of 61 patients diagnosed at birth developed pneumonia before the age of nine months /11,19,23,31, our patient/, three of them (all males) died. Our patient also died of a severe bronchopneumonia. After the second year of life bronchopulmonary infections are not mentioned. In the 60 reported cases there were two (1 male, 1 female) stillborn and three (males) died before the fifth week of life from unknown causes /9,14,27/.

Respiratory failure after birth, which may even lead to tracheostomy, and recurrent severe respiratory tract infections in infancy are typical for some congenital myopathies /8/.

This point is emphasized by some deaths in infancy (3 of 60 patients referenced in the bibliography and our patient) and improvement after the second year of life. Recently Vanek considered the possibility of a primary myopathy in 1986 because he found centrally placed nuclei and moth-eaten necrosis, hypotrophy of type-I- and hypertrophy of type-II-muscle-fibres, and swollen mitochondriae in the biopsy material of two patients. He interpreted these findings as congenital fibre dysproportion /8,27/.

The long philtrum is probably caused by the characteristic shape of the mouth. Other symptoms, however, such as the supraorbital crease, the steep fossa cerebri anterior /11/, the high arched palate, the hypoplasia of the alae nasi, the small oar-blade-like ribs, and particularly the mental retardation, the seizures, and the microcephaly cannot be explained on this basis and imply a more complex pathogenesis of this syndrome.

The reports of mostly autosomal dominant as well as recessive inheritance /1,9,14,25/ and of sporadic cases suggest a genetic heterogeneity. In 1982 Hall /13/ figured out 23 autosomal dominant and 18 sporadic cases in the total number of 41 reported patients. The stigmata of the mother and the maternal grandfather of our patient support the autosomal dominant inheritance of the WFS. Chromosome analysis were normal in most cases /5,6,10,14,22,24,25,30/, but one XO configuration (Turner syndrome) /3/ and one abnormally long paracentric secondary constriction in one of the C-group chromosomes is described /23/.

Despite microcephaly, mental retardation and lack of movement, the presence of contractures, ulnar deviation, whistling face, and normal tendon reflexes at birth exclude a hypoxic cause in this condition.

The following disorders should be excluded when the diagnosis of WFS is considered:

- Distal arthrogryposis (DA): In 1982 Hall /13/ designated the DA type I and type II (A-E, with additional findings). She found out at least 4 of the reported WFS patients to be cases of DA. Thus, because of an overlap of manifestations with the WFS the DA (particularly type I and II B) has to be ruled out carefully.
- Congenital dystrophia myotonica: This condition with generalized muscle hypotony has no characteristic facial abnormalities. Since the biopsy findings may somehow resemble a WFS, it should be excluded by EMG.
- The mitochondrial myopathies should remain in consideration because of the reported mitochondrial abnormalities in the WFS /27/.

Treatment for the WFS remains symptomatic. It seems important to deal adequately with the early respiratory complications. There are no primary skeletal changes of the extremities but marked posture abnormalities at birth. Thus, the secondary changes may develop due to the "skelet forming power of the muscles" (Moss's theory, cited by Vanek /27/. Patients develop joint contractures which may be resistent to

therapy, as seen particularly in myopathies /8/. If physiotherapy cannot prevent a contracture, it has to be corrected surgically.

In our case we missed the opportunity for a muscle biopsy. However, it should be performed in all suspected cases, and biochemical and histochemical studies should be done to further define the aetiology of WFS.

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Acta Paediatrica Hungarica 31 (3), pp 291-297 (1991)

CONGENITAL HYPERAMMONEMIA: SYMPTOMATIC CARRIER GIRL PATIENT AND HER ASYMPTOMATIC HETEROZYGOUS MOTHER FOR ORNITHINE TRANSCARBAMYLASE (OTC) DEFICIENCY: SPECIFIC ENZYME DIAGNOSTIC AND KINETIC INVESTIGATIONS FOR THE DETECTION OF HETEROZYGOUS GENOSTATUS

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Received 30 May 1990

Activities of the specific enzymes of the inherited hyperammonemic syndromes (carbamoyl-phosphate synthetase CPS), ornithine transcarbamylase (OTC), arginine-succinate-synthetase (ASS), arginine-succinate-lyase (ASL) and arginase (ASE) were measured in a liver biopsy specimen of a 2 years-old girl suffering from chronic hyperammonemia and in the erythrocyte- and leukocyte-homogenisate of her parents. The activity of OTC in liver homogenisate of the patient was 62.9 percent; in the leukocytes of the parents it was 78.5 percent (in mother) and 102 per cent (in the father) as compared to the controls.

Our patient proved to be a symptomatic carrier of OTC deficiency and her mother proved to be an asymptomatic carrier.

INTRODUCTION

Ornithine transcarbamylase (OTC) deficiency is an X-linked recessive disorder, usually with lethal hyperammonemia in OTC homozygous males. OTC, one of the five enzymes required for ureagenesis, catalyzes the synthesis of citrulline from carbamyl phosphate and ornithine. Female heterozygotes, however, have variable phenotypic expression depending on the inactivation of the X-chromosomes containing the normal and mutant gene. Some females have hyperammonemic episodes that result in mental retardation, but others may never show the manifestation of this disease, however mild form /1,4,5/.

Batshaw /2/ detected cerebral dysfunction in asymptomatic carriers of OTC deficiency.

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Carrier detection in OTC deficiency: Palmer /12/ has cited studies in which protein or ammonium chloride loads were used as a test for heterozygosity in kindreds with OTC deficiency. Hyperammonemia was not noted after the protein load in many of these studies. Hokanson /9/ detected hyperammonemia in only three of four obligate OTC heterozygotes after a protein challenge. The orotate excretion in the heterozygote group was at least by three standard deviations greater than in the control group. Batshaw /2/ investigations confirmed that urinary excretion of orotic acid is a more sensitive indicator of the OTC heterozygotes than is hyperammonemia.

We report here on specific enzyme investigations of urea cycle and K_m values for OTC of a symptomatic carrier girl patient and her asymptomatic carrier for OTC deficiency.

CASE REPORT

A. T. a 20 month-old girl patient was admitted to our clinic with detected hyperammonemia (311 gamma %), hepatomegaly, elevated liver enzyme activities (SGOT 49 U/l, SGPT 134 U/l). the EEG showed encephalopathic signs.

The perinatal anamnesis was uneventful, familial anamnesis: her twin-brother is healthy, as her 5 years-old sister, too. Birth weight was 3200 g. Her motoric and mental development have become slow from the age of 13 month, she was found to be clumsy, she was noted to fall down frequently, generalized muscle hypotonia and ataxia developed.

Neurological investigation revealed right side spastical hemiparesis, progressive neurological deterioration was established.

Subdural hematoma was suspected according to the cerebral scintigraphy, but carotis angiography was negative.

Since her early infancy she has vomited easily and had intestinal symptoms like diarrhoeic episodes.

Alfa-1-antitrypsin deficiency, morbus Wilson, galactosemia, tyrosinemia, lysinuric protein intolerancy and glycogenosis had been excluded.

She proved to be OTC heterozygote according to our specific enzyme analysis.

After the introduction of the low protein diet (1.5-1.0 g/kg/day), and of the sodium- benzoate (250 mg/kg/day), folic acid, vitamine B_6 therapy the plasma ammonia level decreased to 45 gamma %.

Urinary amino acid chromatography, purine and pyrimidine metabolites and orotic acid were normal. During the protein

TABLE I

Activities of the specific enzymes of the urea cycle of T. family and controls

Liver tissue of patient T.A. and controls
Enzymes in the case of CPS and OTC in citrulline μmol, in ornithine in the cases of ASS, ASL and ASE μmol/h/g liver

	Normal value	T.A. patient	%
1. Carbamoyl-phosphate synthetase (CPS)	264 ± 54	284	107.6
2. Ornithine carbamoyl-transferase (OTC)	6178 ± 1234	3884	62.9
3. Arginine-succinate-synthetase (ASS)	87 ± 18	98	112.6
4. Arginine-succinate-lyase (ASL)	216 ± 32	234	108.3
5. Arginase (ASE)	83000 ± 13000	81200	97.8

Enzyme activities of the homogenisate of the erythrocytes (μmol ornithine/h/gHb)

	Normal value	T.father	%	T.mother	%
3. ASS	1.9 ± 0.7	1.8	94.7	1.6	84.2
4. ASL	12.8 ± 5.3	11.6	90.6	10.8	84.4
5. ASE	1250 ± 445	1106.0	88.5	1176.0	94.0

Enzyme activities of the homogenisate of the leukocytes (1., 2. μmol citrulline 2., 4. and 5. μmol ornithine (h/mg prot.)

	Normal value	T.father	%	T.mother	%
1. CPS	7.7 ± 1.6	6.9	89.6	5.4	70.1
2. OTC	92.4 ± 21.1	94.3	102.0	72.5	78.5
3. ASS	0.48 ± 0.12	0.52	108.3	0.55	114.6
4. ASL	0.42 ± 0.14	0.48	114.3	0.50	119.0
5. ASE	26.85 ± 7.20	25.22	93.2	26.26	97.8

loading the orotic acid urinary excretion has elevated.

She has developed psychosomatically well on MILUPA UCD 2 diet and arginine supplementation. She unexpectedly died of hyperammonemic coma (blood ammonia: 720 µmol/l) against of the introduced peritoneal dialysis at the age of 6 years in April 1990.

METHODS

The activities of specific enzymes of the inherited hyperammonemic syndromes (carbamoyl-phosphate synthetase = CPS, OTC, arginine-succinate-synthetase - ASS, arginine - succinate - lyase = ASL and arginase = ASE were measured in the liver biopsy specimen and in the erythrocyte and leukocyte homogenate of her parents /11/.

The value of the enzyme activities of the urea cycle are given in Table I, from the T. Family K_m /ornithine/ and K_m (carbamyl phosphate) of OTC are summarized in Table II.

TABLE II

K_m values of OTC enzyme

From liver biopsy specimen	Normal values fetal	adult	T.A.patient %
K_m (ornithine /mmol/l	3.5	0.42	4.32
K_m (carbamyl phosphate) (mmol/l)	0.2	0.21	1.28

From leukocytes	Normal value		T.father %	T.mother	%
K_m (ornithine µmol/l	3.88	4.12	106.2	10.25	264.2
K_m carbamyl phosphate (mmol/l)	0.48	0.44	91.7	0.52	108.3

RESULTS

The activities of the above-mentioned enzymes in liver biopsy of the hyperammonemic girl patient was diminished (62.9 %) and the activity of OTC in the homogenate of the mother's leukocyte was 78.5 %. The activity of CPS was diminished too, 70.1 %.

The activities of the urea cycle enzymes proved to be normal in the case of the father.

K_m /ornithine/ and K_m (carbamyl phosphate) of the patient's OTC enhanced similarly as her mother's K_m (ornithine) was increased, too (Table II).

DISCUSSION

Goldstein /6/ demonstrated an excessive urinary excretion of orotic acid after an oral protein load in the obligate heterozygotes for OTC deficiency. Others have confirmed the observation that this non-invasive test identifies heterozygotes more reliably than the measurement of the blood ammonia after a protein or ammonia load /9/. Becroft /3/ discussed the failure of protein loading test to identify heterozygosity for OTC deficiency and of the expected increase of orotic acid excretion and of pyrimidine and purine metabolites in the urine.

Hauser /7/ concluded that measurement of urinary orotidine excretion after the administration of allopurinol is a simple and reliable test for the identification of heterozygous women for ornithine carbamoyltransferase deficiency. This test relies on the allopurinol-induced accumulation of orotidine, whose synthesis is stimulated by carbamoyl phosphate, a substrate that accumulates in ornithine carbamoyltransferase deficiency. The mean plasma glutamine and ammonium levels were significantly higher in the carriers for OTC deficiency than in the controls, while the mean plasma arginine and citrulline levels were significantly lower in carriers /8/.

The identification of the different genostatus was possible due to the determination of the specific enzyme activities of the ureacycle from the homogenate of the peripheral leukocytes.

Kinetic abnormalities from patients with OTC deficiency have been published by Gray /8/. There is a great heterogeneity of mutant enzyme structure or expression as indicated by wide variation in K_m (ornithine) and K_m (carbamyl-phosphate) values. In the case of Qureshi /13/ - a 7 years-old girl, suffering from chronic hyperammonemia and orotic aciduria - the K_m (carbamyl-phosphate) of the mutant enzyme was lower as normal, while the K_m (ornithine) was normal. The activity of OTC was only 17 % of that of a control, pH optimum was 8.1 in the patient and the control.

We have found diminished affinity of the OTC enzyme for both of different substrates from the liver tissue in the case of our girl patient with OTC heterozygosity. The OTC K_m values for the ornithine and carbamyl phosphate in the leukocytes of the father were normal, while in the case of the mother - as an asymptomatic heterozygous genotype - the K_m (ornithine) value proved to be highly elevated, more than twice of the normal value.

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Acta Paediatrica Hungarica 31 (3), pp 299-304 (1991)

HIGH CONSANGUINITY RATE IN HUNGARIAN GIPSY COMMUNITIES

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Received 12 September 1990

Reproductive data of 1074 gipsy women between 13 and 52 years with one or more children were obtained by gipsy social workers through anonymous personal interview confirmed by available official documents. Socioeconomic status of five different gipsy communities studied is much lower than the Hungarian average. Their reproductive activity is also different, e.g., first births occur in much younger age. The high endogamy was proved by the gipsy origin of male partners in 90 % of couples. The occurrence of first cousin couples was 16 times higher than that of the Hungarian population at large, however, its range was wide from 0 % to 21 % in different regions studied.

INTRODUCTION

Previously a higher birth prevalence of posterior cleft palate, congenital cardiovascular malformations /4/, congenital talipes equinovarus /2/ was found in the children of Hungarian gipsy couples than in the non-gipsy Hungarian population. The gipsy communities have a different reproductive style, e.g., a higher rate of consanguinity is supposed. The objective of our study was to determine consanguinity rates in different gipsy samples and to test the hypothesis that the higher rate of some congenital abnormalities is dependent on their higher rate of consanguinity.

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MATERIALS AND METHODS

Gipsy communities were examined in five territorial units including one to eight settlements in Hungary. Voluntary participation and the availability of gipsy social workers were selection criteria thus the study material is not representative for the whole Hungarian gipsy population. All women of reproductive age (between 13 and 52 years) with one or more children were planned to recruit. The data of reproductive activity were obtained by gipsy social workers through personal interview using printed from supplemented available documents, e.g., identification card which includes marital status and birth dates of children. Less than 5 % of women with unreliable responses in different samples were excluded by social workers from the study. The children of 1074 gipsy women studied were born from 1124 husbands or partners. Their origin and the relation between women and husbands or male partners were clarified on the basis of women's replies and the pedigree of the couple studied. Names were not recorded. The cooperation was refused in 0-9 % of gipsy women in different samples.

RESULTS

The distribution of age groups and the mean age of female participants (33.9 years) did not differ significantly among five combined territorial samples. The socioeconomic status of women studied was characterised by the number of schooling (Table I). It is extremely low in a comparison with the data of Hungarian female population at large.

TABLE I

Schooling of gipsy women studied and the Hungarian female population in 1982

Group	Years of schooling 0	1-7	8	9-12	13 or more	Total
Gipsy samples	23.4	56.5	19.6	0.5	0.0	100.0
Hungarian female population	2.0	42.5	31.2	20.4	3.9	100.0

The maternal age at the time of first delivery indicated a significantly earlier onset of reproduction (Table II). The average child number of females could not be evaluated because the reproduction has not been finished in the majority of cases. Gipsy females born between 1930 és 1939 had 4.9 children while this figure was about 1.8 in the Hungarian population at large.

TABLE II

Maternal age distribution at the time of first delivery

Group	Maternal age groups					Total
	<15	15-19	20-24	25-29	30	
Gipsy samples	2.2	62.6	29.4	4.4	1.4	100.0
Hungarian population	0.3	25.0	46.8	21.5	6.4	100.0

The gipsy origin of partners was characteristic (Table III), though a relatively wide range (75-95 %) was found in different regions. Of gipsy partners, 78 % originated from with same settlements. It is not characteristic for partners of non-gipsy origin. The occurrences of different consanguinity types are also shown in Table III. Three of first degree relative connections (0.3 %): father-daughter 2, sibs 1, were mentioned. Three couples had an uncle-niece relation. The rate of first cousin couples differed significantly (0-21 %) from each other in combined territorial samples. Of 1142 couples, 96 (8.5 %) had a degree of consanguinity less than first cousins. It was not possible to define exactly the type of consanguinity by the help of pedigree analysis in further 69 couples (6.1 %), but surely it was not a near one.

TABLE III

Origin of male partners and occurrence of consanguineous couples

Region	Number of females studied	Number of partners studied	Origin of male partner (%) gipsy SS	gipsy DS	non-gipsy SS	non-gipsy DS	unknown	Number of consanguineous couples First degree relatives	Uncle-niece	First cousin	Others	Undefined
Baranya	166	181	52	23	7	15	3	0	1	0 (0.0%)	5 (2.8%)	3 (1.7%)
Bács-Kiskun	129	131	83	9	1	5	2	1	0	4 (3.1%)	9 (6.9%)	4 (3.1%)
Budapest	159	179	76	17	0	6	1	0	1	37 (20.7%)	3 (1.7%)	0 (0.0%)
Szabolcs-Szatmár	284	285	61	30	1	6	2	0	1	6 (2.1%)	20 (7.0%)	10 (3.5%)
Szolnok	336	348	79	16	1	2	2	2	0	6 (1.7%)	59 (17.0%)	52 (14.9%)
Total	1074	1124	70	20	2	6	2	3	3	53 (4.9%)	96 (8.9%)	69 (6.4%)
Without Budapest region	915	945	69	20	3	7	2	3	2	16 (1.7%)	93 (10.2%)	69 (7.5%)

SS = same settlement

DS = different settlement

DISCUSSION

Of the Hungarian population of 10.6 million, the estimated size of the gipsy population is about half a million. Exact figure is not known because there is no official statistical record concerning ethnical origin in Hungary. The gipsy communities live different socio-cultural circumstances. On the one hand the organization of their community is based on the traditional large families (clans; in gipsy language: "nyamo"). About 30 clans establish a community ("compania") and the family solidarity is strong. They prefer to get married within this community, however, sometimes these marriages are not recorded officially. It explains the high rate of endogamy. Furthermore the "blood-relationship" among couples is not a shame as it is in non-gipsy communities. Hopefully this tradition improves the validity of information concerning the consanguinity because other supplementary possibilities for its confirmation were not available. However, the proportions of other and undefined consanguineous couples also showed a significant difference and this deviation may indicate an investigator bias. On the other hand the socioeconomic status of gipsy communities is much lower than the national average and it is connected with the level of education.

The main purpose of this study was to collect data concerning consanguinity rate in different gipsy communities. Our data indicate a higher rate both in endogamy and consanguinity in gipsy couples which are not characteristic for the Hungarian population at large /7/. The occurrence of incest is not known in Hungary, the estimated yearly number based on courts' registration is 2-3 per 100 000 births. It is in an agreement with international estimations (1 per 10 000-100 000 births) /1, 8/. The occurrence of relations between second degree relatives, e.g., uncle-niece is rare (0.01 %) in Hungary. The rate of first cousin marriages is about 0.3 % in Hungary /5/. Thus, the combined figure of first cousin gipsy couples (4.7 %) exceeds 16 times the national average, however, significant regional differences were found. Without the

Budapest sample which had an extremely high figure, the rate of first cousin gipsy couples is 1.7 % and it is 5.7 times higher than the Hungarian population figure. In the case of congenital abnormalities with multifactorial-polygenic origin, consanguinity of parents is expected to be slightly increased. The proportion of familial genes in common increases slightly and this goes together with a decrease of genetic variability. In the offspring of first cousins the risk may increase about 3-fold. It is reasonable because of a flattening of the normal distribution of polygenic liability together with a higher proportion of suprathreshold field /6/. Thus the higher rate of consanguinity may explain the higher birth prevalence of some congenital abnormalities with multifactorial origin in gipsy communities /3/.

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Acta Paediatrica Hungarica 31 (3), pp 305-319 (1991)

HYPERLIPEMIA AND HYPERLIPOPROTEINEMIA /HLP/ SCREENING AMONG THE CHILDREN FROM PREMATURE MYOCARDIAL INFARCTION RISK FAMILIES

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Received 28 February 1990

Serum lipids and lipoproteins were investigated in the premature myocardial infarction (PMI) risk families before 45 years of age with the aid of screening for hyperlipemia and hyperlipoproteinemia (HLP): in the case of 174 persons from Csongrád County from the Departments of Internal Medicine I and II and of 42 patients (fathers) suffering from PMI and their 79 "high risk" children from Heves County.

In the investigated three groups of "high risk" children the genetically determined antiatherogenic HDL-Ch level diminished in 34.8, 52.3, 40.5 per cent.

Significant negative correlation was detected between the serum HDL-Ch and beta-lipoprotein; significant positive correlations were found between the HDL-Ch and the serum lipase activity; between the beta-lipoprotein and the phospholipid level; significant negative correlation was proved between the HDL-Ch and the phospholipid level in the group of PMI patients and their offsprings. The Ch/Tg, and the HDL-Ch ratios were significantly diminished in the PMI patients' group against the risk children' group, while the Ch/HDL-Ch rate was significantly elevated.

INTRODUCTION

Plasma lipoproteins and apolipoproteins are subjects of interest because of their association with coronary artery disease. Elevations of total and low-density lipoprotein (LDL) cholesterol and the main apolipoprotein constituent of LDL, apolipoprotein B, are associated with an increased risk of

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coronary artery disease. Similarly, elevated plasma levels of Lp/a/ are seen more frequently in patients with premature coronary artery disease. Conversely, low levels of high-density lipoprotein (HDL) cholesterol and its major protein constituent, apolipoprotein A-I, are also associated with an increased risk of coronary artery disease /4b, 12a/.

Regression analyses were performed by Rosenbaum et al /26b/ with the value of the cardiovascular risk factor variable for the child as the dependent variable and race, sex of child and either mother's values, father's values, or both mothers's and father's values as the independent variables. The most significant relationship between parents and their children was for height; parental serum lipids and lipoprotein tended to increase with the child's age. Child-father regression coefficients and child-mother regression coefficients were generally significant after age of 2 years for total cholesterol. Less association was noted for triglycerides and lipoproteins. Parental diastolic blood pressure was a poor predictor of children's values; the regression coefficients for systolic blood pressure were higher and more significant /26/b/.

Serum lipid and lipoprotein levels at the age of 7 years were associated with previously measured levels as early as 6 months of age and infants with unfavourable levels were likely to have similar adverse levels at 7 years of age. In addition, increases in obesity between 6 months and 7 years of age were positively associated with increases in levels of serum triglycerides /5/a/.

As generally known the hyperbeta-lipoproteinemia (HBlp) and hypertriglyceridemia (HTg) are risk factors for premature myocardial infarction (PMI) /28, 3, 14, 19/. High density lipoprotein-cholesterol (HDL-Ch), low density lipoprotein-Ch (LDL-Ch) and the very low density lipoprotein-Ch (VLDL-Ch) proved to be genetically determined /20, 26/ among the risk factors.

Heinle et al /8/ found 25 per cent HLP type IV and 29 per cent HLP type II among their coronary sclerotic patients. Pados et al /23/ detected different types of HLP of the men's and

women's group - 59.3 per cent and 40.7 per cent resp. - suffering from PMI. HLP type IV or HLP type IIb and IIa were found to be the most frequent ones in the myocardial infarct patient's group /12/. According to the data of Szabó et al. /30/ HLP was 61.3 per cent among the infarct patients and 30.9 per cent in their offsprings.
It seemed to be useful to investigate the serum lipid and lipoprotein parameters in families at high risk for PMI.

MATERIALS AND METHODS

73 persons, 26 PMI patients and 47 "high risk children" 1.5-17.5 years from 26 families of the I. and II. Department of Medicine, Medical University of Szeged, 101 persons 24 PMI patients and 77 "high risk children" from 44 families of the Department of Internal Medicine, Hospital of Szeged and 121 persons 42 PMI patients and 79 offsprings from 42 families from the County Heves with high risk of PMI (under 45 years) were investigated for serum lipids [total Ch, Tg, phospholipid (=Phl)] and for lipoproteins (HDL-Ch), beta-lipoprotein (=Blp) and for lipase activity.
Serum cholesterol (Ch) and triglycerides (Tg) were measured by Goedecke-UV test, enzaChol-F (Goedecke), (EnzGlycid GPO) Goedecke.
The antiatherogenic HDL-Ch was measured after precipitation with Na-phosphowolframat and $MgCl_2$, the phospholipide (Phl) was determined fluorimetrically (1,6-Diphenyl-1,3,5-hexatriene (SIGMA), the serum lipase activity was measured by Boehringer-Lipase test (No 262358, No 263346 Lipase Monotest 10). The distribution of the hyperlipoproteinemias (HLP) types was given. The linear correlations between the serum lipids and lipoproteins were estimated in the patient-group originating from County Heves.

RESULTS

In the first group among the 26 PMI patients there was 15.3 per cent HLP II.a type (Ch > 6.5 mmol/l,hyperbeta-lipoproteinemia = NBLP = beta - lipoprotein > 8.5 g/l), 15.3 per cent HLP type II.b (HCh + HTg/Tg > 2.5 mmol/l), 3.8 per cent HLP type IV. in 31.8 per cent the HDL-Ch level diminished

under 1.2 mmol/l. The HDL-Ch diminished in 34.8 per cent in the high risk children's group (n = 47), hyperbeta-lipoproteinemia was 4.3 per cent, HLP type IV. was the same per cent.
Ch/Tg ratio elevated over 8 in 30.6 per cent of patients in 57.8 per cent of them this ratio was informative for hypercholesterolemia. In the 17.7 per cent of the investigated families the total Ch/HDL-Ch rate was elevated (> 7.0), in all of them with 100 per cent informative for hypercholesterolemia.

In the second group 101 persons from 44 families were investigated for lipid risk factors. Among the PMI (n = 24) there were 20.8 per cent HLP type IIb, 16.6 per cent HLP type IV and 8.3 per cent HLP type IIa, 20.8 per cent hypercholesterolemia without hyper -beta-lipoproteinemia, 4.2 per cent HTg, 8.3 per cent HBlp. The antiatherogenic HDL-Ch diminished in 25 per cent of cases.

In the high risk offsprings' group (n = 77) the HDL-Ch was diminished in 52.3 per cent (< 0.85 mmol/l under 14 years and < 1.1 mmol/l over 14 years), 2.6 per cent HCh /2/77/, 6.5 per cent /5/77/ HTg, 5.2 per cent /4/77/ HBlp, 1.3 per cent /1/77/ HLP IIb, and there were no HLP type IIa and type IV (Table Ia) cases.

In the third group (County Heves) the mean values of the serum lipids, of the lipoproteins, the Ch/HDL-Ch ratio were in the normal range, while the HDL-Ch was under 1.5 mmol/l (Table I) in the high risk offspings' group. Lipase activity was low in 14.9 per cent (20-40 U/l). The Phl level was more than 3 mmol/l in 25.3 per cent /22/87/.

The PMI patients proved to be HCh, HTg, HBlp, and hypoHDL-Cholesterolemic ones according to the mean values (Table I).

In the PMI group the frequency of HLP type IIa was 38.1 per cent /16/42/, the same as HLP type IIb, HLP type IV was only 4.8 per cent /2/42/, HCh was 14.3 per cent /6/42/ without HBlp, HDL-Ch diminished in 42.0 per cent.

In the high risk offsprings' group there was 3.8 per cent /3/79/ HLP type IIa, 2.5 per cent /2/79/ HLP type IIb, 3.8 per cent HCh without HBlp, 6.3 per cent /5/79/ HBlp. The antiatherogenic HDL-Ch diminished under 1.2 mmol/l in 40.5 per cent /32/79/ (Table Ia).

TABLE I

Serum lipid and lipoprotein values in premature myocardial infarct patients (County Heves)

	Cholesterol mmol/l	Triglycerid mmol/l	HDL-Ch mmol/l	Beta-lipoprotein g/l	Lipase E/l	Phospholipid mmol/l
n = 42						
$\bar{X}$ =	6.71	2.6	1.2	9.39	65.6	3.86
S.D.$\pm$	1.59	2.46	0.45	4.39	22.0	1.71
			High risk children			
n = 79						
$\bar{X}$ =	4.85	0.95	1.35	5.41	52.5	2.43
S.D.$\pm$	0.82	0.49	0.32	2.41	25.4	0.44

TABLE I.a

Percentual incidency of the hyperlipemia and hyperlipoproteinemia in the high risk premature myocardial infarct families

	HCh	HLP II.a	II.b	IV.	HBlp	decreased HDL-Ch	%
1. group (I.and II. Department of Internal Medicine, Med.Univ.Szeged)							
PMI patients (n = 26)	0	15.3	15.3	3.8	0	31.8	
High risk children (n = 47)	0	0	0	4.3	4.3	34.8	
2. group (Dept. of Internal Medicine, County Hospital, Szeged)							
PMI patients (n = 24)	20.8	8.3	20.8	16.6	8.3	25.0	
High risk children (n = 77)	2.6	0	1.3	0	5.2	52.3	
3. group (County Heves)							
PMI patients (n = 42)	14.3	38.1	38.1	4.8	0	42.0	
High risk children (n = 79)	3.8	3.8	2.5	0	6.3	40.5	

HCh = hypercholesterolemia without HLP
HBlp = hyperbeta-lipoproteinemia without HLP

TABLE I.b

Lipid and lipoprotein atherogenic ratios

Groups		Ch/Tg	Ch/HDL-Ch	HDL-Ch/Ch
I.	PMI	23	14	14
	$\bar{X}$ =	4.01	4.43	0.24
	S.D.±	3.03	1.38	0.07
	p	= 0.057	> 0.05	> 0.05
	Risk children	35	31	31
	$\bar{X}$ =	5.31	3.86	0.28
	S.D.±	2.12	1.30	0.08
II.	PMI	21	19	19
	$\bar{X}$ =	3.83	7.97	0.13
	S.D.±	2.19	2.35	0.04
	p	< 0.001	< 0.001	< 0.001
	Risk children	79	73	73
	$\bar{X}$ =	5.02	5.37	0.20
	S.D.±	2.26	1.80	0.07
III.	PMI	38	38	38
	$\bar{X}$ =	4.15	5.92	0.19
	S.D.±	2.51	2.70	0.06
	p	< 0.05	< 0.001	< 0.001
	Risk children	68	68	68
	$\bar{X}$ =	6.03	6.67	0.29
	S.D.±	2.82	1.06	0.07
Controls	n =			17
3 - 6 year	$\bar{X}$ =			0.19
	S.D.±			0.04
6 -10 year	n =			14
	$\bar{X}$ =			0.187
	S.D.±			0.04
10-14 year	n =			18
	$\bar{X}$ =			0.2
	S.D.±			0.05

Significant positive linear correlation was proven between the serum Ch and Tg, Ch and BLp, Ch and Phl, significant negative correlation was proven between the HDL-Ch and Blp, between the Phl and HDL-Ch in the investigated third group. There was a positive correlation between the HDL-Ch and lipase activity, between the Blp and Phl in the total group (PMI patient and their high risk children) (Table II).

There was no correlation between the Blp and Ch, and between the HDL-Ch and the lipase activity in the group of PMI patients (Table III).

The correlations between the Ch and Tg, HDL-Ch and lipase activity, HDL-Ch and Phl were absent in the high risk offsprings' group (Table III). The lipid and lipoprotein atherogenic rations are seen in the Table I.b according to the different groups. The Ch/Tg, Ch/HDL-Ch and the HDL-Ch (Ch ratios significantly changed in the II. and III.groups; the Ch/Tg and the HDL-Ch/Ch ratios were signicantly diminished in the PMI patients' group against the high risk children's group, while the Ch/HDL-Ch rate was significantly elevated. These correlations did not change significantly in the cases of the first group.

DISCUSSION

Andersen et al /1/ found among 1407 Danish children whose fathers have died from ischemic heart disease before age of 45, 15 per cent HCh 8 per cent HTg, 1.8 per cent familial HLP.

Glueck et al /6/ among 233 children of 70 parents with a myocardial infarction before age of 50 years found 2.5 per cent with HCh. Blumenthal et al /4/ found 13.8 per cent with HCh, Hennekens et al /10/ found 16.1 per cent high risk children with elevated serum Ch. Rissanen and Nikkilä /25/ among 213 children of 104 men with angina pectoris before age of 56 years found hyperlipemia in around 23 per cent as opposed to 13 per cent in the control group.

TABLE II

Linear correlation coefficients between the serum lipids and lipoproteins (high risk families, premature infarct patients /parents/ and their children)

n=83 (29+54)	Cholesterol r	Triglycerid r	HDL-Ch r	Beta-lipoprot. r	Lipase r	Phospholipid r
Cholesterol	1.00	0.55^{x}	-0.10	0.53^{x}	0.15	0.61^{x}
Triglycerid	0.55^{x}	1.00	-0.40^{x}	0.68^{x}	0.06	0.81^{x}
HDL-Ch	-0.10	-0.40^{x}	1.00	-0.44^{x}	0.25	-0.30^{x}
Beta-lipoprotein	0.52^{x}	0.68^{x}	-0.44^{x}	1.00	0.11	0.75^{x}
Lipase	0.15	0.06	-0.24^{x}	0.11	1.00	0.07
Phospholipid	0.61^{x}	0.81^{x}	-0.30^{x}	0.75^{x}	0.07	1.00

^{x} $p < 0.05$

TABLE III

Linear correlation coefficients between the serum lipids and lipoproteins (premature myocardial infarct patients)

n = 29	Cholesterol r	Triglycerid r	HDL-Ch r	Beta-lipoprot. r	Lipase r	Phospholipid r
Cholesterol	1.00	0.48^{x}	-0.09	0.32	-0.10	0.39^{x}
Triglycerid	0.48^{x}	1.00	-0.52^{x}	0.66^{x}	-0.03	0.78^{x}
HDL-Ch	-0.09	-0.52^{x}	1.00	-0.57^{x}	-0.27	-0.44^{x}
Beta-lipoprotein	0.33	0.66^{x}	-0.57^{x}	1.00	-0.06	0.72^{x}
Lipase	-0.09	-0.04	-0.27	-0.06	1.00	-0.14
Phospholipid	0.39^{x}	0.78^{x}	-0.44^{x}	0.72^{x}	-0.14	1.00
Linear correlation coefficients between the serum lipids and lipoproteins (children with high risk)						
n = 54						
Cholesterol	1.00	0.13	0.14	0.33^{x}	0.06	0.61^{x}
Triglycerid	0.13	1.00	-0.29^{x}	0.66^{x}	-0.17	0.65^{x}
HDL-Ch	0.14	-0.29^{x}	1.00	-0.28^{x}	-0.18	0.02
Beta-lipoprotein	0.33^{x}	0.66^{x}	-0.28^{x}	1.00	0.00	0.67^{x}
Lipase	0.06	-0.17	-0.18	0.00	1.00	-0.08
Phospholipid	0.61^{x}	0.65^{x}	0.02	0.67^{x}	-0.08	1.00

^{x} $p < 0.05$

In the literature there are data about the negative correlation between the HDL-Ch value and the severity of the myocardial infarction, and positive correlation between the LDL-Ch level /8/. Apolipoprotein A-I (Apo-A-I) proved to be a marker for coronaria-sclerosis /18/. Apo-A-I is an important functional part of the HDL-Ch. The LDL-Ch was significantly diminished, the HDL-Ch significantly elevated in the physically trained group /2/; by the elevation of the activity of lipoprotein lipase in muscles and in the fat tissue.

Franzen and Fex /5/ showed a positive correlation between the Tg level and Apo-A-I/HDL-Ch ratios; a negative correlation between the HDL-Ch and Tg. There was a strong correlation between the HDL-Cl level of the high risk sons and their fathers suffering from PMI.

Lees and Lees /16/ published significantly less Apo-A-I value than in the control group in the first degree relatives of PMI patients and significantly higher Apo-B values. In our own material the total Ch/HDL-Ch ratio was 100 per cent informative for HLP and HCh, while the Ch/Tg ratio was false positive in 42.4 per cent, originating from the low Tg levels with normal Ch values. We have detected compensatoric HDL-Ch elevation in 28.5 per cent of the PMI patients. Apo-B value proved to be the best discriminative factor between the male family members and Apo-A-I between the female family members and the control group /13/.

Oberhänslie et al /20/ found the HDL/serum Ch and HDL/LDL/Ch ratio to be useful as the indicator for PMI. Heldenberg et al /9/ published elevated Ch and HDL-Ch levels in the high risk children of PMI fathers.

Goldstein et al /7/ detected 60 per cent primary HLP, Ibsen /11/ detected more frequently familial HLP in the high risk family members. In Somogyi's /29/ material there was 24.3 per cent HLP type IIa in the high risk children's group, 60.3 per cent HLP in the PMI patients' group and 31 per cent HLP in their offsprings.

Longitudinal assessment of children with elevated lipid and lipoprotein levels may permit early identification of risk factors which increase the risk to coronary heart disease in

adulthood (Ch, Tg, LDL-Ch) or decrease it (HDL-Ch) /15/.

To establish the value of screening children for hypercholesterolemia predicting adult-age risk for the same condition /21/ stated that cholesterol screening in childhood proved to be predictive for adult HCh.

Familial hypercholesterolemia is based on the structural mutation in the LDL-receptor gene, which is one among the most common inborn errors of metabolism /24/.

Romics et al /27/ observed significantly elevated Ch, Tg, LDL and VLDL-Ch, diminished HDL-Ch, VLDL-LDL ratio in the group of PMI persons.

We found 72.5 per cent hyperlipemia or HLP in PMI patients, the antiatherogenic HDL-Ch was under 1.2 mmol/l value in 52.3 per cent of the 77 high risk children from 44 PMI families.
Decreased plasma HDL-Ch and Apo-A-I levels have been associated with premature coronary disease (PCAD). Ordovas et al /22/ detected Apo-A-I gene polymorphism associated with PCAD and familial hypo-alpha-lipoproteinemia.
In our third investigated group from County Heves the antiatherogenic HDL-Ch level diminished in 42.0 per cent of the PMI patients and in 40.5 per cent of their descendants. The Ch/Tg and the HDL-Ch/Ch ratios were significantly diminished in the PMI patients' group against the high risk children's group, while the Ch/HDL-Ch rate was significantly elevated in our own first and second groups investigated.

Szamosi et al /30/ and Czinner et al /4a/ screening the Hungarian high risk families for arteriosclerosis have got significantly higher Ch and lower HDL-Ch levels among the risk children of PMI patients.
According to literature data and our own results the screening for lipid and lipoprotein parameters among the children of high risk families seems to be useful inspite of the poor preventive and therapeutic possibilities. Rational diet and sufficient physical activities could be more advisable as any drugs in this early age.

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Acta Paediatrica Hungarica 31 (3), pp 321-336 (1991)

THE REDISTRIBUTION OF THE BLOOD FLOW UNDER NIFEDIPINE TREATMENT IN THE SHEEP FOETUSES

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Received 7 February 1990

The Ca^{++}-antagonist nifedipine has been succesfully employed in the treatment of non-gravid hypertension, and was found to inhibit uterine contractions in the perimenstrual period, as well as during premature labour in animal models.

The use of antihypertensive drugs in pregnancy introduces the possibility of iatrogenic foetal distress. It has been established that nifedipine crosses the placental barrier in the sheep and causes a fall in mean arterial pressure and tachycardia in both the ewe and the foetus. This paper examines the effects of nifedipine on the foetus when administered to the pregnant ewe. Catheters and electrodes were implanted by surgical procedures in 15 ewes and foetal lambs between days 118 and 122 of gestation. The redistribution of foetal blood flow was measured by the radioactive microsphere injection technique. The infusion of nifedipine caused a 9 % increase in the combined ventricular output (CVO) from 446 to 509 ml/min/kg in the foetus. Foetal lung blood flow increased from 29 $\pm$ 6 to 69 $\pm$ 14 ml/min/kg while figures for the skeletal muscle flow were 109 $\pm$ 34 and 141 $\pm$ 41.6 ml/min/kg. Heart and brain blood flow, expressed as percentages of CVO showed variations of 4.3 and 5.6 percent, respectively.

Blood flow in the gut, placental membranes, skin, kidney and spleen was reduced.

The present results show that nifedipine, in addition to its known effects causes a redistribution of the foetal circulation.

INTRODUCTION

Severe hypertension is a major cause of maternal mortality and morbidity during pregnancy, and is associated with significant foetal complications /2/. The use of antihyper-

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tensive drugs in pregnant women introduces the possibility of iatrogenic foetal distress. The calcium antagonist nifedipine has been used succesfully in the treatment of non-gravid hypertension /12, 22/ and it is being considered for trials in pregnant women, particularly in cases of severe acute hypertensive episodes /30/. Some studies have reported on its effects on the pregnant ewe and foetus /11, 24/.

It has been established that nifedipine crosses the ovine placenta and causes a fall in mean arterial pressure and tachycardia in both the ewe and foetus. The mechanisms by which these effects occur are unclear.

This paper examines some of the consequences on the control of foetal circulation during maternal administration of nifedipine. The redistribution of foetal blood flow under the influence of nifedipine has been measured by a radioactive microsphere injection technique /15, 25/. Two foetal organs were of particular interest, viz. the lungs and the placenta. Premature closure of the ductus arteriosus has been implicated in cases of persistent neonatal pulmonary hypertension after the treatment of pregnant women with prostaglandin synthetase inhibitors /17, 19/. A possible effect of nifedipine on the ductus arteriosus must therefore be considered. The maintenance of placental perfusion in the presence of lowered arterial pressure was also examined. The relative levels of activity of the foetal sympathetic and parasympathetic nervous systems in controlling the cardiovascular responses of the foetus to nifedipine were studied using specific blocking agents /6, 27, 29/.

Propranolol was used to block sympathetic stimulation of the foetal heart, and atropine methonitrate to block parasympathetic activity. Finally the variability of the foetal heart rate was measured.

This is of clinical importance as the foetal heart rate is the most accessible and widely used parameter of foetal wellbeing.

MATERIALS AND METHODS

Catheters and electrodes were implanted in fifteen ewes and foetal lambs 118-122 days of gestation. The surgical procedures have been described previously /7, 29/ and were performed at laparatomy using aseptic techniques with halothane anaesthesia. A maternal carotid artery, jugular vein and utero-ovarian vein were catheterized and a recording electrode was saturated into the myometrium. A carotid artery, jugular vein and the trachea were catheterized in each foetus and recording electrodes implanted subcutaneously along the sternum for ECG detection and bilaterally onto the parietal dura to record the electrocorticogram. The amniotic sac was also catheterized. In foetuses for blood flow measurements with radioactive microspheres, catheters were inserted also into a brachial artery, femoral artery and tarsal vein. All catheters and leads were passed through the ewes flank. The ewe received 2 ml Streptopen i.m. (penicilline 2500 000 IU/ml + streptomycin 250 mg/ml, Glaxo) before the operation and for four days there after. The foetus was given Penicilline (300 000 IU i.v.) and 1 ml Streptopen was injected into the amniotic fluid after the operation and for four days. All the arterial catheters were continuously infused with sterile heparinized saline (250 IU/ml) at a rate of 0.2 ml/hr. The foetal arterial, tracheal and amniotic catheters were connected to pressure transducers. The true foetal arterial and tracheal pressures were calculated on a Devices 6 channel recorder. Blood samples were analysed for PO_2, PCO_2 and pH using a Corning Blood gas analyser (England) calibrated at 39 ^{o}C.

Nifedipine preparation - Nifedipine was supplied in a sterile 100 mg/ml solution in 90 % ethanol. It was diluted with 60 % ethanol and infused using a Braun pump. A dose of 10 µg/kg maternal weight/min was administered in 15 ml 60 % ethanol per hour for two hours. All syringes, infusion lines and the maternal jugular vein catheter, as far as it was visible, were wrapped in black PVC tape as were all tape connections to exclude all light.

After one hour of a two-hour 10 µg/kg/min nifedipine infusion to the ewe, atropine methonitrate (Sigma) 0.5 mg/kg foetal weight was injected i.v. into 6 foetuses. Propranolol (Inderal, ICI) was administered to 5 foetuses, 2 mg i.v. followed by an infusion of 15 µg/min for 1 hour, after one hour of a two-hour 10µg/kg/min infusion of nifedipine to the ewe.

Data handling

Arterial samples were taken from the ewe and the foetus one hour before, every 15 mins during and one hour after infusions. Foetal heart rate and mean arterial pressure were measured at the same intervals. Foetal breathing movements and electrocorticogram records of high voltage activity were compared with the 6-hour control period preceding the experiment. All the record analysis was done by hand. The results are expressed as mean $\pm$ SD, statistical analysis was done with paired "t" test.

Microspheres and blood flow measurement

The reference sample method described by Rudolf and Heyman (1967) was used. Blood samples of 1 ml were drawn from the carotid artery before each blood flow measurement to determine blood gases, pH and packed cell volume. Blood flow measurements were made with isotoplabelled, 141 Cr, 46 Sc, 113 Sn, 103 Ru and 95 Nb, 15μm/diameter polystyrene microspheres, coated with polymeric resin and suspended in 10 % dextran with 0.01 % polyoxyethylene 80 sorbonit mono-oleate (Tween 80) to reduce aggregation. The specific activity was determined on 5.000 spheres, leaking into the suspending medium was minimal.

Microspheres (mean 2.5 $\pm$ 0.3 million) were injected over 45 sec through a mixing chamber into the caudal vena cava. Reference samples were withdrawn over 75 sec from the femoral and carotid or brachial artery for the upper and lower body, respectively, and were simultaneously replaced by dextran. Two injections were given during a control period, then an infusion of 10 μg/kg/min nifedipine was started.

Two further injections were given at least 90 mins after the start of the infusion. A final injection was given at least two hours after the end of the nifedipine infusion. Injections were given during episodes of high and low voltage foetal electrocortical activity alternatively. After the microsphere injections had been completed the ewe was killed with pentobar itone and the foetus perfused with 300 ml 15 % formalin. The foetal organs were placed into vials after weighing. Four separate samples each of skin, skeletal muscle and lung tissue were counted, otherwise duplicate samples were taken whenever the tissue was too big for total counting. The number of microspheres in each samples always exceeded 800.

For counting an Auto Gamma Spectrometer (Packard 5320 Mudumatic II) with 2 iodine crystals, optically coupled to two photomultiplier tubes, was used. This was connected to a multichannel pulse height analyser, interfaced on line to a programmable computer stored the data on floppy discs. The programme corrected the count rates for geometrical and spectral distribution and background; the filling height was derived from the organ weight by linear regression ($r = 0.965$). Loss of counts due to coincidence of nuclear disintegrations was negligible since the specific activities used were low (about 5 mC/g) and the count rate of the samples never exceeded 500.000 cpm.

Heart rate variability

An AIM-65 microprocessor was used to measure R-R intervals of the foetal ECG before and during maternal infusions of nifedipine and to calculate the mean heart rate (HR), the standard deviation over 2 minute periods (long term variability LTV) and the mean beat-to-beat deviation from the heart period trend (short term variation STV).

RESULTS

Propranolol

The infusion of propranolol alone for one hour caused an 11 % fall in heart rate from 171.5 ± 9 to 153.7 ± 16 beats/min. There was no significant effect on arterial pressure, blood gases or pH. However, when propranolol was infused into five foetuses during the maternal infusion of nifedipine the foetal heart rate fell from its increased rate of 190 ± 18 to baseline values of 145 ± 17 beats/min /baseline was 149 ± 18 beats/min within 15 min.

The foetal heart rate remained very stable at this level for 3-4 hours after the end of the infusions (Fig 1a). The foetal carotid PO_2 fell from a mean of 20 ± 0.79 to 17.8 ± 0.55 mmHg ($p < 0.01$), and the pH fell from 7.33 ± 0.017 to 7.287 ± 0.028 ($p < 0.01$), by the end of the second hour of infusion (Fig 1b).

The mean fetal arterial pressure remained at the reduced level, having fallen with nifedipine from 49.6 ± 1.95 to 42.8 ± 4 mmHg after 60 min.

Atropine methonitrate

The injection of atropine methonitrate into the foetal lamb caused an increase in foetal heart rate of 16 % from 151 ± 8.8 to 176 ± 16 beats/min. There was no change in arterial pressure, PO_2, PCO_2 or pH. The injection of atropine methonitrate into six foetal sheeps after 1 hour of a 2-hour infusion of 10 µg/kg/min nifedipine into the ewe caused a lowering of the raised heart rate from 198 ± 26.7 to 178 ± 34.7 beats/min ($p < 0.001$) and a further 2 mmHg fall in carotid arterial pressure from 44.8 ± 2.6 to 42.2 ± 4.7 mmHg (Fig 2a), while there were no significant changes in blood gases or pH (Fig 2b).

The results showed that the infusion of nifedipine for at least 90 min caused a 9 % increase in combined ventricular output (CVO) from 466 ml/min/kg foetal weight to 509 ml/min/kg. Two hours after the end of the nifedipine infusion the CVO fell by 28 % of the control value to 335 ml/min/kg. As can be seen from Fig 3a the increased blood flow was diverted to the lung

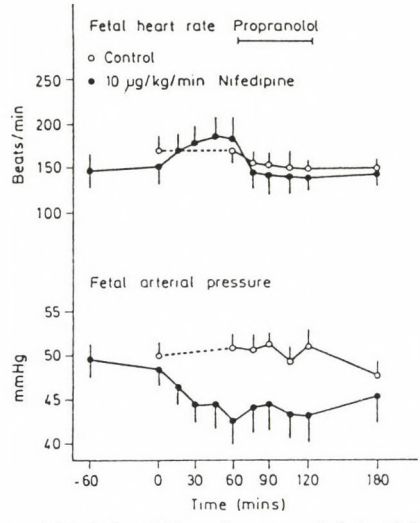

Fig. 1a. Effects of propranolol infusion (○) and propranolol + nifedipine infusions (●) on foetal heart rate and on mean arterial pressure

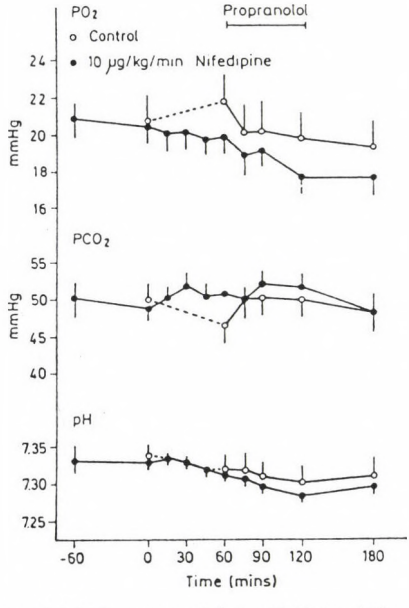

Fig. 1b. Effects of propranolol (○), propranolol + nifedipine (●) on foetal PO_2, PCO_2 and pH

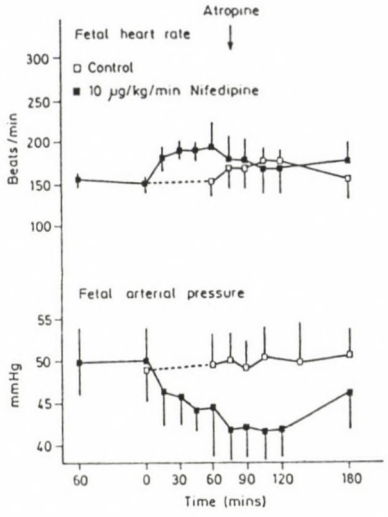

Fig. 2a. Effects of atropine methonitrate (□) and atropine methonitrate + nifedipine (■) on foetal heart rate and on mean arterial pressure

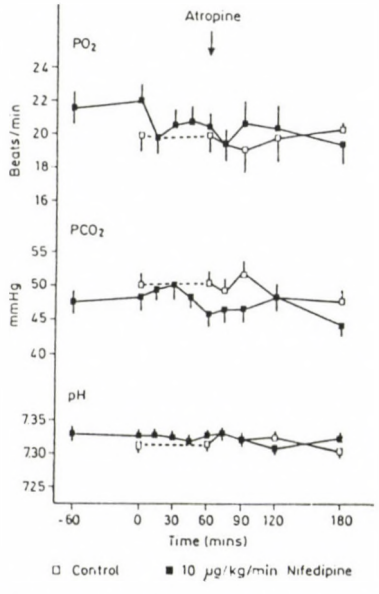

Fig. 2b. Effects of atropine methonitrate (□) and atropine methonitrate + nifedipine (■) on foetal PO_2, PCO_2 and pH

and skeletal muscle. Blood flow to the foetal lungs increased from 29 ± 6 ml/min/kg to 69 ± 15 ml/min/kg, a change in % CVO from 6.2 % to 13.5 %, while the skeletal muscle flow increased by 4 % from 109 ± 34 ml/min/kg to 141 ± 11.6 ml/min/kg. The blood flow as % CVO to the heart rate and brain remained unaffected at 4.0 % and 5.6 % CVO, respectively. The blood flow to the gut, placental membranes, skin, kidneys and spleen were reduced (Fig 3b).

During the recovery period both the CVO and the conductance were reduced by 28 % of the control values. The heart was the only organ to receive an increased % CVO during the recovery period. It increased to 6.7 % of the reduced output of 335 ml/min/kg foetal weight. The flow to the lungs and skeletal muscle fell to 3.7 % and 18.1 % CVO, respectively. The conductance of all the organs, except the heart, fell or remained, while that of the heart remained unchanged at 15 ml/min/mmHg/100g. The blood flow to the cotyledons was maintained during the nifedipine infusion at 155 ± 21 ml/min/kg foetal weight, while during the recovery period this was reduced to 124 ± 12.6 ml/min/kg foetus although the % CVO was maintained at about 35 %. Whether the blood flow through the ductus arteriosus was affected by nifedipine or not, cannot be answered directly. However, the % CVO feeding the lower body changed from a control of 7.4 % to 66.2 % while the upper body flow increased during the administration of nifedipine from 133 ml/min/kg foetus to 172 ml/min/kg foetus.

The extent to which this might be related to any change in the ductus is not clear particularly in view of the observed changes in the pulmonary and skeletal muscle blood flow.

Heart rate variability

During nifedipine administration the values compared with control values were as follows.

Heart rate, 198 ± 7.8; 4.6 ($p < 0.001$)

LTV, 12.5 ± 1.1; 16.8 ± 2.1 ($p < 0.05$)

STV, 1.6 ± 0.3; 2.0 ± 0.4 NS (paired "t" test)

The values during high voltage electrocortical activity compared with low voltage electrocortical activity were:

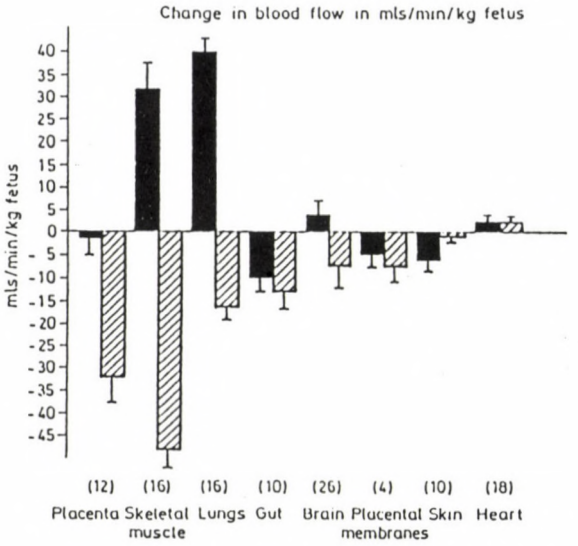

Fig. 3a. The blood flow to the placenta, skeletal muscle, lungs, gut, brain, placental membranes, skin, and heart during nifedipine infusion (■) and after tro hour recovery (▨). (mean ± SD)

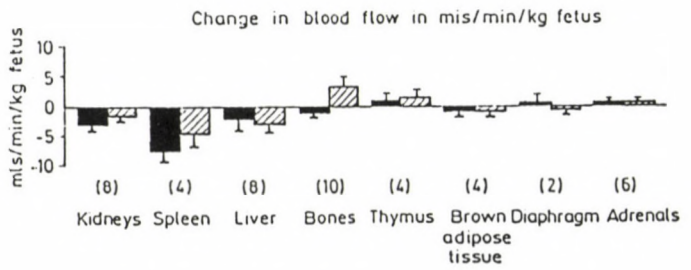

Fig. 3b. Change in blood flow to the foetal organs during nifedipine infusion (■) and after two hour recovery period (▨)

Heart rate, 184 ± 10; 171 ± 9 ($p < 0.002$)
LTV, 14 ± 1.9; 15 ± 2.0 NS
STV, 1.7 ± 0.3; 2.1 ± 0.4 ($p < 0.05$)
The changes during nifedipine administration were similar when high and low voltage electrocortical activity were analysed separately.

DISCUSSION

The measurement of the distribution of combined ventricular output (CVO) in the foetus using the capture of radioactive microspheres by different tissues is a well-documented procedure.

The methodological problems described by other workers, have been overcome by using about half a million 15 μm spheres per kg foetal weight, injected over 45 sec. Thus any circulatory changes caused by the injection of the microspheres are minimized and there are enough spheres per sample for reliable counting. The results concerning the control period injections agree with the general range of distribution of CVO measured at this foetal age. A difference in blood flow between high and low voltage electrocortical activity in elements of the brain and the gut has been described. Measurements taken during high voltage electrocortical activity were used whenever there was a significant difference. The most striking change in blood flow occurred in the foetal lungs which received twice the flow they had during the control period. The increase in skeletal muscle blood flow was not unexpected as it is one of the main sites of action of nifedipine in humans. The increase of 4 % of CVO was not significant, but the increase in blood of 30 ml/min/kg foetus is large enough compared to the normal blood flow to the gut or the brain. The overall increase of 9 % in CVO together with only a 7.2 % increase in vascular conductance suggests that nifedipine has a stimulatory effect on the heart either directly or through the increased production of catecholamines.

Nifedipine itself did not exert a deleterious effect on placental blood flow. However, the circulatory adjustments of

the foetus to compensate for the enforced fall in arterial pressure, seem to have persisted longer than the fall in pressure once nifedipine was removed. This resulted in a fall in placental blood flow lasting up to two hours after the end of nifedipine administration.

The mechanisms controlling this rearrangement of foetal blood flow in response to the hypotensive actions of nifedipine were investigated using two drugs which block different sections of the autonomic nervous system. The infusion of propranolol into the foetal sheep at 128-135 days of gestation caused a slight fall in heart rate. The infusion of atropine methonitrate into foetal sheep of the same age caused a slight increase in heart rate. Neither of the drugs had any significant effect on foetal arterial blood gases or pH, or foetal blood pressure. This agrees with the data of Vapaavouri et al. (1973), van Petten (1975) and Dalton et al. (1978).

The effects of propranolol administered to the foetus during the maternal infusion of nifedipine to reduce the raised heart rate to resting levels indicate that the tachycardia is of symphathetic origin, but it does not distinguish between neural responses and circulating catecholamines. However, in spite of this reduction in heart rate the foetal blood pressure is maintained. The mechanisms available to maintain blood pressure include the beta-receptors of the sympathetic system, any vasoconstriction by the parasympathetic system and other hormonal and circulating factors, for example vasopressin, the activity of which might be augmented by the chemoreceptor response to the fall in PO_2 and pH.

Its significance is obvious for the human foetus, where the treatment, of pregnant, hypertensive women with the combination of nifedipine and propranolol could have serious consequences.

Atropine methonitrate was chosen to block the parasymphathetic nervous system because it does not cross the blood-brain barrier. It is a drug with a well defined action blocking the muscarinic receptors of the parasympathetic nervous system thus preventing the action of acetylcholine at the end-plate. The demonstration of the increase in foetal heart rate following the injection of atropine methonitrate

into the foetus confirms a certain level of vagal tone on the foetal heart at this gestational age as has been described by others /6, 27, 30/.

An increase in arterial pressure is not often seen in foetuses of this age but is documented for older foetuses near term in which the paraysmpathetic system is more developed /30/. The injection of atropine methonitrate into foetal sheep already receiving nifedipine had the opposite effects to those expected.

If there has been any vagal tone left on the foetal heart at its raised rate, then atropine methonitrate would have caused a further small increase in the rate.

If no vagal tone remained, then heart rate should have remained at the raised level rather than fell to a lower rate. The injection of atropine into the foetal sheep during the administration of propranolol increases foetal heart rate, although not quite back to resting levels. The parasympathetic system also acts on the peripheral vasculature. Acetylcholine dilutes the vascular bed of the foetal lung /17/.

Effects on the vascular beds of other foetal organs, particularly the skeletal muscles, are not clear. It is doubtful therefore whether atropine methonitrate could have had any effect on the foetal lungs, as it would have tended to induce a vasoconstriction and raise the foetal arterial pressure rather than cause a further fall as was in fact measured. If the parasympathetic system is mediating the vasoconstriction of the vasculature of the gut, skin, spleen, and placental membranes (the tissues in which there was a fall in blood flow, as measured by the microsphere studies, during the administration of nifedipine), then atropine methonitrate could block this and cause a further fall in blood pressure in addition to that caused by nifedipine. If the parasympathetic system is not mediating this vasoconstriction the atropine methonitrate should have no effect of foetal blood pressure.

Previous observations have shown that a fall in arterial pressure is accompanied by tachycardia in the foetus, the administration of the antagonist phentolamine causing a sustained increase in foetal heart rate. An increase in the

foetal arterial pressure causes a lengthening of the heart cycle as described by others using either the infusion of phenylephrine or methosamine. Sometimes a change in arterial pressure has no effect on the heart rate, particularly if it is transient or not large enough /8/. Whether a further fall in heart rate caused by atropine methonitrate is the driving force in causing a fall in arterial pressure is not clear, but in the propranolol experiments with nifedipine the fall in heart rate was much greater than that with atropine methonitrate, and nifedipine had no effect on arterial pressure.

A possible situation in which a fall in heart rate and arterial pressure could occur together would be if atropine methonitrate blocked the autonomic ganglia of the sympathetic nervous system. There are two aspects of this theory to be considered firstly that the automatic ganglia might be affected by the nifedipine already circulating and secondly the existence of atropine sensitive neurons in the rat autonomic ganglia. The effects of nifedipine on ganglionic transmission have not been assessed throughly. The release acetylcholine in the rat sympathetic superior cervical ganglion is dependent on the presence of calcium. There is a calcium dependent potassium current which nifedipine might interfere with. Furthermore, there are atropine sensitive neurons in the autonomic ganglia of the rat cervical ganglion, which modulate the normal nicotinic ganglionic function.
Therefore, it is not impossible that atropine might have blocked an already existing system.

The above findings might be important in the human for two reasons. First it appears from the analogous sheep experiment that the combination of nifedipine and propranolol might have serious consequences for a human foetus if administered to the pregnant woman. Secondly we have demonstrated that a combination of drugs might not have the same effect as simply adding these effects of the two drugs, particularly for the foetus, in which the details of cardiovascular function are not fully understood and direct comparisons with the adult do not apply.

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Acta Paediatrica Hungarica 31 (3), pp 337-355 (1991)

EFFECTS OF PROSTAGLANDIN E_2 ON THE NEWBORN RESPIRATORY SYSTEM

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Received 7 February 1990

To test the hypothesis that prostaglandin (PG) E_2 is a respiratory depressant in the newborn lamb, 12 chronically catheterized, unanesthetized lambs (age 2 to 6 days) were infused with progressively increasing doses of PGE_2 (0.1, 0.5, 1.0 and 5.0 ug/kg/min: 30 min for each dose) into the ascending aorta. PGE_2 caused significant, progressive decrease in ventilation (due to decreased tidal volume and breathing rate) heart rate, blood pressure and percent of the time spent in low voltage electrocortical activity (LVA). PGE_2 also caused respiratory acidosis, hypoxemia and increased frequency and duration of apneic events (> 3 sec). During the infusion, there was a dose related increase in plasma concentration of PGE_2. At 30 min post-infusion, all measured variables showed recovery, although arterial pH carbon dioxide tension and plasma PGE_2 remained significantly different from control values and the percent time in LVA was even higher than during control. Infusion of the vehicle alone (n = 5) caused no significant changes in any of the measured variables. The results, taken in combination with previous fetal studies, indicate that PGE_2 has marked inhibitory effects on breathing movements both before and after birth.

INTRODUCTION

The physiological factors responsible for the control of the fetal breathing movements (FMB) and the onset of continuous breathing and ventilation at birth have not been completely defined, although it is likely that several factors are involved in this process /11,25,32/. In sheep, FBM are intermittent, normally occur only during low voltage

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electrocortical activity (LVA), and are absent during high voltage electrocortical activity (HVA) /8/. Experiments in fetal sheep indicate that prostaglandins (PG), especially PGE_2, may be important in the control of FBM. Infusions of PGE_2 temporarily inhibit FMB: after end of the infusion, there is rapid return of normal FBM /14/. Conversely, infusion of prostaglandin synthetase inhibitors (either meclofenamate or indomethacin) causes a marked decrease in plasma concentration of PGE_2, /19,31,32/ and an associated increase in the incidence and amplitude of FBM /12,13,15,19,32/, so that they occur almost continuously, even during HVA. There is recent evidence that endogenous PGE_2 participates in the regulation of FBM, acting primarily by decreasing the incidence of FBM during HVA /31/. Furthermore, the incidence of FMB correlates inversely with the plasma concentration of PGE_2 both during infusions of different doses of PGE_2 /31/ and during preterm labor induced by administration of ACTH /23/. During spontaneous labor, PGE_2 concentration increases /5/ and the incidence of FBM decreases /1/. At birth when breathing becomes continuous and ventilation is established, the concentration of PGE_2 decreases rapidly to very low level /5,7/.

There is evidence that E-type prostaglandins can affect the control of breathing and ventilation after birth. Apnea has been reported to occur with the infusion of PGE_1 and PGE_2 both in newborn swine /30/ and in human infants with cyanotic heart disease /21/. In addition, PGE_1 decreases the output of the phrenic nerve in newborn swine that have been anaesthetized, paralyzed and maintained with assisted ventilation /16/. However there have been no previous systematic studies of the effects of PGE_2 on ventilation in spontaneously breathing, unanaesthetized newborns.

The present study was designed to determine the effects of PGE_2 infusion on the control of breathing in newborn lambs, more specifically, to test the hypothesis that PGE_2 is a respiratory depressant in newborn lambs.

METHODS

We studied a total of 12 unanaesthetized chronically instrumented newborn lambs.

To be able to perform the experiments as soon as possible after birth, we operated on 7 fetuses (gestational age 140 to 143 days, term 140 ± 5 days) using sterile technique with the ewe and fetus under general anaesthesia /31,32/. Polyvinyl catheters (1.5 mm ID, 75 cm long) were inserted into a carotid artery and an axillary artery, the tip of the carotid catheter was placed in the ascending aorta just above the aortic valve. The catheter tip was localized by pressure measurements, it was advanced into the left ventricle and then withdrawn into the aorta. A balloon-tipped catheter for measurement of intrathoracic pressure was inserted into the pleural space through an incision in the fifth or sixth intercostal space in the midaxillary line. To record the electrocorticogram (ECoG), stainless steel screw electrodes attached to a shielded cable (Cooner Wire Company, California) were inserted through each parietal bone to rest on the dura. The electrodes were insulated from the fetal scalp with dental acrylic compound and grounded to the scalp of the fetus. The vascular catheters were filled with a solution of heparin (1.000 USP units/ml), the catheters and the electrodes were put into a pocket under the fetal skin and exteriorized after birth.

We defined an apneic event as a pause in breathing for 3 or more seconds and we divided the apneic events in three categories: 3 to 6 seconds, 7 to 10 seconds and longer than 10 seconds. We measured heart rate and blood pressure through the axillary arterial catheter connected to a P23DB (Statham) transducer. We recorded ECoG from the subdural electrodes with a preamplifier and we defined LVA as an amplitude less than 40 μv /26/. The recording was analyzed to determine the percent of the time spent in LVA and in HVA. We recorded continuously the cardiorespiratory variables and ECoG on a Devices MX6 polygraph (Devices Instruments Ltd. U.K.). Zero reference point for all pressures was atmospheric pressure at the midchest level of the lamb.

An axillary arterial blood sample (1 ml) was taken every 15 minutes to measure pH, carbon dioxide tension ($PaCO_2$), oxygen tension (PaO_2) and oxygen saturation in a blood gas analyzer (Corning 165 pH/Blood Gas Analyzer, U.K.). The samples were placed on ice immediately and were analyzed within 15 min after collection, values were corrected to 39°C. To measure plasma PGE_2 concentration, another axillary blood sample (5 ml) was taken every 15 minutes and collected in a cold, heparinized syringe, transferred to a cold test tube containing indomethacin (2 μg/ml), and centrifuged at 5°C. Plasma was collected and frozen at -25°C, plasma PGE_2 was later extracted with cyclohexane and ethyl acetate, purified over silicic acid columns, and assayed with specific antibody directed toward PGE_2 /7/. To account for possible losses during extraction /9/, all samples had an internal standard of ^3H-PGE_2. To replace blood withdrawn for measurements of pH, $PaCO_2$, PaO_2 and PGE_2 concentration, the lamb was transfused with 12 ml of maternal blood at the end of each 30 min period.

Infusion: each study lasted 3 hours, the studies were performed between 09.00 to 16.00 h. After a control period of 30 min, all 12 lambs were infused independently of the electrocortical state, with continuous and increasing doses of PGE_2 (Upjohn) through the carotid arterial catheter with the tip above the aortic valve. The doses given were 0.1, 0.5, 1.0 and 5.0 µg/kg/min, each dose was given for 30 min. The amount of PGE_2 needed for each study was dissolved in 100 ml of sodium chloride (9 g/l, pH = 6.84). Variables were also measured during a 30 min post-infusion period. As a control, 5 of these lambs were also infused, on a different day, with sodium chloride (9 g/l) for a similar 3 hour period. These experiments were done at room temperature (approx. 23°C). Plasma PGE_2 concentration was measured in 7 of the 12 lambs infused with PGE_2 and in 4 of the 5 lambs infused with saline.

Data analysis: for statistical analysis of each cardiorespiratory variable, we used the average of the last 3 minutes of each 30 min period for each animal. The reported values of pH and arterial blood gas tensions are the last samples taken during each 30 min period. The time spent in LVA and HVA expressed as percent of time for each of each 30 min period. Apneic events observed in each 30 min period were expressed as apneic events/hour. The plasma concentration of PGE_2 represented an average of the values obtained at 15 and 30 min of each period. Data from post-infusion period were compared to control data using Student's t-test for paired data.

Linear regression analysis was used to determine the relationship of the dose of PGE_2 infused (independent variable) with breathing rate, tidal volume, minute ventilation, arterial pH and blood gas tensions, heart rate, arterial blood pressure, percent of the time spent in LVA and plasma concentration of PGE_2 (dependent variables). For these analyses, the independent variable was transformed as the cube root of the dose, and control period was considered a dose "0". Chi square analysis was used to compare the association between incidence and duration of apnea with the dose of PGE_2 infused and the incidence of apnea during HVA and LVA. Linear regression analysis was also done between the plasma concentration of PGE_2 and the measured variables, where the observations were plotted independently of the period or dose infused. For the lambs infused with the vehicle alone, similar analyses were done using data from the corresponding 30 min periods of the experiments. Statistical analyses were done using the statistical programs "SYSTAT" with an IBM-PC computer and "STATVIEW 512+". with a MACINTOSH computer. The data are expressed as mean $\pm$ SE. A p value < 0.05 was considered significant.

RESULTS

In all 12 lambs, the infusions of PGE_2 had marked effects on the respiratory and the cardiovascular systems (Table I), the maximal effect of each dose occurred between 15 and 30 min after the start of the dose. Ventilation decreased progressively and significantly due to decreases in both the rate of breathing and tidal volume, this resulted in progressive respiratory acidosis and hypoxemia. Blood pressure and heart rate declined progressively throughout the infusions.

ECoG was recorded in 7 lambs. LVA decreased significantly during the infusion periods. The plasma concentration of PGE_2 was measured in 7 of the 12 lambs. During the control period, the concentration of PGE_2 was very low, and 4 of the measurements were below the assay range. In all 7 there was a significant, progressive increase in plasma PGE_2 concentration during the infusion.

By 30 min after the end of the infusion there was recovery of all the measured variables. The post-infusion values for ventilation, breathing rate, tidal volume, PaO_2, oxygen saturation and heart rate were similar to the levels observed during control period. The post-infusion values for pH, $PaCO_2$ and plasma PGE_2 concentration remained significantly different from the control values. The post-infusion value for time spent in LVA was significantly higher than during control.

Infusion of the vehicle caused no significant changes in the measured variables (Table II). In this group, the plasma concentration of PGE_2 was measured in 4 of the 5 lambs. No significant change was observed, 6 of the 24 measurements were below the assay range.

Infusion of progressively increasing doses of PGE_2 also caused a progressive increase in frequency and duration of apneic events (Table III). The most frequent type of apneic events was the shortest (3 to 6 sec), during most of the apneic events, associated hypotension and bradycardia occurred. During the infusion of PGE_2, the apneic events were observed during both LVA and HVA but they were twice as frequent during the time spent in LVA (Table IV). The presence of trembling and diarrhea was also noted during the infusions.

TABLE I

Effects of prostaglandin E_2 infusion on cardiorespiratory variables, incidence of low voltage electrocortical activity and plasma concentration of prostaglandin E_2 in 12 newborn lambs

	Dose of PGE_2 (μg/kg/min)						
	Control	0.1	0.5	1.0	5.0	Post-infusion	rx
Ventilation (ml/kg/min)	506±42	385±26	337±31	292±31	240±22	470±37	-0.64^{++}
Breathing rate (breath/min)	59±6	55±5	49±4	48±4	43±4	55±6	-0.31^{+}
Tidal volume (ml/kg)	9.0±0.6	7.3±0.5	6.8±0.4	6.2±0.4	5.7±0.5	8.7±0.5	-0.55^{++}
pH	7.41±0.01	7.37±0.02	7.35±0.02	7.32±0.02	7.27±0.02	7.35±0.02	-0.66^{++}
$PaCO_2$ (Torr)	47±2	53±3	54±3	60±3	66±4	53±2 q	$+0.54^{++}$
PaO_2 (Torr)	70±3	65±4	58±3	58±3	55±4	68±2	-0.40^{++}
O_2 Saturation (%)	94±1	90±3	86±3	85±3	79±3	92±1	-0.53^{++}
Heart rate (beat/min)	231±9	239±7	215±8	204±9	179±8	236±8	-0.56^{++}

Table I. cont.

Blood pressure							
(torr)	74±4	65±4	61±3	60±4	55±4	70±4 q	-0.44++
LV ECoG							
(% of the time) A	51±8	47±10	39±14	19±5	15±5	78±3 q	-0.54++
Plasma PGE_2							
(PG/ml) B	1.4±0.9	45.0±10.5	71.9±18.1	59.7±14.9	91.5±24.6	14.3±6.7 q	+0.57++

Values are means ± SE LV ECoG, low voltage electrocortical activity. PGE_2, prostaglandin E_2 post-infusion period includes values at 30 min after the end of infusion. A, N = 6 lambs; B, N = 7 lambs. rx, regression value; regression includes control period as dose "0" and it does not include post-infusion period.

\+ $p < 0.05$
++ $p < 0.005$ q $p < 0.05$ for control vs. post-infusion by student's t-test for paired data

TABLE II

Effects of sodium chloride (9 g/l) infusion on cardiorespiratory variables, incidence of low voltage electrocortical activity and plasma concentration of prostaglandin E_2 in 5 newborn lambs

	Duration of infusion (hours)						
	Control	0:30	1:00	1:30	2:00	Post-infusion	rx
Ventilation (ml/kg/min)	467±52	505±152	478±147	483±149	524±165	470±62	0.11
Breathing rate (breath/min)	53±13	52±12	51±13	52±13	56±15	52±5	0.09
Tidal volume (ml/kg)	9.0±0.7	9.7±1.6	9.4±1.7	9.3±1.4	9.4±2.0	9.1±0.8	0.06
pH	7.41±0.02	7.42±0.02	7.41±0.03	7.41±0.03	7.39±0.03	7.40±0.02	-0.27
$PaCO_2$ (torr)	46±5	45±4	46±4	46±6	47±6	47±2	0.08
PaO_2 (torr)	80±4	78±3	80±3	80±5	81±5	79±2	0.09
O_2 Saturation (%)	92±2	91±2	90±4	91±4	90±5	91±1	-0.16
Heart rate (beat/min)	230±9	234±12	239±27	228±26	238±21	224±10	0.10

Table II. cont.

Blood pressure (torr)	72±9	72±10	72±8	73±9	72±9	70±4	0.01
LV ECoG (% of the time) A	40±8	50±10	37±7	52±11	57±9	42±4	0.35
Plasma PGE_2 (pG/ml) B	1.8±0.6	2.2±0.6	1.5±0.4	1.3±0.3	5.9±0.7	3.9±0.7	0.30

Value are means ± SE. LV ECoG, low voltage electrocortical activity. PG, prostaglandin. Post-infusion period includes values at 30 min of the end of infusion. A, N = 3 lambs; B, N = 4 lambs. rx, regression value; regression includes control period as dose "0" and it does not include post-infusion period. None of these results was statistically significant.

TABLE III

Effects of prostaglandin E_2 infusion on incidence of apneic events in 12 newborn lambs

	Prostaglandin E_2 (μg/kg/min)					
Apneic events (sec)	Control	0.1	0.5	1.0	5.0	Total
3 - 6	6	21	146	202	241	616
7 - 10	0	1	22	21	65	109
10	0	0	2	4	12	18
Total	6	22	170	227	318	743

Data are apneic events/hour $x^2 = 22.9$, DF = 8, $p < 0.005$

TABLE IV

Total number of apneic events/hour observed during the different electrocortical stages in 6 newborn lambs infused with prostaglandin E_2

	Prostaglandin E_2 (μg/kg/min)					
Electrocortical stages	control	0.1	0.5	1.0	5.0	Total
High voltage activity	2	6	61	103	72	244
Low voltage activity	4	4	80	153	207	448
Total	6	10	141	256	279	692

Data are total number of apneic events/hour.
Pearson $x^2 = 20.34$, DF = 4, $p < 0.001$

Plasma PGE_2 concentration (42 observations in 7 lambs), plotted independently of the dose infused, showed a significant inverse correlation with ventilation, pH and blood pressure and a significant positive correlation with $PaCO_2$, although the actual data showed variability (Figures 1 and 2). Plasma PGE_2 concentration did not correlate significantly with heart rate (Fig.1), incidence of apneic events ($r = 0.08$, $y = 7 + 0.02x$), PaO_2 ($r = 0.20$, $y = 62 - 0.05x$, or oxygen saturation ($r = -0.10$, $y = 88 - 0.02x$).

In the 4 animals with both ECoG recording and measurements of PGE_2 concentrations, there was a significant inverse correlation of PGE_2 concentration with the incidence of LVA (Fig. 1).

DISCUSSION

Our results indicate that infusion of increasing doses of PGE_2 has marked inhibitory effects on ventilation in newborn lambs. PGE_2 induced a progressive decrease in ventilation (due to decreases in both breathing rate and tidal volume) and increases in the incidence and duration of apneic events. Although there have been no previous systematic studies on the effects of prostaglandins on ventilation in newborn swine /30/ and newborn human infants with cyanotic heart disease /21/, and PGE_1 decreases the output of the phrenic nerve in piglets that have been anesthetized, paralyzed and maintained with assisted ventilation /16/. The inhibitory effects of PGE_2 on ventilation in newborn animals and infants, in the present study and the others quoted above, are analogous to previous studies in fetal sheep where PGE_2 inhibited FBM (14,15,19,20,31). However, these results are in contrast to studies in adults in which E-type prostaglandins caused an increase in ventilation in dogs /28/, rats, cats /17/, guinea pigs /18/ and awake human males /4/. Thus, there appears to be a change in the effects of E-type prostaglandins on ventilation with maturation after birth. The

factors responsible for this change and the age at which it occurs are not known.

In the present study, the inhibitory effects of PGE_2 on ventilation correlated both with the dose infused and with the plasma concentration of PGE_2, these results are similar to those reported for fetal sheep /31/. Of concern is that the measured concentrations of PGE_2 in this study were only about 1/100 of the expected values, based on the amount of PGE_2 infused and an assumed cardiac output of 400 ml/kg/min /27/. The reasons for this discrepancy are not known. Possible explanations include conversion of PGE_2 to PGF_2 in sheep blood /3/, streaming of blood away from the axillary artery in the brachiocephalic trunk or loss of PGE_2 during its extraction and purification /9/.

The site at which PGE_2 exerts its respiratory effects in newborn lambs is not known. In fetal sheep, the site of action on FBM is in the central nervous sytem /32/, most likely in the pons or lower medulla /12,15,20/. Because PGE_2 has an inhibitory effect on breathing both in the fetus and the newborn, it seems reasonable to assume that the site of action is similar in both. However, there are some differences in the effects of PGE_2 on the respiratory system in the newborn compared to he fetus. In fetal sheep, PGE_2 infusion inhibits FBM but does not affect arterial pH or $PaCO_2$ /14,15,19,20), also, PGE_2 does not inhibit FBM stimulated by hypercarbia /15/. Conversely, in the present study in newborn lambs, PGE_2 decreased ventilation and caused hypercarbia and acidosis,

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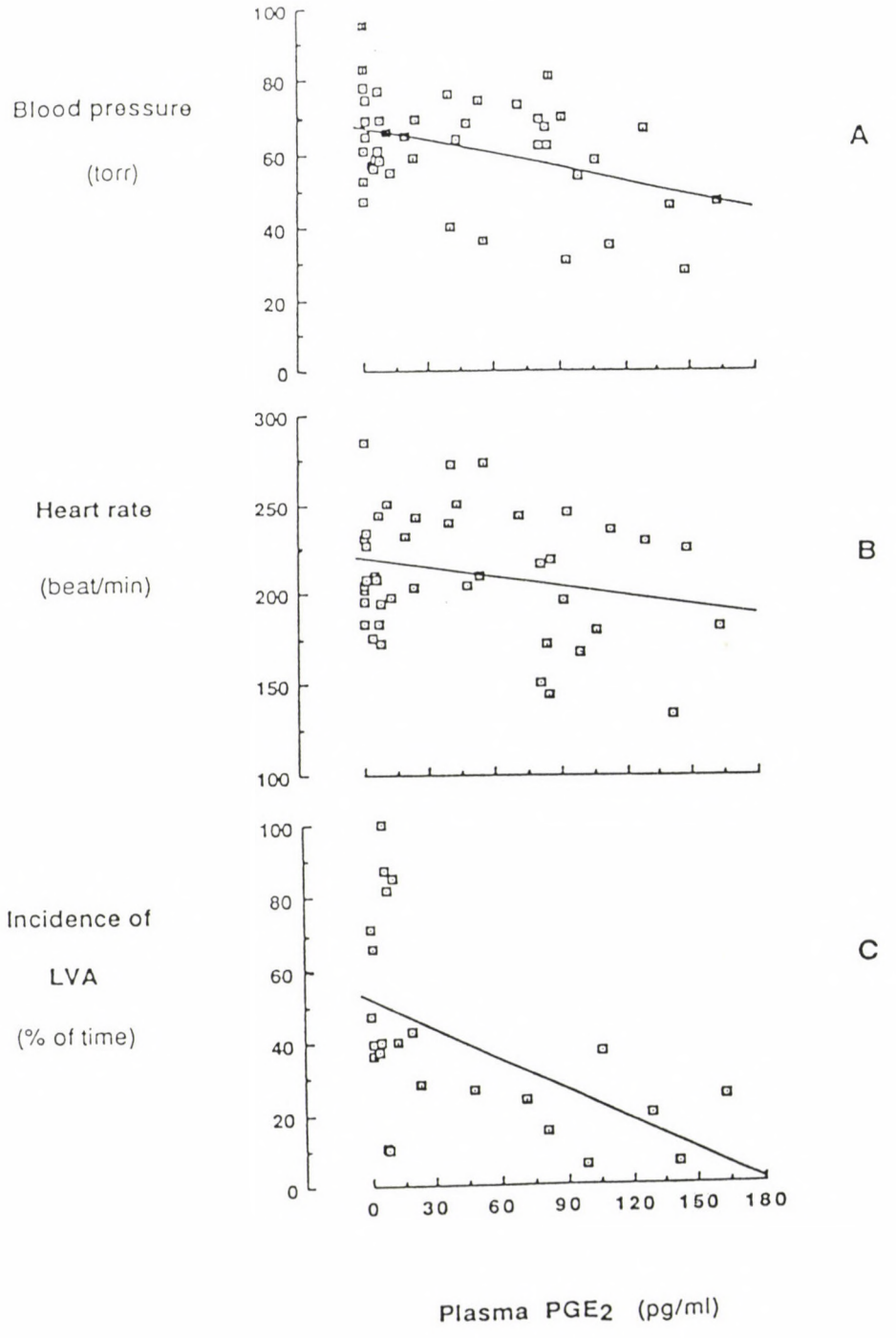
Fig. 1. Relationship of plasma concentration of prostaglandin (PG) E_2 with mean arterial blood pressure (A), and heart rate (B) in 4 lambs, and with the time spent in low voltage electrocortical activity (LVA; C) in lambs. PGE_2 concentration showed significant inverse correlations with blood pressure ($r = -0.41$; $y = 67 - 0.12x$; $p < 0.005$) and incidence of LVA ($r = -0.53$; $y = 52 - -0.28x$; $p < 0.001$), but did not correlate with heart rate ($r = -0.23$; $y = -212 - 0.16x$).


Blood pressure
(torr)
100
80
60
40
20
0
A
Heart rate
(beat/min)
300
250
200
150
100
B
Incidence of
LVA
(% of time)
100
80
60
40
20
0
C
0
30
60
90
120
150
180
Plasma PGE2 (pg/ml)

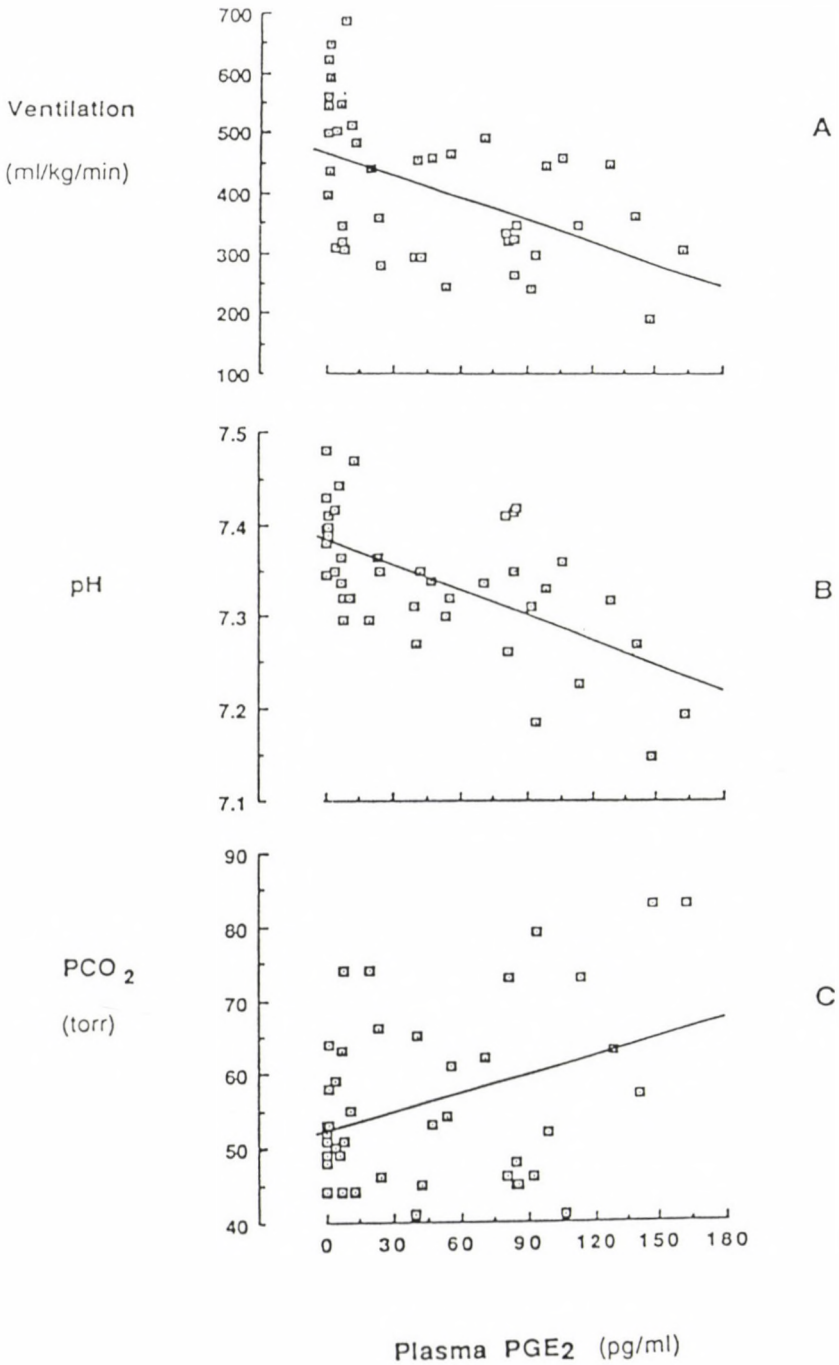
conditions which normally stimulate ventilation in the newborn /12,25/. Thus, PGE_2 appears to be a potent inhibitor of respiration in the newborn lamb and can overcome the effects of the strong physiological respiratory stimulants, hypercarbia, and acidosis. The mechanism by which PGE_2 affects the respiratory system is not known, although there are some data suggesting that PGE_2 may act as a neurotransmitter or modulate other neurotransmitters /24/.

An unexpected finding in the present study was the marked decrease in incidence of LVA during PGE_2 infusion (Table I), this occurred in association with hypoxemia, hypercarbia and acidosis. These findings differ from the effects of PGE_2, in fetal sheep in which PGE_2 causes only a slight decrease in incidence of LVA from 54 % to 47 % of the time, with no changes in arterial pH or blood gas tensions /20/. Furthermore, in fetal sheep, hypoxemia decreases /2/, hypercarbia increases /2,11/, and acidosis has no effect on the incidence of LVA /10/. The reasons why PGE_2 causes such a marked decrease in incidence of LVA in newborn lambs are not apparent. A possible explanation is that the decrease in LVA is due to a combined effect of PGE_2 and hypoxemia, both of which independently decrease LVA in fetal sheep /2,20/, and this effect is stronger than the tendency for hypercarbia to increase the incidence of LVA. Further studies are needed to resolve this issue.

In this study, PGE_2 caused progressive decreases in heart rate and blood pressure. These effects are similar to those reported by Olley et al /22/. They found that the infusion of

⟶

Fig. 2. Relationship of plasma concentration of prostaglandin (PG) E_2 with ventilation (A), arterial pH (B) and PCO_2 PCO_2 (C) in 7 lambs, PGE_2 concentration showed significant inverse correlations with ventilation ($r = -0.50$; $y = 466 - 1.2x$; $p < 0.005$) and pH ($r = -0.63$; $y = 7.39 - 0.001x$; $p < 0.001$), and a significant positive correlation with PCO_2 ($r=0.35$; $y = 52 + 0.08x$; $p < 0.025$).


Ventilation
(ml/kg/min)
700
600
500
400
300
200
100
A
pH
7.5
7.4
7.3
7.2
7.1
B
PCO 2
(torr)
90
80
70
60
50
40
C
0
30
60
90
120
150
180
Plasma PGE2 (pg/ml)

E-type prostaglandins caused a decrease in cardiac output in lambs which were anaesthetized and given assisted ventilation to maintain arterial pH and blood gas tensions in the normal range. These cardiovascular effects may be due to effects on the peripheral vessels, on the myocardium /22/, on the central nervous system /6/, or a combination of these. These findings are in contrast to studies in fetal sheep, in which the infusion of PGE_2 has no effect on heart rate or blood pressure /14/. These differences may be explained by the differences in the circulation of the fetus and the newborn /27/. It is unlikely that the respiratory changes observed during the infusion of PGE_2 were due to these cardiovascular changes. Sola et al reported that, in lambs spontaneously breathing room air, a withdrawal of 50 % of their blood volume caused hypotension, bradycardia, decreased myocardial blood flow and cerebral oxygen delivery, and metabolic acidosis /28/, but these animals were hypocarbic and normoxic, suggesting minute ventilation increased.

In summary, we conclude that infusion of PGE_2 has marked inhibitory effects on ventilation in newborn lambs. These findings taken in combination with previous fetal studies /12,14,15,19,20,31/ indicate that PGE_2 has depressant effects on the respiratory system both in the fetus and in the first few days after birth.

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Acta Paediatrica Hungarica 31 (3), pp 357-364 (1991)

ERYTHROCYTE DAMAGE IN NEWBORN BABIES CAUSED BY HYPERBILIRUBINAEMIA AND HYPOXIA

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Received 16 October 1990

Erythrocyte damage of newborn babies suffering from hyperbilirubinaemia and hypoxia was compared with a control group. In the cases of serum bilirubin level higher than physiological icterus lipid peroxidation of erythrocytes decreased probably due to the antioxidant effect of bilirubin. Moreover, an increase in potassium and protein outflow from patients' red blood cells was observed indicating a membrane damage both in hyperbilirubinaemic and hypoxic groups. Superoxide dismutase activity of serum and erythrocytes did not show significant difference in patients compared with healthy newborns. However, the low serum coeruloplasmin level in the hypoxic group and the low serum transferrin level of babies both with hypoxia and hyperbilirubinaemia suggest an insufficient antioxidant defence against free radicals.

INTRODUCTION

The role of hyperbilirubinaemia is a very thoroughly studied area of neonatology. However, we could find only a few data about the effect of bilirubin on erythrocytes /1,3,10/. During our previous study on the antioxidant system of premature babies /6/ arose the question: what is the influence of hyperbilirubinaemia and hypoxia on erythrocytes. In order to answer this question we measured lipid peroxidation (LPO), K^+ outflow as well as protein release in red blood cell suspension of healthy, hyperbilirubinaemic and hypoxic newborns. In addition, the level of three components of serum antioxidant system - superoxide dismutase, coeruloplasmin and transferrin - was investigated in all the three groups.

Akadémiai Kiadó, Budapest

MATERIALS AND METHODS

Patients

Twenty-five healthy newborns with birth weight 3378 ± 507 g (SD) and gest. age 38.8 ± 1.8 weeks (SD) were enrolled into the control group (I.). Birth weight of 30 hyperbilirubinaemic newborns (II.) was 1805 ± 655 g (SD), their gestational age was 31.8 ± 3.4 weeks (SD), and their serum bilirubin level (145-320 μm/l) was higher than that of physiological icterus.

The birth weight of 10 newborns (III.) - suffering from hypoxia - was 2010 ± 1244 g (SD), their gestational age was 33.2 ± 4.0 weeks. Their blood gas parameters during the last 24 hours before drawing blood were the following: oxygen saturation was below 85 %, pCO_2 was higher than 40 Hgmm (AVL 995 Automatic Blood Gas Analyser). All the patients received usual perinatal medical treatment in our perinatal intensive care unit.

Lipid peroxidation (LPO) in erythrocytes

Freshly drawn heparinised blood was washed three times with 10 volume of 0.15 M NaCl solution centrifuged at $0^{o}C$ for 10 min, with 2500 r.p.m. The parameters a.-c. (Table I) are referred to the hematocrit of this 3-times washed erythrocyte suspension. One ml from 10 % hemolysate of this suspension was left to stand for 2 hours at $0^{o}C$. After adding 1 ml of 15 % trichloroacetic acid (TCA) to haemolysate malon-dialdehyde - an end-product of lipid peroxidation - was measured /4,15/ with 0.5 ml 1 % thiobarbituric acid (in 0.05 M NaOH) at $100^{o}C$. After having determined the differences in absorbance between 532 and 600 nm, the MDA concentration was calculated using the molar extinction coefficient given by Sinnhuber and Yu /12/ as 1.56×10^{5}.

In vitro inhibition of lipid peroxidation (LPO) in erythrocytes

To the three times washed healthy erythrocyte suspension 25-250 μm/l bilirubin (Reanal) was added in 0.15 M NaCl solution (containing 20 % ethanol to gain better solubility). The mixture was left to stand for 1 hour at $0^{o}C$ and erythrocytes were centrifuged at $0^{o}C$ (10 min, 2500 r.p.m.). (The treatment of erythrocytes with 0.15 M NaCl - containing 20 % ethanol - did not cause any considerable inhibition effect in LPO). Measurement of LPO in these erythrocytes was carried out as described above.

Protein and K^{+}- outflow from erythrocytes

0.2 ml 0.15 M NaCl solution was added to 0.2 ml of three times washed erythrocyte suspension. The protein content of the supernatant was estimated at t=0 time and after 2 hours incubation at $0^{o}C$ according to Lowry's method /7,9/. Concentration of K^{+} in the same supernatant samples was determined with a Radelkis OP-266/1 type biological Alkali Microanalyser. Superoxide dismutase activity in serum and erythrocytes was determined with method described by Misra and Fridovich /8/.

Serum transferrin and coeruloplasmin level was measured with a Beckman II-type Immunochemistry Analyser.

TABLE I

Newborn babies	I. Healthy (25)	II. Hyperbilirubinaemic (30)	III. Hypoxic (10)
Birth weight (g)	3378 ± 507 (SD)	1805 ± 655 (SD)	2010 ± 1244 (SD)
gest. age (week)	38.8 ± 1.8 (SD)	31.8 ± 3.4 (SD)	33.2 ± 4.0 (SD)
a. LPO in erythrocytes (μM/l)	1.99 ± 0.26	0.91 ± 0.33	1.61 ± 0.31 (n.s.)
b. Protein outflow from erythrocytes (mg/ml)	0.06 ± 0.02	0.132 ± 0.022	0.138 ± 0.04
c. K^+ outflow from erythrocytes (mM/l)	0.81 ± 0.12	1.13 ± 0.17	2.7 ± 0.70
Serum antioxidants:			
coeruloplasmin in se. (mg/l)	180 - 450	273 ± 125 (n.s.)	133.8 ± 67.3
transferrin in se. (mg/l)	2040 - 3600	1996 ± 655	2114 ± 574

n.s. no significant difference
LPO lipid peroxidation

RESULTS

To determine the effect of hyperbilirubinaemia and hypoxia on erythrocytes of newborns we investigated the following parameters in the case of 25 healthy (group I), 30 hyperbilirubinaemic (group II) and 10 hypoxic newborn babies (group III) (Table I).

a.) The amount of thiobarbituric acid reactive substances, which formed from washed and incubated erythrocytes during lipid peroxidation, was significantly lower in hyperbilirubinaemic newborns (0.91 $\pm$ 0.33 μM/l) than in the healthy group (1.99 $\pm$ 0.26 μM/l) ($p < 0.001$ with unpaired t-test). In the case of hypoxic newborns this value (1.61 $\pm$ 0.31 uM/l) did not show a significant difference in comparison with the healthy group (Table I).

In vitro incubation of normal erythrocytes with different amount of exogenous bilirubin resulted in dose dependent decreasing in lipid peroxidation (LPO). Thus, a 250 μM/l of exogenous bilirubin solution was able to reduce LPO with 30 % (Fig.1).

b.) Protein outflow from the washed erythrocyte in vitro proved to be significantly higher with 0.132 $\pm$ 0.022 mg/ml in hyperbilirubinaemic group (II) than in the healthy group (I) with 0.06 $\pm$ 0.02 mg/ml ($p < 0.05$ with unpaired t-test, and $p < 0.01$ with Mann-Whitney's test). This parameter was significantly higher in the case of hypoxic newborns (III). with 0.138 $\pm$ 0.04 mg/ml.

c.) Potassium ion outflow changed similarly to protein outflow. K^+ outflow was higher (1.13 $\pm$ 0.17 mM/l) in the case of hyperbilirubinaemia (II) than in the healthy group (I) with 0.81 $\pm$ 0.12 mM/l ($p < 0.01$ with Mann-Whitney's test, and $p < 0.01$ with Student's t-test referring to log of the mean values of K^+ outflow). In the hypoxic group (III.) this value was considerably higher (2.7 $\pm$ 0.70 mM/l) comparing with the healthy group (I).

d.) Superoxide dismutase activity in the serum and erythrocytes of newborn babies did not show significant difference in the I. II. and III. groups.

e.) Serum coeruloplasmin level in the hyperbilirubinaemic group (273 ± 125 mg/l) did not show significant difference related to normal range (180-450 mg/l). Serum coeruloplasmin level was significantly lower (133.8 ± 67.3 mg/l) in the case of hypoxic babies (III.) on the 4th day of life ($p < 0.001$). A wide range of coeruloplasmin reference values can be found in the literature /2,11/.

f.) Serum transferrin level was significantly lower in the hyperbilirubinaemic group(1996 ± 655 mg/l) as related to the normal range: 2040-3600 mg/l ($p < 0.01$). Similarly, in the hypoxic group (III.) this value was significantly low, 2114 ± 574 mg/l on the 4th day of life ($p < 0.01$ calculated with one sample t-test).

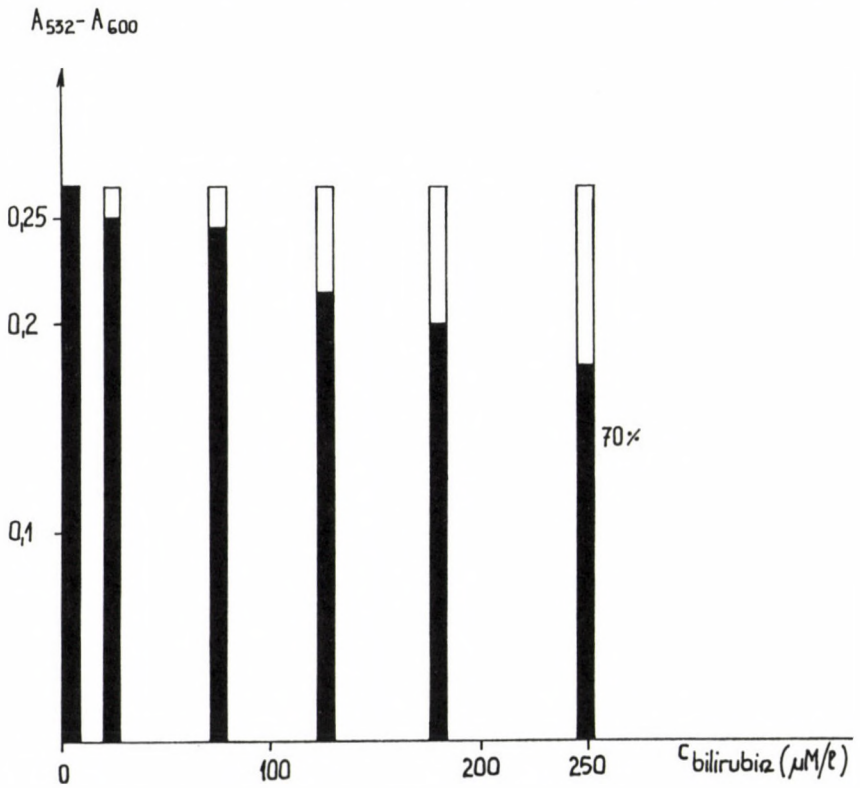

Fig. 1. Dose-dependent lipid peroxidation in erythrocytes of healthy newborn babies following 1 h in vitro incubation with exogenous bilirubin at 0°C. (Mean of three determinations)
$A_{532} - A_{600}$: absorbance of thiobarbituric acid reactive substances

DISCUSSION

In vivo and *in vitro* decrease in erythrocyte lipid peroxidation of newborn seems to support the theory - well-known in literature /14/ - that bilirubin possesses a remarkable antioxidant property beside its cytotoxic effect. The base of antioxidant effect is that bilirubin can donate its H atom attached to the C-10 of the tetrapyrrole ring, forming the resonance stable bilirubin radical. This radical is able to react with oxygen as well as alkyl-peroxyl radicals resulting in nonradical products. In this way bilirubin, as an effective free radical scavenger reduces the chain-breaking of unsaturated lipids in the membrane. This can be the reason for decreasing erythrocytes membrane lipid peroxidation in the hyperbilirubinaemic newborns as well as *in vitro* test.

Probably other erythrocyte surface membrane compounds (e.g. proteins, glycoproteins) are more sensitive to free radicals than lipids. We suppose that membrane damage initiated by free radicals and some interaction between bilirubin and erythrocytes' cell surface ingredients resulted in an increased degree of protein and K^+ outflow from erythrocytes. On the basis of our investigation (using the method of E.J. van Kampen /5/) a considerable portion of the released proteins proved to be haemoglobin fragments. This protein and K^+ release might follow an erythrocyte membrane damage in hyperbilirubinaemic and in hypoxic group, too.

Our present and previous study being in accordance with the results of Sullivan /13/ showed that in case of newborn babies, particularly in prematures the low level of serum coeruloplasmin and transferrin cannot protect erythrocytes effectively against free radical attack.

ACKNOWLEDGEMENT

The authors thank Dr. S. Imre, Dr. Cs. Kiss, Dr. M. Mikita, Mrs. M. Szabó and Mrs. Czapp for cooperation.

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Acta Paediatrica Hungarica 31 (3), pp 365-377 (1991)

AN AETIOLOGICAL STUDY ON 6 TO 14 YEARS-OLD CHILDREN WITH SEVERE VISUAL HANDICAP IN HUNGARY

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Received 5 October 1990

A population-based aetiological study was carried out on 6 to 14 years-old severely visually handicapped children in Hungary. Of the 547 recorded cases 491 (90 %) were included in the analysis. Eleven aetiological groups were separated: isolated cataracts (16.7 %), congenital abnormalities of the eye (15.1 %), high myopia ± retinal detachment and other cases (13.4 %), retinopathy of praemature (11.0 %), choroidoretinal degenerations (10.0 %), syndromes (9.6 %), nystagmus and/or hypermetropia (9.0 %), isolated and complicated optic atrophy (6.7 %), postnatal causes (4.9 %), retinoblastoma (1.8 %), praenatal causes (1.8 %). A significantly higher rate of previous induced abortions was found in the group of retinopathy of praemature. Perinatal damage syndrome and Mendelian monogenic defects are the two most common aetiological categories in the origin of severe visual handicaps in Hungary.

INTRODUCTION

In Hungary the recorded prevalence of primary school age (6-14) children with severe isolated visual handcap shows a nearly permanent rate about 0.42 per 1000 (0.40 and 0.45 between 1974 and 1987). However, this figure may be an underestimate of the true prevalence for two reasons: (i) it is lower than the well-established rates (0.5-1.3 per 1000) in industrialised countries /8, 9/ and (ii) the territorial distribution of prevalences shows significant differences /4/. The two highest rates were recorded in Hajdu-Bihar county (0.79 per 1000) and Budapest (0.58 per 1000) where the three institutions of these handicapped children exist while the lowest rate of 0.12 was in

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three territorial units. The difference between the highest and the lowest rate is 6.6-fold. The higher figures may reflect the true prevalence, however, lower figures can be explained mainly by the lack of children with low vision.

The present epidemiological study was undertaken to examine the aetiological factors in severe visual handicaps and to compare, to the extent possible, the results with those of Fraser and Friedmann /5/ in the U.K. The severe visual handicap includes blindness (categories 3,4 and 5 of the WHO /10/) and low vision (categories of 1 and 2 of the WHO /10/). The study samples comprised children from the three educational institutions in which mainly "isolated" visual handicap occurs. Thus, those with multihandicaps, e.g., the combinations of mental retardation and visual handicap were excluded.

MATERIALS AND METHODS

Of 547 recorded 6-14 year-old cases, 491 (89.8 %) were included in the study. The reasons for the exclusions of 56 cases were (i) appropriate ophthalmological data were not available in 33 cases of the Institute of Blind Children, Budapest; (ii) the ophthalmological examination could not be performed due to the lack of cooperation of cases and anesthesia was not allowed (8 cases); (iii) lack of parental permission (2 cases) and (iv) acute illness during the study period (13 cases).

Following initial visits to the institutions, the available personal, medical (mainly ophthalmological) and educational data were obtained and recorded in the individual files of index cases. The technical facilities for the study were then organized. Invitation letters were sent to the parents of cases requesting them to give permission for the examination of cases, to take part personally in the study with the sibs of cases and to bring all available medical documents of cases to us.

In the second stage, cases with low vision from two special schools were examined:

1. Complete ophthalmological examination (E.T.) involving all parts of the eye. Unfortunately, the director of the Institute of Blind Children did not allow the ophthalmological examination within the study. Thus, for these cases only data made available to us were used. It explains some obvious difference in the aetiological categories (Table I).
2. Paediatric examinations (J.K.). There were two main purposes: (i) to separate children with suspected or confirmed syndromes, i.e., multimalformed children with extraocular congenital anomalies; the so-called complicated cases where

TABLE I

The main aetiological groups of severe visual handicaps in the Hungarian and English /5/ study samples

Main aetiological groups	School for children with low vision, Budapest		School for children with low vision, Debrecen		Institute of Blind Children, Budapest		Hungarian cases together		English cases	
	No.	%	No.	%	No.	%	No.	%	No.	%
Choroido-retinal degenerations	26	15.4	19	14.4	4	2.1	49	10.0	116	15.0
Retinoblastoma	0	0.0	0	0.0	9	4.7	9	1.8	43	5.5
Optic atrophy	11	6.5	10	7.6	12	6.3	33	6.7	56	7.2
High myopia ± retinal detachment, etc.	23	13.6	34	25.7	9	4.7	66	13.4	47	6.1
Cataract	33	19.5	21	15.9	28	14.7	82	16.7	107	13.8
CAs of eye	21	12.4	17	12.9	36	18.9	74	15.1	94	12.1
Syndromes	22	13.0	12	9.1	13	6.9	47	9.6.	42	5.4
Nystagmus and/or hypermetropia	17	10.1	11	8.3	16	8.4	44	9.0	-	-
Praenatal agents	1	0.6	2	1.5	6	3.2	9	1.8	17	2.2
Retinopathy of premature	8	4.7	3	2.3	43	22.6	54	11.0	177	22.8
Postnatal causes	7	4.2	3	2.3	14	7.4	24	4.9	77	9.9
Total	169	100.0	132	100.0	190	100.0	491	100.0	776	100.0

ocular defect associated with the symptoms of central nervous system and "isolated" ocular cases; (ii) to refer cases for further necessary special medical (e.g., neurological) or laboratory examinations.

3. Oto-laryngeal and speech examination (G.Sz.) because eye disorders may associate with partial deafness.

4. Anthropometric examinations (M.V.). Body weight, height, head circumference, head maximum length and wideness, cranial index, face length and width, facial index were measured and calculated. (These data are not included in the present paper.)

5. Laboratory examinations. Urine test for aminoacidurias and kidney function, and special examinations (e.g., chromosome) in selected cases.

Participating parents and sibs were also examined by the ophthalmologist. The data of epidemiological questionnaire were obtained through personal interview by social workers and parents were asked to fill in a sociological data sheet.

Further necessary data were obtained by correspondance. In data analysis, 11 aetiological groups were distinguished (L.G.D.).

RESULTS

Overall findings

The percentages of cases with blindness and low vision were 61.3 and 38.7 %, respectively. There is a general male preponderance in the study sample but the sex ratio differs in aetiological groups (Table II). The proportions of low and very low birth weight and preterm births indicate an important role in the origin of some aetiological groups (Table III). The analysis of maternal and paternal age, furthermore, the socioeconomic status of parents did not show significant deviation from the data of population at large. The rate of consanguinity (first cousin) in the parents of cases was 1.9 %.

Aetiological aspects

The distribution of 11 aetiological groups in three institutions is shown in Table I.

The criteria for choroido-retinal degenerations were (i) characteristic ophthalmological findings, e.g., limited peripheral pigmentary change, confirmed to slight "dappling" with limited or no macular change in the type 1 of retinal aplasia /5, 7/, (ii) family history and (iii) case history including the onset of disorder. The proportion of this

TABLE II

Sex ratio and proportion of familial cases and affected sibs

Main aetiological groups	Sex		Sex ratio	Familial (F)	Non-familial (NF)	Ratio (NF/F)	Sibs		Segregation (A/A+N)
	Male	Female					Normal (N)	Affected (A)	
Choroido-retinal degenerations	25	24	0.510	13	36	2.8	49	12	0.20
Retinoblastoma	3	6	0.333	1	8	8.0	3	0	-
Optic atrophy	17	16	0.515	11	22	2.0	24	1	0.04
High myopia ± retinal detachment, etc.	38	28	0.576	21	45	2.1	70	15	0.18
Cataract	45	37	0.549	31	51	1.6	72	9	0.11
CAs of eye	33	41	0.446	13	61	4.7	46	6	0.12
Syndromes	28	19	0.596	4	43	10.8	49	6	0.11
Nystagmus and/or hypermetropia	26	18	0.591	8	36	4.5	51	4	0.07
Praenatal causes	4	5	0.444	0	9	-	15	0	-
Retinopathy of premature	28	26	0.519	4	50	12.5	46	3	0.07
Postnatal causes	15	9	0.625	2	22	11.0	17	0	-
Total	262	229	0.534	108	383	3.5	442	56	0.13

TABLE III

Mean birth weight and gestational week, the rate of low and very low birth, and preterm birth

Main aetiological groups	Average birth weight (g)	<2500 g %	<1500 g %	Average gestation time (week)	<37 week %
Choroido-retinal degenerations	3003	19.2	1.9	39.7	11.5
Retinoblastoma	3350	0.0	0.0	40.2	0.0
Optic atrophy	3076	13.6	0.0	40.2	0.0
High myopia ± retinal detachment, etc.	2718	32.1	13.2	38.2	28.3
Cataract	2821	25.4	6.8	39.2	13.6
CAs of eye	2952	19.1	6.4	39.7	17.8
Syndromes	3153	5.9	2.9	40.2	8.8
Nystagmus and/or hypermetropia	3176	17.6	5.9	40.3	11.8
Praenatal causes	2530	14.3	14.3	39.1	14.3
Retinopathy of premature	1275	94.4	83.8	31.1	89.2
Postnatal causes	3390	0.0	0.0	39.9	0.0
Total	2783	27.3	14.1	38.8	21.8

aetiological group was 10 % however, it is worth stressing here that the majority of cases were diagnosed by the ophthalmological examination within study in the two schools for children with low vision (Table I). The distribution of different types was as follows: retinal aplasia (type I-III) 30; macular lesions ± peripheral involvement, e.g., Stargardt's juvenile macular dystrophy, "central" retinitis pigmentosa, retinitis pigmentosa with macular dystrophy 8; choroideremia 1; choroido-retinal degeneration of uncertain classification 10 cases.

Retinoblastoma was diagnosed in 9 cases, only one was familial.

Optic atrophy is a heterogeneous group; here cases affected with isolated, complicated and perinatal optic atrophy were evaluated. Postnatal and syndromatic cases are classified into other groups. Of 33 cases, 11 isolated familial cases had autosomal dominant origin with a male preponderance.

High myopia ± retinal detachment and other high myopia is again a heterogeneous group. The uncomplicated myopia rarely causes sufficient visual handicap in childhood to necessitate certification as blindness or low vision. About one-third of cases affected with high myopia ± retinal detachment might be the consequence of perinatal damage syndrome. The family history indicated autosomal dominant origin in another one-third. However, the retinal detachment may not occur in all affected members of these families indicating a variable expressivity. Congenital and infantile retinal detachment was diagnosed in 3 cases, pseudoglioma in 2 cases and retinoschisis in 1 case.

The group of cataract includes visual defects of heterogeneous origin, here the isolated cases are evaluated. Complex cases are classified into the congenital abnormality (CA) group while some other cases into the syndrome and praenatal groups, respectively. Of 82 cases, the family history indicated an autosomal dominant origin in 31 cases. An important category was the cataract as a symptom of perinatal damage syndrome including about one-quarter of cases. It explains the low mean birth weight and shorter gestational time

(Table III).

CAs of the eye were diagnosed in 74 cases. Isolated, complex and secondary cases were separated (Table IV). The family history indicated obvious genetic origin only in 13 cases. Buphthalmos of autosomal recessive origin occurred in 6 sibs of cases with healthy parents.

The following syndromes were identified: Marfan 13, different types of oculocutaneus albinism 10 including one Hermansky-Pudlak, neuronal ceroid lipofuscinosis 2, Bardet-Biedl 2, Coats 2, Gillespie (oculo-dento-digital) 2, Lowue 1, Loken-Senior 1, Knobloch 1, galactosemia 1 case. A syndrome could not be identified in 12 multimalformed cases.

Cases affected with nystagmus and/or hypermetropia were classified into one group. Of 44 cases, 23, 8 and 12 had congenital nystagmus, congenital nystagmus and hypermetropia, and hypermetropia, respectively.

Praenatal causes were confirmed only in 9 cases. Congenital rubella syndrome was diagnosed in 3 cases. The component CAs of these cases were (i) cataract, patent ductus arteriosus, microcephaly, (ii) cataract-microphthalmos and partial deafness with seroconversion during pregnancy, (iii) cataract-microphthalmos, patent ductus arteriosus, partial deafness with seroconversion during pregnancy. This praenatal cause was suspected in more cases, however, these did not fit the diagnostic criteria. Congenital toxoplasmosis was diagnosed in 6 cases, mainly on the basis of characteristic ophthalmological finding. The consequence of lues and gonorrhoea were not detected. The role of other teratogenic factors (physical, chemical, occupational, etc) could not be confirmed though all kinds were studied.

One of the most important aetiological groups is retinopathy of premature. (The classification of Fraser and Friedmann /5/ used term retrolental fibroplasia.) These cases do not include other ocular consequences of perinatal damage syndrome (e.g., high myopia $\pm$ retinal detachment, cataract, optic atrophy, buphthalmos). Of 54 cases, birth weight did not exceed 2500 gram in 51 cases (94.4 %) and 1500 gram in 45 cases (83.3 %). The mean birth weight was 1275 gram while the mean gestation

TABLE IV

Distribution of isolated, complex and secondary CA-s of the eye

CA-groups	No.	%	%*
Isolated			
An-microphthalmos	7	9.5	1.4
Buphthalmos	18	24.3	3.7
Ectopic lentis	2	2.7	0.4
Aniridia	2	2.7	0.4
Coloboma	8	10.8	1.6
Cong. corneal distrophy	1	1.4	0.2
Microcornea	1	1.4	0.2
Optic nerve hypoplasia	1	1.4	0.2
Cong. ptosis	3	4.1	0.6
Subtotal	43	58.1	8.8
Complex CAs			
Cataract and microphthalmos	11	14.9	2.2
Cataract and aniridia	5	6.8	1.0
Cataract and coloboma	2	2.7	0.4
Cataract and myopia	4	5.4	0.8
Cataract and ectopia lentis	1	1.4	0.2
Cataract and microcornea	2	2.7	0.4
Microphthalmos and coloboma	3	4.1	0.6
Coloboma and myopia	1	1.4	0.2
Subtotal	29	39.2	5.9
Secondary CAs			
Hydrocephaly	2	2.7	0.4
Total	74	100.0	15.1

*Calculated for the total study sample

time was 31.1 weeks. Thus, the proportion of preterm babies was 89 %. In agreement with these findings, the rate of twins was 13 % instead of expected 2 % based on the Hungarian population figure. Our study showed three interesting correlations.(i) Retinopathy of premature were connected relatively frequently with "CA-association of low birth weight" /3/. (ii) Of 54 cases, 3 had affected sibs. This "familial" cluster may indicate an aetiological role of permanent maternal factors. It is important to know them in order to prevent sib-occurrences. (iii) There was a significantly higher rate of induced abortion in the previous pregnancies of mothers in this aetiological group ($X^2_1 = 4.33$; $p<0.05$) Table V). Another significantly higher rate of induced abortion in previous pregnancies in the group of retinoblastoma was explained by two induced abortions in the familial case. The higher induced abortion rate is a well-known phenomenon after the affected child.

The majority of cases with postnatal origin had optic atrophy. Their aetiological types were: trauma 8, infections 3, uveitis 8, keratitis 1, tumours 4.

DISCUSSION

This first Hungarian population-based epidemiological study designed to detect causes, faced some technical difficulties. (i) The majority of ophthalmologists are satisfied with clinical diagnosis and make no effort to achieve a nosological-aetiological one. (ii) The ophthalmological examination within the study was not allowed in the cases of the Institute of Blind Children (38.7 %), thus only the available ophthalmological data could be evaluated in these cases. (iii) At the time of school age it is difficult to diagnose the primary pathological process due to the progression of the condition and/or medical treatment (e.g., surgery).
However, in general, available previous medical documents helped us to establish the diagnosis of primary disease.

A comparison between the distribution of aetiological groups in the English and Hungarian studies shows a similarity in the

TABLE V

The number of previous and subsequent pregnancy outcomes in the mothers of cases and the proportion of induced abortions

Main aetiological groups	No. of previous pregnancies	Induced abortion		No. of subsequent pregnancies	Induced abortion	
		No.	%		No.	%
Choroido-retinal degenerations	43	10	23.3	43	10	23.3
Retinoblastoma	11	4	36.4	3	2	66.7
Uptic atrophy	21	5	23.8	27	9	33.3
High myopia ± retinal detachment, etc.	63	14	22.2	43	12	27.9
Cataract	33	22	26.5	67	21	31.3
CAs of eyes	53	15	28.3	39	11	28.2
Syndromes	45	7	15.6	33	12	36.4
Nystagmus and/or hypermetropia	63	14	22.2	52	12	23.1
Praenatal causes	15	1	6.7	2	0	0.0
Retinopathy of praemature	95	34	35.8	44	20	45.5
Postnatal causes	15	2	13.3	16	8	50.0
Total	507	128	25.2	369	117	31.7

group of praenatal causes, optic atrophy and choroidoretinal degeneration (in the latter if one excludes cases of the Institute of Blind Children) (Table I). The differences are small in the groups of cataract and CAs of the eye. The deviation in other groups can be explained mainly by the differences of diagnostic criteria and classification.

The purpose of this study was the delineation of causes in the origin of Hungarian childhood visual handicaps. In general, our findings confirm those available in the literature on the aetiology of blindness and low vision in childhood. To our knowledge, our study was the first one which detected the role of previous induced abortion in the origin of retinopathy of praemature. In Hungary the classical D+C method was used for the termination of pregnancy and it may cause cervical insufficiency /1/. The latter may mediate the preterm birth with low birth weight causing a predisposition for this cause of visual handicap. The second case of Knobloch and Layer /6/ syndrome was found in the study sample.

Obviously the two most common general aetiological categories are the perinatal damage syndrome and the monogenic Mendelian inheritance. The former category involved retinopathy of praemature, several cases with high myopia $\pm$ retinal detachment, cataract, optic atrophy, buphthalmos. The existence of latter category could be demonstrated by the pattern of familial cases and the consanguinity. The 1.9 % occurrence of cousin parents is 6-times higher than the general Hungarian figure /2/. Chromosome aberrations were not detected in these children affected with severe isolated visual handicap. The knowledge of causes may help us in the prevention of severe visual handicaps in the future.

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Acta Paediatrica Hungarica 31 (3), pp 379-395 (1991)

IN VITRO EFFECT OF ANTITUMOR DRUGS ON LYMPHOCYTIC BLASTOGENESIS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND NON-HODGKIN'S LYMPHOMA (NHL)

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Received 28 May 1990

"In vitro" sensitivity of lectin (PHA, Con A)-stimulated lymphocytes to antitumor drugs (ARA-C, ADR, VM_{26}, MTX, CP, VCR, Vepesid, ACLA) and the clinical efficiency of the complex therapy was compared in 7 patients with ALL and 2 patients with NHL. H^3-thymidin incorporation of lymphocytes labelled prior to the drug exposure was used as "in vitro" method. A fairly good correlation was found between the "in vitro" test and the clinical response to the drug administered. These results suggest that this "in vitro" test is useful in choosing the drugs to be administered in case of malignancies of children.

INTRODUCTION

In the last 20 years several institutions and researchers have developed new treatment protocols for childhood lymphomas. The best results have been achieved with patients suffering from stage I and II disease /34/. Curability for these early stages is in the range of 90%, except when immunosupression develops due to complications of chemotherapy or an onset of an unrelated disease, which can cause death. In selecting the optimal individual drug combination with special care to avoiding drug resistancy during relapse, a determination of drug sensitivity in vitro would be helpful.

Morphological, cytogenetic /14, 17/ and immunological /7, 11, 26/ findings urged us to develop a new classification and therapeutic strategies /13/ for childhood malignancies. This approach was promoted by widening the methodologies using in vitro human cell cultures /18, 37, 38/. However, the

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antineoplastic effects of cytostatic drugs are not the same "in vitro", as "in vivo" /37/, therefore this technique has certain limitations to the preclinical screening of chemotherapeutics. The valuation of their antiproliferative effect on the lectin [photohaemagglutinine (PHA) and concavalin-A (con-A)] - stimulated cells /6, 9, 10, 15, 23, 35/ during the established treatment regimen has some advantage in a modified approach of treatment with regard to the patient's individual situation and drug sensitivity.

When the lectin-binding ability (response to PHA and con-A) of leukemic lymphocytes, which may play an important part in changing the surface receptors, is altered it can also influence the humoral and cellular immune-responses. The multiplication of tumor cells depends on the lectin-binding spots and the success of therapy may be influenced by the behavior of blastic or normal (intact) lymphocytes. This method can obviously not cover all aspects of cellular responses. Only local reactions i.e. drug-cell interactions are determined independent of the host, giving some important information about the effect of drugs on the cells' proliferative response. This kind of approach in selecting the optimum treatment may allow for avoiding the cumulative toxic effect of drugs in effective on the growthcontrol of the malignant cell-proliferation. In our former study we found that the incidence of infections in ALL patients increased in the case of lower helper and Pan-T cell levels during the consolidation and maintenance stage of the therapy /2/.

In this paper we have measured the 3H-thymidine incorporation rate after the lectin (PHA and conA) stimulatory effect on proliferating lymphocytes in childhood malignancies of the lymphoid system (ALL, NHL), in the presence and absence of different chemotherapeutic agents, especially before the stage of attempting to treat relapses /8/.

MATERIALS AND METHODS

Whole blood was obtained by venipuncture into heparinized tubes (0.01 ml heparin/10 ml syringes). The blood was then diluted with Hank's balanced solution to a ratio of 3:1. The lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation /4/. At first, the donor sera was heat inactivated and filtered, prior to use in self-culture at a concentration of 30%. Parallel to the self-cultured samples we used fetal calf serum (GIBCO) to optimise culture conditions. After establishing the clinical status, the surface antigen phenotype of the lymphocytes in the separated cells was determined by monoclonal antibodies (Behring) /26/, and then a specimen was prepared by the modified Matutes' method /20/ for electron-microscopic investigation.

To stimulate blastogenesis of T-lymphocytes, phyto-hemagglutinin-M (PHA) was used in each culture [0.03 ml/10 ml RPMI-1640 medium (GIBCO)], without antibiotics. The lymphocyte suspension was adjusted to one million cells/ml of medium. Cultures from each sample were divided into subgroups:

- Controls, 2 tubes containing RPMI-1640 medium supplemented with 30% fetal calf serum and no PHA,
- PHA stimulated groups, cells cultured in the presence of PHA (0.025 ml/ml) dissolved in RPMI-1640 medium,
- PHA (0.025 ml/ml RPMI-1640 + different cytostatics agents; detailed protocol see below.
- Concavalin-A (Con-A) group 2 tubes containing Con-A/100 µg/ml dissolved in RPMI medium /6, 10, 32, 36/.
- The experiments were repeated in two parallels with doses of cytostatic agents included in treatment protocol, the doses were calculated to body surface and serum volume, and adjusted in a volume of 100 µl. Controls were applied in the same way.

The cells were incubated for 42 hours at 37°C in humidified CO_2 (7.5% in air), using a LABOR-MIM incubator (Hungary).

In the final 6-12 hours of incubation, 3H-thymidine (Amersham), diluted to a final concentration of 1 µCi/ml, was added to each culture. Then cultivation was stopped by adding 0.1 M cold citric acid. Thereafter 50 µl of the cell suspension was pipetted into 5 ml of the scintillation cocktail and radioactivity was assayed by scintillation counting (Hewlett-Packard) to determine (3H)-thymidine incorporation /33/. The cell suspension treated with the cytostatic agents were incubated for 60 minutes in a CO_2 incubator, then the cells in each tube were washed twice in Hank's buffer solution resuspended in a mixture of 0.7 ml of PHA and 0.3 ml of fetal calf serum Phylaxia, Hungary, and placed back in the incubator for 36 h. Then the above-described procedure was performed. The cells were fixed with a mixture of methanol-acetic acid-formaldehyde and centrifuged with 600 g. 50 µl of fixed cell nuclei, then they were air dried to the surface of a slide where the scintillation value for a single cell was calculated with the help of the cell count measured in a Buerker chamber to 50 µl. Labelling indexes were obtained on the basis of the measurements of radioactivity in the DNA after treatment with

various drugs and were calculated for a single cell, according to the next formulas:

In group one: $\frac{\text{(contr.)cpm}}{\text{(PHA)cpm}}$

In group two: $\frac{\text{(contr.) cpm}}{\text{(Con-A)cpm}}$

In group three: $\frac{\text{(Con-A)cpm}}{\text{(PHA)cpm}}$

and in the groups of investigated $\frac{\text{(PHA+Cytostatic agent*)cpm}}{\text{(PHA)cpm}}$

Patients

The study was carried out on five children with newly diagnosed malignancy and four children with relapse before restarting therapy. The patient's data were compared to those of healthy children of the same age (Table I). During the study the patients were referred to by codes. Some major features of the history were presented to facilitate the evaluation of the results obtained.

Case Nº B_3: The histopathological investigation of the right axillary lymph node confirmed a lymphoblastic lymphoma at clinical stage I in 1988, consequently NHL stage I. treatment was applied. Remission ensued, at present maintenance treatment is being performed.

Case Nº B_4: An enlarged right lateral jugular lymph node was observed in 1988. Toxoplasmosis was suspected and corresponding treatment was performed, however, the node enlarged. Histopathology revealed lymphoblastic lymphoma. As there were also alterations detectable in the kidneys and a proliferation of cells in the CSF, the child was classified as a stage III patient and treated according to the LSA_2L_2 protocol. Treatment was well tolerated, at present the child is symptom-free and maintenance therapy is being pursued.

Case Nº B_9: The high malignancy ALL patient (girl, time of diagnosis: 1987.) was treated according to the "HPOG-MMI-86" protocol. After the CNS relapse the Induction Protocol E was applied.

* (according to treatment protocols: see Table I and list of abbreviations)

TABLE I

Data of patients and healthy children of the same age and sex. They were referred by codes numbers.

Patients	Characterisation of the investigated groups							
Healthy controls	age (years)	sex	dg	risk factors			treatment	present state
				BFM	FAB	Imm.		
B_1	4	♂						
B_2	14	♀						
B_4	9	♂						
B_{11}	10	♂						
B_{13}	5	♂						
B_{16}	7	♂						
B_{18}	3	♂						
Newly diagnosed cases								
B_3	9	♂	NHL	St. I.			Murphy prot.	compl. rem.
B_5	14	♂	NHL	St. III.			Wollner prot.	compl. rem.
B_9	4	♀	ALL	RF>1,7	L_2	O	HPOG-86	nonresponder
B_{12}	7	♂	ALL	RF>1,7	L_1	CALLA	ALL-88	compl. rem.
B_{14}	4	♂	ALL	RF<1,7	L_1	CALLA	ALL-88	compl. rem.
Isolated CNS and medullary relapses								
B_8	6	♀	ALL CNS	RF<1,7	L_1	CALLA	ALL-83 Block R1+R2	nonresponder
B_{10}	11	♂	ALL	RF<1,7	L_2	CALLA	ALL-83	remission
B_{15}	11	♂	ALL CNS	RF<1,7	L_1	CALLA	ALL-83 Block R1+R2	nonresponder
B_{17}	3	♀	ALL	RF>1,7	L_1	O	ALL-83 ("E")	remission

Case N^{o} B_{12}: This ALL case had large lymph nodes in the right lateral submandibular region. During ALL-88 Prot.I.subtotal lymphadenoctomy was performed, and maintenance therapy, prescribed for high risk cases continued.

Case N^{o} B_{14}: In the case of this child with C-ALL, the ALL'88 treatment was initiated. Maintenance therapy was pursued. Complete remission was recorded.

Case N^{o} B_{8}: In 1988 CNS relapse was observed in the ALL patient of average malignancy. The child was treated according to the BFM ALL meningeal relapse protocol of 1983. In 1989 recurrent meningeal relapse, after several weeks a systemic relapse was recorded.

Case N^{o} B_{10}: In 1982 ALL -a late systemic relapse-was diagnosed in this boy, so the BMF protocol of 1983 was applied. His present condition is statisfactory.

Case N^{o} B_{15}: In a boy under treatment for ALL of average malignancy, CNS relapse was diagnosed in May 1988, thus the BMF meningeal relapse protocol of 1983 was attempted. After 6 months, decerebration symptoms, paraplegy and sensormotor aphasy developed.

Case N^{o} B_{17}: ALL systemic relapse was diagnosed in an infant in the 52nd week of "HPOG-MMI-86" therapy, and BFM'83 ALL-relapse induction protocol ("E") was initiated. At the beginning of the treatment his overall condition improved then high fever developed and he died due to pancytopenia and cardiovascular-respiratory failure.

RESULTS

The characterisation of the groups investigated is illustrated in Table I. Results (Fig. 1) are related to the mathematical means (dotted line) of healthy children.

The RPMI/PHA index* (Fig. 1) is higher than the mathematical means of healthy children.

The change in the RPMI/Con-A ratio (Fig. 1) shows a similar trend, while the ratios of the two lectins, Con-A and PHA were practically identical or lower than the mathematical means of the corresponding healthy values or controls.

* of ALL, NHL patients

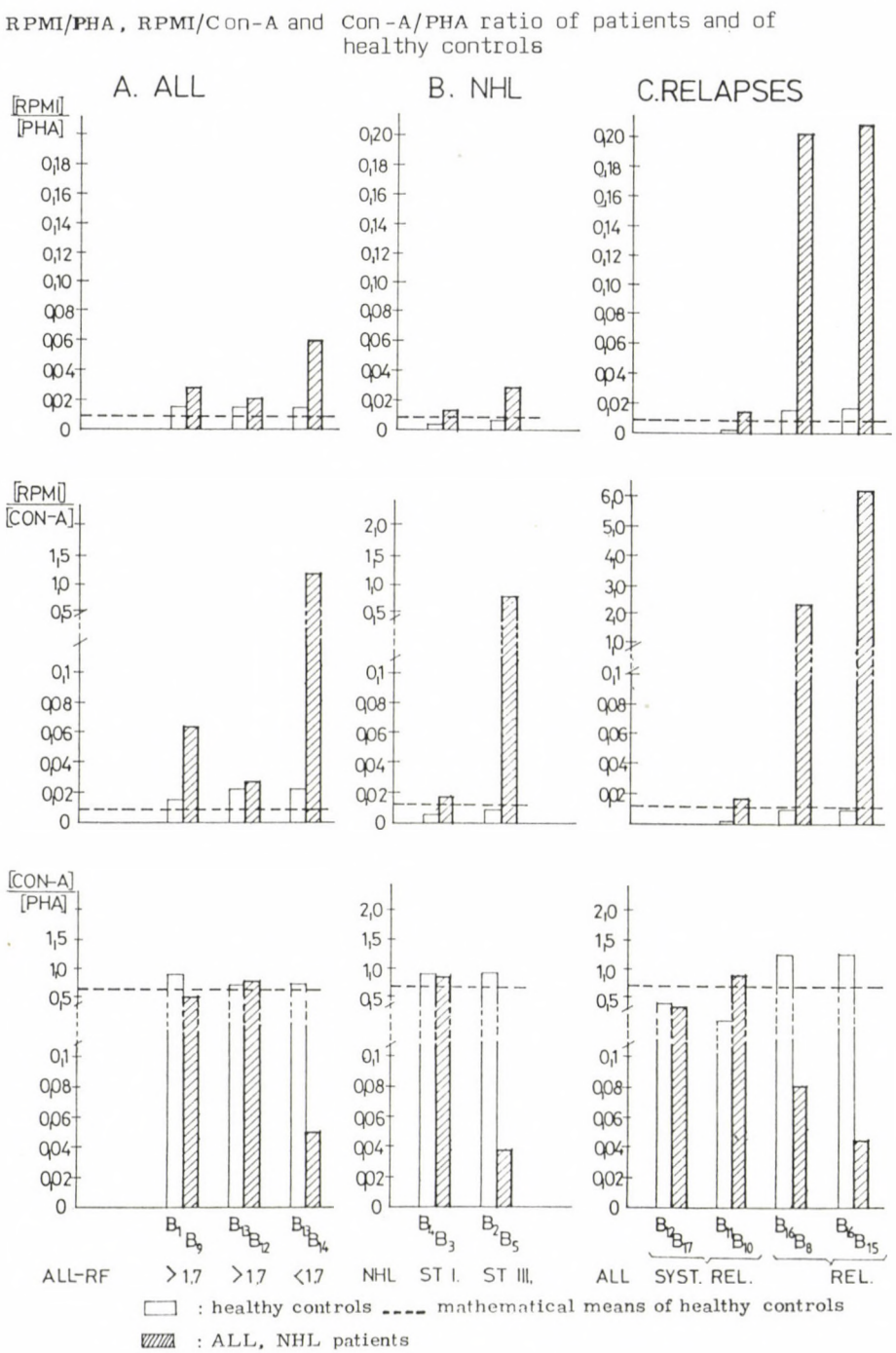

Fig. 1. RPMI/PHA, RPMI/Con-A index and Con-A/PHA ratio patients related to the matemathical means of healthy children (dotted line). There are matched controls.

The results obtained with the cytostatic agents mentioned in the protocol, as well as the data of healthy children were corrected to the excepted level of PHA stimulation rate with the PHA values (Figs. 2, 3, 4).

ALL (Fig. 2):

B_9: From the drugs listed in the "HPOG-MMI-86" protocol, reduction was induced by C-ARA, VM_{26} and CP.

B_{12}, B_{14}: From the agents of the "ALL'88" protocol C-ARA, CP and ASP caused reduced levels compared to the control in these two children. In case B_{14}, VCR also resulted reduced level.

NHL (Fig. 3):

B_3: From the agents of treatment NHL stage I., the suppressive effect of MTX, C-ARA and ASP was distinct also at in vitro conditions.

B_5: Studying the agents of the LSA_2L_2 therapy, except for CP, the scintillation activity of the cell culture, corrected with the value assayed in PHA medium, showed a decrease in each case.

Isolated CNS and medullary relapse:

B_8, B_{15}: Fig. 4 demonstrates that treatment constituents VM-26, MTX and CP C-ARA, IFO and VCR induced reductions in the scintillation activity of lymphocytes compared to the controls in children submitted to ALL'83 CNS relapse therapy.

B_{10}, B_{17}: Fig. 5. Summing up the effect of therapy on the systemic ALL relapse in B_{17}, each constituent of the BFM'83 protocol based on induction, inhibited the blastic transformation of the lymphocytes of the patient, while in the case of patient B_{10}, reduction in the scintillation activity was induced only by MTX.

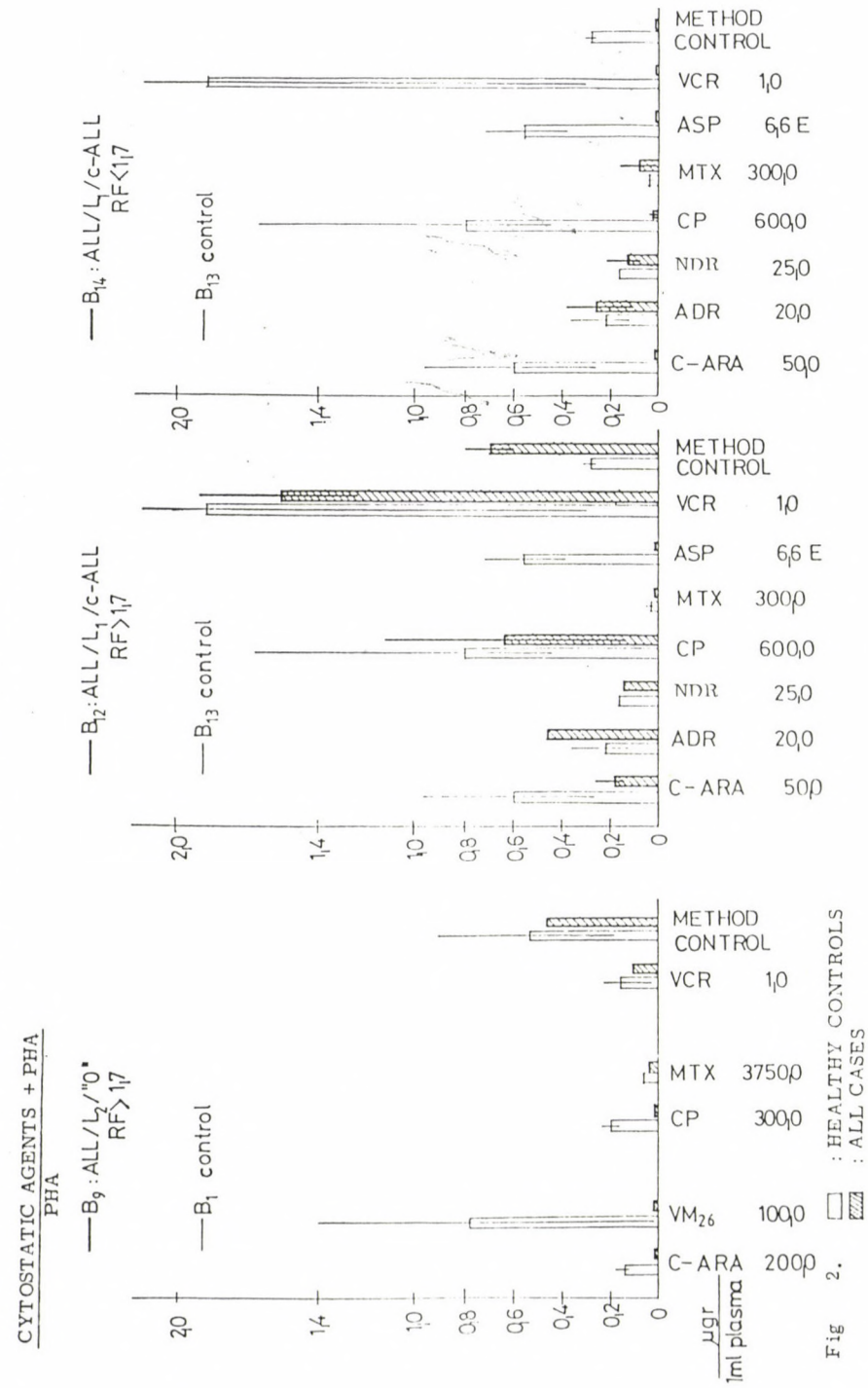

Fig 2. Results obtained with the cytostatic agents-data of patients and controls, corrected to the basic PHA values

Results obtained with the cytostatic agents-data of patients and controls, corrected to the basic PHA values

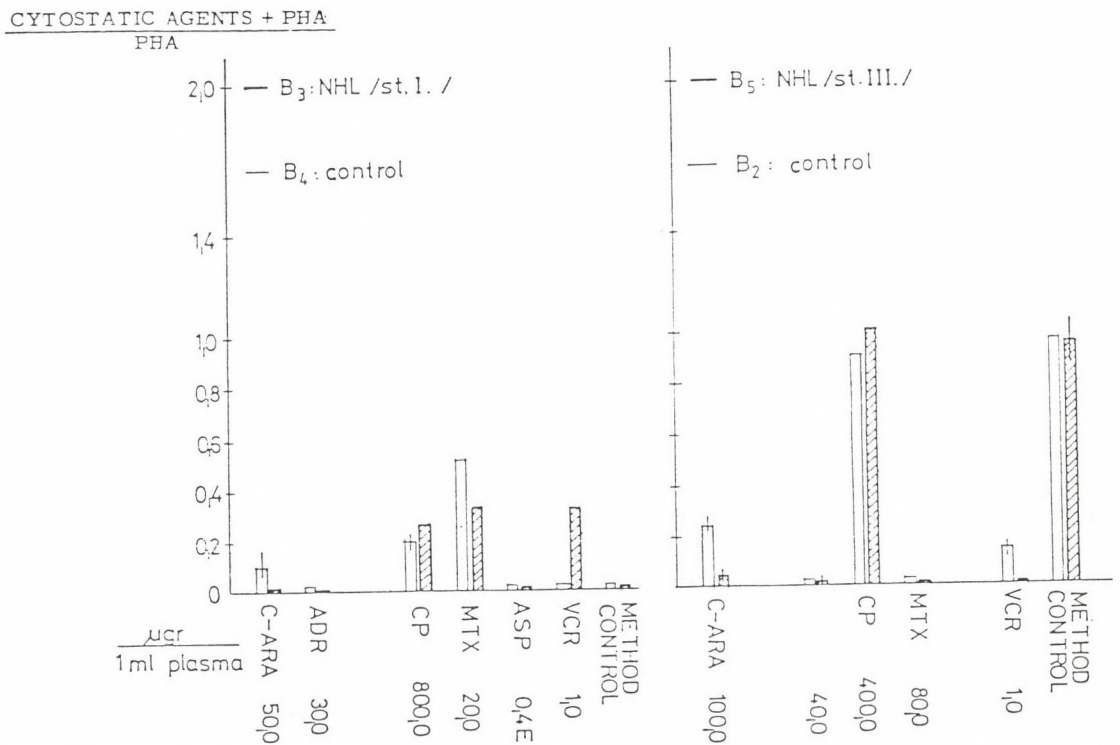

Fig 3. ☐ : HEALTHY CONTROLS
▨ : NHL CASES

Results obtained with the cytostatic agents-data of CNS relapses and controls, corrected to the basic PHA values

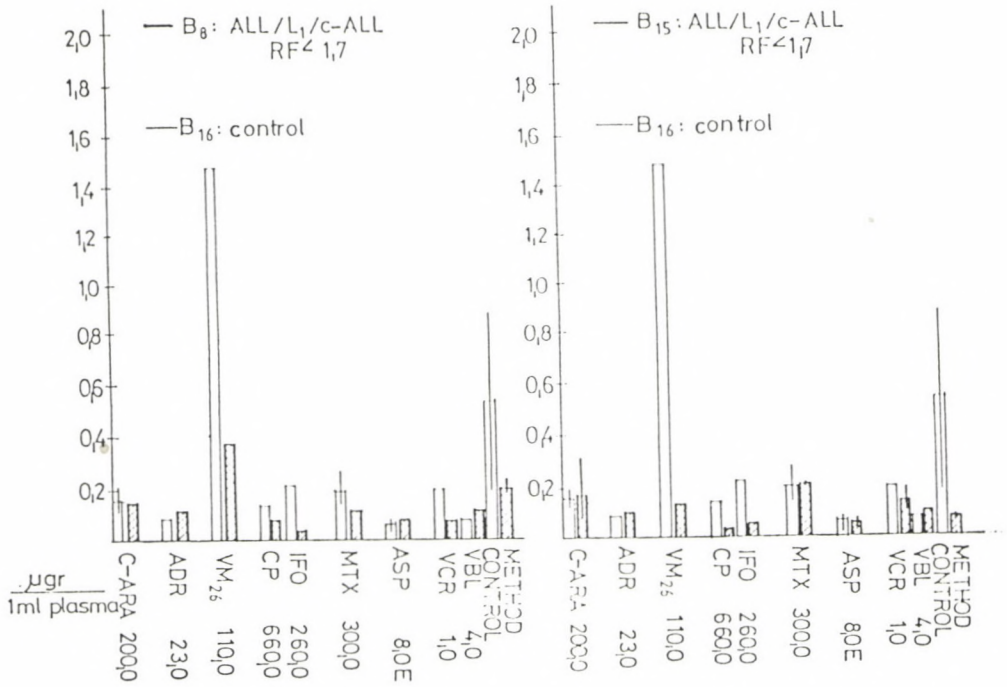

Fig 4. ☐ : HEALTHY CONTROLS
▨ : ALL, NHL PATIENTS

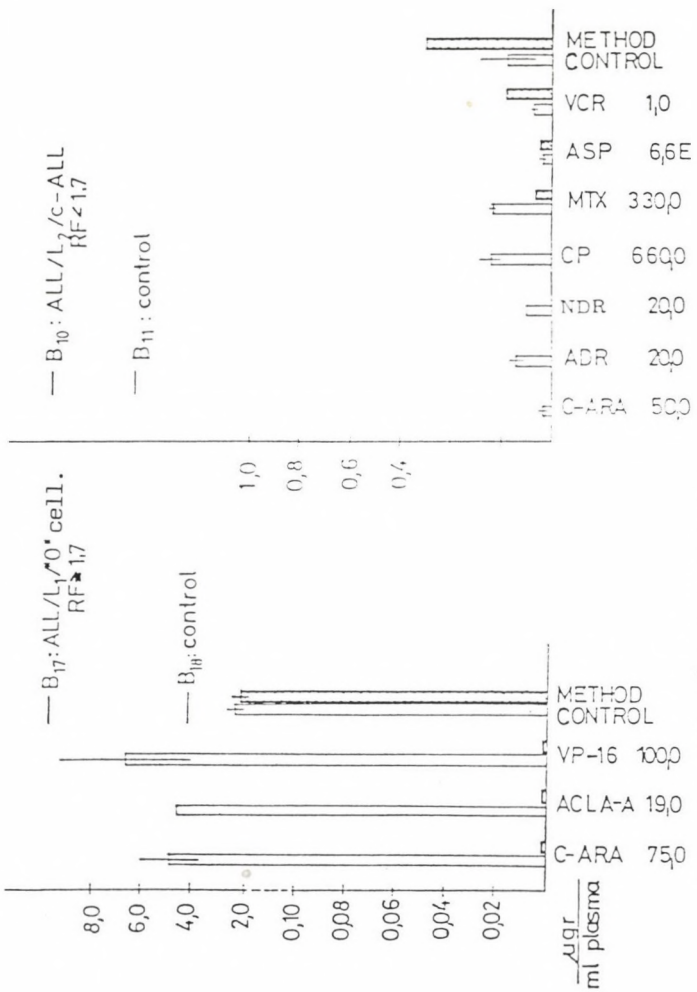
Results obtained with the cytostatic agents-data of CNS relapses and controls, corrected to the basic PHA values
— B17: ALL/L1"O" cell. RF≥1.7
— B18: control
— B10: ALL/L2/c-ALL RF<1.7
— B11: control
8,0
6,0
4,0
2,0
0,10
0,08
0,06
0,04
0,02
1,0
0,8
0,6
0,4
µgr / 1ml plasma
METHOD CONTROL
VP-16 100,0
ACLA-A 19,0
C-ARA 75,0
METHOD CONTROL
VCR 1,0
ASP 6,6E
MTX 330,0
CP 660,0
NDR 20,0
ADR 20,0
C-ARA 50,0
: HEALTHY CONTROLS
: ALL, NHL PATIENTS

Fig 5.

DISCUSSION

Several studies confirm the significance of the immune system in patients with ALL and NHL, proving a correlation between clinical therapeutical effects and in vitro prediction of tumor cell chemosensitivity /15, 22/. In this study in vitro reactivity of T-lymphocytes to PHA /22/. Con-A /6, 32, 36/ and some cytostatical drugs /24, 25, 28/ was investigated in ALL, NHL patients and healthy control children.

Attention should be drawn to the relationship between the change recorded in PHA medium and the basic activity (RPMI). We have concluded that in ALL patients /27/ lymphocytes showed a higher initial blastogenic activity assayed in RPMI medium with a higher RPMI/PHA ratio compared to the healthy control, while in ALL of high malignancy (RF > 1.7) the rate of blastic transformation induced by PHA is higher, the RPMI/PHA ratio being consequently lower, compared to the corresponding parameters of patients of average malignancy (RF < 1.7).

Similar observations have been made when data of NHL patients were compared to those of a healthy child of the same age /30/. In the present study the increased RPMI/PHA ratio was considered as a sign of relapse in patients with meningeal symptoms /8/, suggesting the presence of the increased number of lymphoblasts in the pheripheral blood. In the case of systemic relapse /3/, the rate of PHA stimulation rate was higher than those of ALL-patients with high malignancy (RF > 1.7), resulting a lower RPMI/PHA ratio.

In conclusion, our study showed the usefulness of "in vitro" determination of blastogenic response of T-lymphocytes in forecasting the responsiveness of the leukemic cells to the cytostatic drugs /25/ in the stage of relapses /8/. Although the CP and VCR treatment required metabolic activations to be active in the cells /28/, the resistance to this agent /12, 16, 31, 39/ can be achieved by a previous treatment of metabolizing cells or using the sera of already treated patients. An other possibility to estimate the changes in the response to these

drugs, is to compare the PHA stimulation rate of the first treatment time with the response measured relapse. This method has the advantage of being able to monitor the T-cell ability to blastogenic transformation in the presence of different lectins (PHA and conA) and to compare it to the changes of stimulation rate in the presence of different cytotoxic drugs. The protocol makes it possible to prevent the undesirable toxic side effects of the drug combination, /21, 28, 29/ and to peel up the ineffective component of the treatment trial. In the future we shall also attempt to perform the necessary metabolic activation of certain drugs, needed in the case /1/ prior to commencing the treatment. By exploiting the method of using in vitro systems /37/ to model the pathomechanisms of various differences /32/ in the cell-drug relationship /19/, the result can trigger an efficient antileukemic therapy with a consideration for the individual differences in the response /13, 35/.

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Acta Paediatrica Hungarica 31 (3), pp 396 (1991)

BOOK REVIEW

György Fekete: Congenital chromosome aberrations and tumour predisposition. Akadémiai Kiadó, Budapest, 1991. 162 pages.

The nicely presented book gives a survey on the main guidelines of clinical observations and scientific research concerning the links between congenital chromosome aberrations, malformations and tumour development.

The book contains three chapters illustrated with 44 figures, list of abbreviations, references and subject index.

In the first chapter the frequency of congenital chromosome aberrations and of the malignant tumours accompanying them, as well as the characteristic clinical and cytogenetic symptoms are reviewed.

The second chapter deals with those mechanisms which, to our present knowledge, may probably be responsible for the connection between chromosome aberrations and carcinogenesis.

The third chapter comprises some practical conclusions which may help the work of tumour-related genetic counselling.

The list of references on pages 119-150 comprises the most relevant works up to 1989.

The book by reviewing the topic and providing abundant references, yields useful information to paediatricians or other specialists who dispose of well-established knowledge on the field. It can be suggested to the library of every paediatricians and paediatric departments.

Miklós Miltényi M.D.

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Index: 26.021

Acta Paediatrica Hungarica

VOLUME 31, NUMBER 4, 1991

Akadémiai Kiadó, Budapest

ACTA PAEDIATR HUNG HU ISSN 0231-441X

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A Quarterly of the Hungarian Academy of Sciences

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Acta Paediatrica Hungarica is abstracted/indexed in Biological Abstract, Chemical Abstracts, Excerpta Medica, Index Medicus

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Contents

Acta Paediatrica Hungarica 31 (4), pp 397-402 (1991)

BALANCED CHROMOSOME REARRANGEMENTS AND ABNORMAL PHENOTYPE

G. KOSZTOLÁNYI[1], Katalin BAJNÓCZKY[2], K. MÉHES[1]

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Received 12 November 1990

Analysis of the results of 3411 routine cytogenetic examinations initiated by abnormal phenotype or family history revealed that out of 44 cases with balanced structural aberration 12 patients had an abnormal phenotype. Of the 12 cases, there were four reciprocal translocations, three Robertsonian translocations, and five pericentric inversions. Eight rearrangements were inherited, one had occurred de novo, and three were of unknown origin. Each carrier parent was apparently healthy. In all of the four cases with reciprocal translocation the rearrangements were of paternal origin. None of the clinical abnormalities could be assigned specifically to the breakpoints. Explaining the association of balanced chromosomal rearrangement and clinical abnormalities, possibilities of causal relationship and by chance coincidence are discussed.

INTRODUCTION

Balanced chromosomal rearrangements usually do not cause abnormal phenotype. However, they have been reported repeatedly in association with mental retardation and/or congenital anomalies over the past years /1,2,3,4,5,8/. To explain the relationship between the abnormal phenotype and the apparently balanced karyotype, several hypotheses have been suggested, viz. 1/ submicroscopical aneuploidy, 2/ a gene mutation at the breakpoint, 3/ position effect /6/, but this association deserves further clarification. We therefore decided to analyse our cases with balanced chromosomal rearrangement found in routine diagnostic cytogenetic activity and tried to find out whether the clinical symptomatology could be related to the hypotheses mentioned.

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MATERIAL

The study group comprises 3411 patients referred for chromosome analysis to the Pediatric Department of University Medical School, Pécs between 1974 and 1989, and to the Pediatric Department of County Hospital, Győr between 1980 and 1989. Chromosome analyses were indicated according to the generally accepted clinical practice, viz. by abnormal phenotype, by reproductive failure, or by having a first degree relative with structural chromosome aberration. Chromosome analyses performed in any screening program were excluded.

RESULTS

A total of 615 cases with abnormal karyotype were found among 3411 patients whose cytogenetic examination was initiated by phenotype or history suggesting chromosome aberration. Out of the 615 constitutional chromosome abnormalities, 499 (81.1%) were numerical and 116 (18.9%) were structural aberrations. The structural aberration was "balanced" in 44 cases, i.e. in 7.2% of the total (Table I).

TABLE I

Type of aberration in 3411 patients referred for chromosome analysis because of suspected chromosome anomaly

Aberration	No.	%
Numerical	499	81.1
Structural	116	18.9
unbalanced	72	11.7
balanced	44	7.2

Table II shows that the majority of balanced structural aberrations were discovered in the course of family studies initiated by unbalanced aberration in a member of the family, i.e. in the index patient. Surprisingly, there were only a few

cases discovered on the base of repeated spontaneous abortions. The low ratio may result from referral bias (both laboratories are functioning in pediatric clinics). Twelve cases, i.e. 1.9% of all aberrations were diagnosed in patients who were referred for examination because of abnormal phenotype (Table II).

TABLE II

Reason of referral for chromosome analysis in 44 cases with balanced structural aberration

Indication	No.
Family study	23
Spontaneous abortions	9
Abnormal phenotype	12

Table III summarizes the cytogenetic findings and the main clinical features of patients whose cytogenetic examination was initiated by abnormalities in the phenotype. Neither the mental retardation, nor the major/minor malformations of these patients could be explained by any other clinical and laboratory tests or by pre-, peri-, or postnatal history. It is interesting that in all of the 4 cases with balanced reciprocal translocation the aberrations were of paternal origin, but the number of cases does not allow to draw any conclusion. In the majority of cases the balanced aberration was familial (in 3 cases the parents, or any of them, were not accessible for chromosome analysis). Neither of the transmitting parents showed phenotypic abnormalities.

TABLE III

Balanced chromosome aberration with abnormal phenotype

Case No.	Chromosome aberration	Clinical abnormalities
Case 1.	t(5/14) pat	some features of Turner syndrome
Case 2.	t(6/12) pat	some features of Turner syndrome
Case 3.	t(3/19) pat	fetal hydrops
Case 4.	t(11/13) pat	esophageal atresia
Case 5.	t(D/D) ?	mental retardation, syndactyly on feet, facial dysmorphism
Case 6.	t(13/15) mat	mental retardation, microcephaly
Case 7.	t(14/21) pat	mental retardation, facial dysmorphism
Case 8.	inv(2) mat	mental retardation, facial dysmorphism
Case 9.	inv(5) de novo	hypogonadism, micropenis
Case 10.	inv(9) ?	some features of Turner syndrome
Case 11.	inv(9) pat	ano-uro-genital malformation
Case 12.	inv(9) ?	hypogonadism, obesity

DISCUSSION

One quarter of our patients with "balanced" structural aberration had phenotype abnormalities. This could be the result of submicroscopic aneuploidy caused by a tiny deletion or duplication prior to chromosomal rearrangement. However, none of the clinical abnormalities could be assigned specifically to the breakpoints. In addition, in 8 cases the probands' rearrangement was also found in one of the parents who had no clinical abnormalities. Although meticulous examination of "healthy" subjects - which was not done in every carrier parent in this retrospective study - may reveal latent features of microdeletions or trisomies /7/, the parent/offspring difference in the clinical manifestation of an

apparently same rearrangement might be explained by secondary duplications or deletions arising at meiosis leading to phenotype abnormalities in the child, but also this possibility can be excluded by the lack of specific karyotypic/phenotypic correlation.

Clinical abnormalities in the offspring and normal phenotype in the parent with the same rearrangement could be explained by gene mutation at the breakpoint, if we suppose heterozygosity at the locus in the other parent, and hence, homozygosity in the offspring. However, the clinical manifestation did not suggest a known autosomal recessive disorder in any of the index patients.

The most plausible explanation seems to be a by chance coincidence of a chromosomal rearrangement and clinical abnormalities of unknown reason without any causal relationship. Since most chromosome analyses are done because of phenotypic abnormalities, an ascertainment bias favoring the discovery of a rearrangement in a subject with an abnormal phenotype certainly contributes to an unduly high rate of mental retardation and/or malformation among carriers of apparently balanced rearrangements. However, if we want to rely on this explanation in genetic counseling for estimating the reproductive risk of a person with a "balanced" chromosomal rearrangement, further studies on larger population are needed.

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Acta Paediatrica Hungarica 31 (4), pp 403-406 (1991)

FAMILIAL EXTRA BISATELLITED MICROCHROMOSOME AND DOWN SYNDROME

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Received 11 March 1991

Authors report on a Down infant, whose chromosome complement includes an extra small bisatellited marker chromosome. This marker was also found in four healthy members of the family.

INTRODUCTION

The detection of an accessory small marker chromosome may present a difficult diagnostic problem and especially familial cases may call attention to new aspects of the question /2, 3, 4, 5, 6, 7/. Recently we observed a girl with trisomy 21 who had an additional bisatellited marker chromosome which was also found in four healthy family members in three generations.

CASE REPORT

The newborn female infant was the first child of the healthy, nonconsanguineus parents. The mother aged 22 years, the father 21 years. The proposita's birth weight was 2900 g, length 47 cm. She had a typical phenotype of Down's syndrome.

Analysis of her lymphocyte mitoses revealed regular trisomy 21 plus an accessory small chromosome in each of the 50 cells examined. This marker was smaller than the members of the G-group; in 70% of the mitoses it was associated to the satellites of one of the acrocentrics. An attempt for its identification was done by means of C and G-banding, DA/DAPI and NOR methods, and it was regarded as inv dup 15p (Fig. 1).

The same marker was identified in four out of ten healthy, mentally normal family members (Fig. 2). At least 20 mitoses were analysed in each case and no mosaicism was detected.

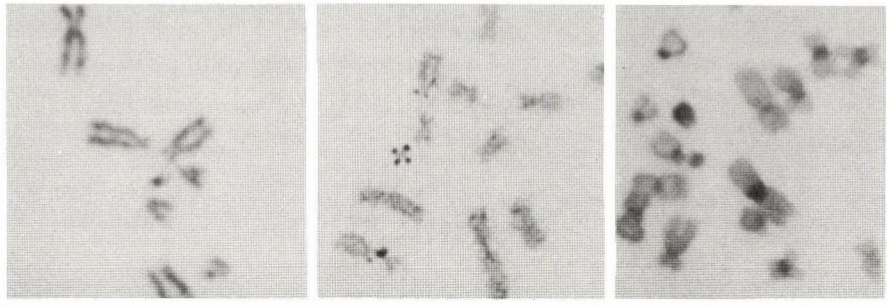

Fig 1. G-banded (left), silver-stained (middle) and C-banded (right) marker chromosome

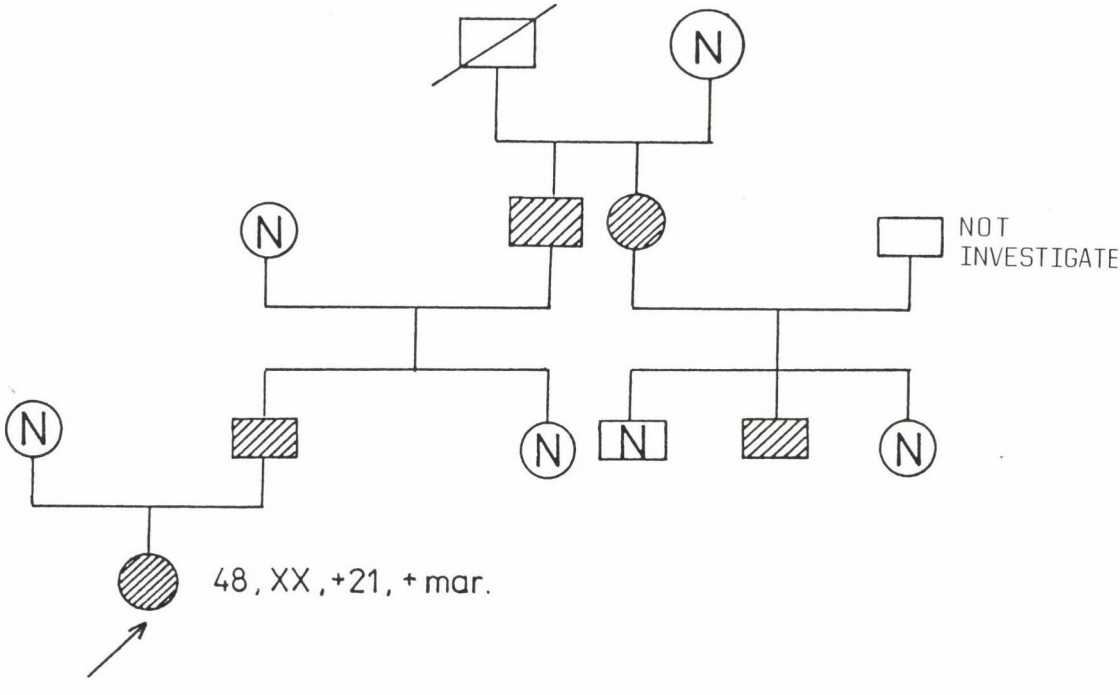

Fig. 2. Pedigree of the family

DISCUSSION

The significance of supernumerary marker chromosomes was extensively reviewed by Buckton et al /1/. The familial case reported here confirms most of their conclusions, however, we call attention to two particular points:

1. The simultaneous occurrence of trisomy 21 and inv. dup /15/ may be merely incidental, but it also may suggest that the presence of a marker chromosome may interfere with the normal process of chromosome pairing and disjunction of acrocentric chromosomes.
2. The present case provides further evidence for the assumption that the risk of abnormality to a fetus carrying a marker is minimal if the phenotypically normal carrier parent has that marker in a non-mosaic form.

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Acta Paediatrica Hungarica 31 (4), pp 407-413 (1991)

SHORT CLINICAL REPORT: A NEW CASE WITH DE NOVO PARTIAL 9_p MONOSOMY

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Received 22 June 1990

A female patient is described with a karyotype 46,XX,del (9) (p22) showing characteristic dysmorphic phenotype: trigonocephaly, prominent forehead, long philtrum, small mouth, high arched palate, low set ears, short neck, widely spaced nipples, long fingers and toes, omphalocele.

The first Hungarian case of 9p monosomy syndrome is reported here.

INTRODUCTION

The 9p monosomy syndrome was first delineated by Alfi in 1973 using two cases /2/ and was further described on the basis of six cases /3/. Until 1986, fewer than 60 cases were reported /15/. The clinical features of the syndrome are consistent and characteristic /3, 8, 22, 24/. The most common manifestations are summarized in Table I.

Life expectancy does not seem significantly diminished /8, 19/.

The 9p monosomy occurs more often in female than male patients /8, 24/.

In most of the cases the aberration is de novo /1, 2, 3, 9, 11, 15, 18, 23/, but it may be inherited /2, 12, 20/ and/or combined with other aberrant chromosome /7, 10, 12, 17/. One case was reported having ring (9)/del (9p) mosaicism /15/.

Akadémiai Kiadó, Budapest

TABLE I

The main clinical features in patients with 9p- deletion syndrome

Clinical findings from previous reports	Present case
Moderate mental and developmental retardation	+
Hypertonia	-
Trigonocephaly	+
Prominent forehead	+
Flat occiput	+
Up-slanting palpebral fissures	+
Epichantal folds	+
Ocular hypertelorism	+
Exophthalmos	+
Flat nasal bridge	+
Anteverted nostrils	+
Long philtrum	+
Small mouth	+
Micrognathia/retrognathia	+
High-arched palate	+
Flat abnormal auricles	+
Low-set ears	+
Short broad neck	+
Widely spaced nipples	+
Cardiovascular malformations	-
Square hyperconvex nails	-
Long fingers and/or toes	+
Omphalocele	+

CASE REPORT

The proband, a girl born on 25th of June 1989, is the first child of healthy unrelated parents. Mother was 20 years old, and the father was 24 at the time of her birth. The pregnancy and delivery were uneventful. The birth weight was 4350 g. Apgar score was 8/9. Maternal serum AFP level was higher than normal during the 16th week of pregnancy, but there was not shown any abnormalities by ultrasound. Omphalocele was seen immediately after birth, therefore she was sent for operation.

Cytogenetic examination was performed because of her multiple congenital abnormality including craniofacial dysmorphism (Fig. 1 and Table I). Muscle tone was normal. There was no hyperreflexia.

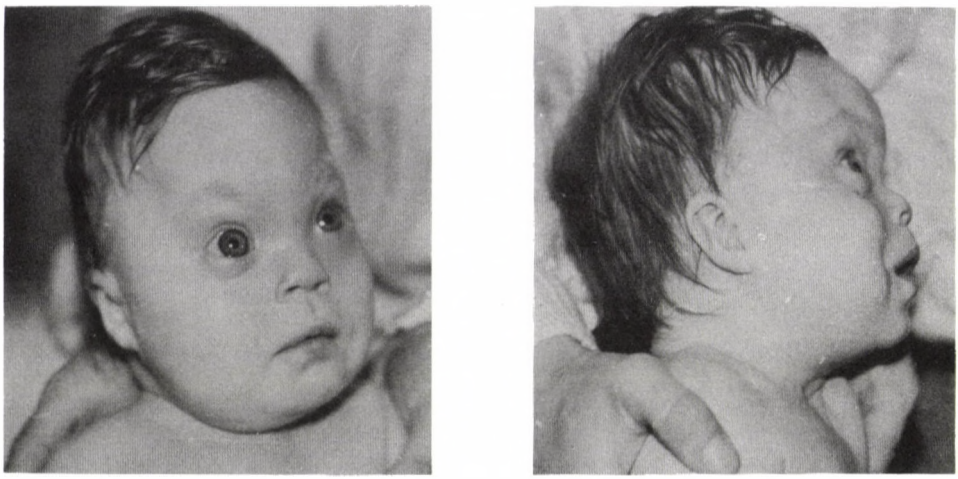

Fig. 1. Frontal /a/ and lateral /b/ views of the patient aged 4 months.

Psychological test /13, 14/ was performed at the age of 9 months. According to this test she was delayed mentally and developmentally. She was functioning at six months level. However she is amiable, affectionate and sociable.

Cytogenetics

Chromosome investigations of the patient and her parents were performed on peripheral blood leukocytes using standard phytohemagglutinin-stimulated whole-blood cultures. Cytogenetic analysis of the proband's cells demonstrated a terminal deletion of the short arm of chromosome 9 at band p 22, identified by G- and C-banding (Fig. 2). The karyotype was 46,XX,del (9) (p22). Parents' chromosomes were normal.

DISCUSSION

Clinical features of 9_p- syndrome are consistent and characteristic. However, unusual clinical features can be found in some cases, i.e. advanced osseus maturation, marked congenital vertebral anomalies /21/ and redundant posterior neck skin, absence of exophthalmos /15/. This may be related to the fact, that patients have other chromosome aberration beside 9p deletion /10, 15/. Our case shows the most characteristic phenotype of the syndrome (Table I) and she does not have any distinctive features.

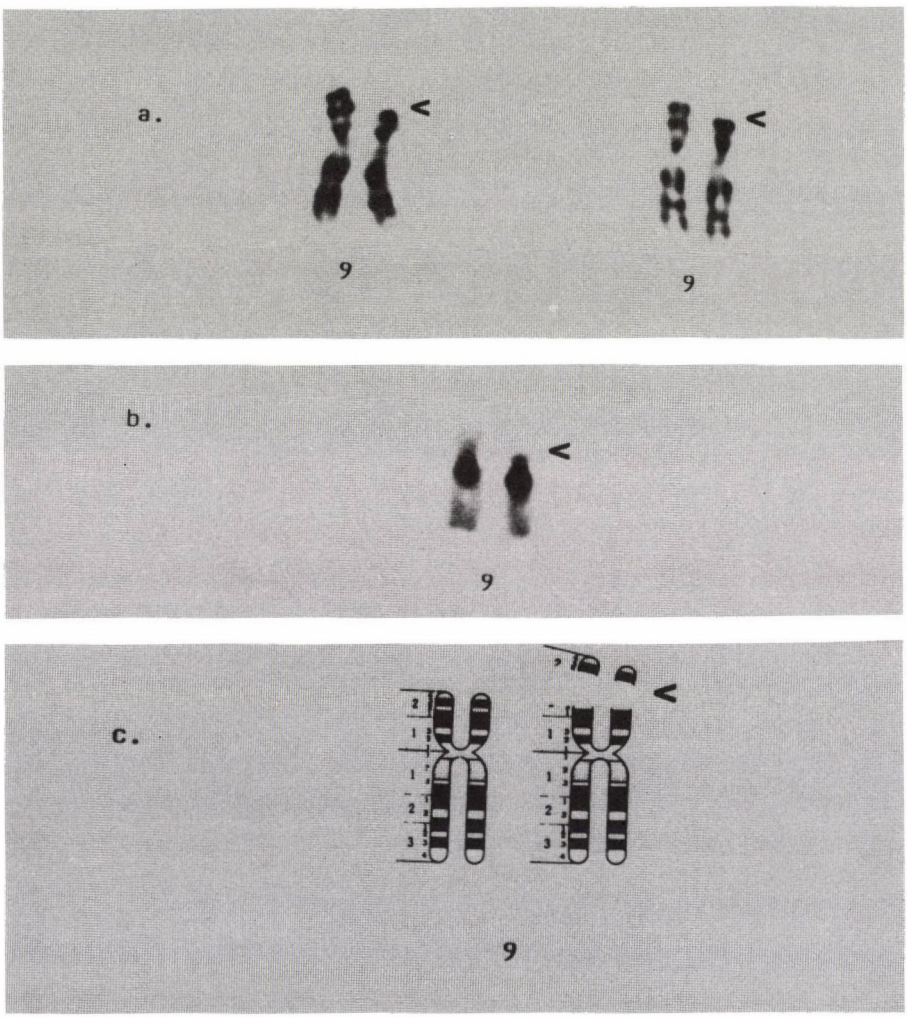

Fig. 2. Three partial karyotypes of the patient using G-banding /a/ and C-banding /b/ techniques, and the idiogram of the aberration /c/. Arrowheads show the breakpoint on the short arm of chromosome 9 at band p22.

Patients' apparently long fingers due to relative shortness of metacarpals /23/ were found in some patients /1/.

The GALT (galactose-1-phosphate uridyl transferase) activity was studied in two patients with 9p deletion. Findings

suggested that locus of the GALT gene is on the short arm of chromosome 9 in band 9p21 /5/.

9p deletion was found in patients with acute lymphoblastic leukemia (ALL) as well /4, 6, 16/.

Coordinated studies will be necessary to clarify the importance of chromosomal material at 9p21-22.

ACKNOWLEDGEMENTS

The authors are indebted to Maria Feuer for her helpful comments on psychological and developmental aspects of the case.

They are grateful to Judit Gramantik for her photographic help and to the patient's family for their willing collaboration.

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Acta Paediatrica Hungarica 31 (4), pp 415-422 (1991)

THE INFLUENCE OF METOCLOPRAMIDE ON THE COMPOSITION OF HUMAN BREAST MILK

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Received 4 February 1991

The breast milk prolactin (PRL) has been claimed to play a role in the control of electrolyte composition of the milk. Since metoclopramide has been shown to increase milk production in humans, we have made an attempt to investigate the production, the PRL and sodium concentrations in milk with (group I) and without (group II) maternal metoclopramide treatment (5 days, 30 mg/day). Both groups consisted of 11 mothers and their full-term newborn infants. The daily milk production was significantly higher in the treated group (276.4 ± 36.6 vs 150.9 ± 25.3 ml/day, $p<0.01$). The PRL measured by RIA was similar in the milk samples of the metoclopramide treated and control groups (80.5 ± 17.7 vs 90.7 ± 27.3 ng/ml). The sodium concentration in the milk of mothers taking metoclopramide was 22.1 ± 1.6 mmol/l and 24.3 ± 3.2 mmol/l in the control group ($p=0.59$). On the 5th postnatal day the plasma PRL of the newborns of mothers treated with metoclopramide does not differ from the values of the control babies (29.8 ± 2.6 vs 30.7 ± 2.4 ng/ml) indicating that the amount of metoclopramide transferred into the milk has no apparent influence on the hypothalamo-hypophyseal axis of the neonate. In conclusion: the maternal metoclopramide treatment augments the milk production without having any effect on the PRL and sodium concentration of human "mature" milk.

INTRODUCTION

Breast milk is undoubtedly the best food for infants /7, 20/ and mothers are advised to feed their babies with their own milk at least for 3 months. Prolactin (PRL) plays a critical role in the initiation and maintenance of lactation in humans /18, 19/. In some cases deficient lactation is caused by

inappropriate secretion of PRL and metoclopramide stimulates PRL secretion by occupying hypothalamic dopaminergic receptors and blocking dopamine's action as an inhibitor of PRL secretion /4, 5, 9, 11, 14, 15, 16/. Maternal metoclopramide treatment has been shown to augment milk production by elevating basal PRL level /12/, but it may also interfere with the hypothalamic response to suckling, and milk expression induced PRL release may not occur /5/.

PRL in milk is biologically potent /8, 10/ and has been claimed to play a role in the control of electrolyte composition of the milk. Irrespective of the length of gestation, the daily PRL excretion into the milk showed significant negative correlation with milk Na level and Na/K ratio /6/. Metoclopramide is transferred into breast milk and in a few cases elevated neonatal PRL levels were reported after maternal metoclopramide treatment /13/.

In the present study we decided to investigate the daily milk production, the daily milk PRL excretion, and the Na content of milk after maternal metoclopramide treatment. In addition, the pituitary response of the newborn to metoclopramide was evaluated by measuring the plasma concentration of PRL.

MATERIALS AND METHODS

Two groups of healthy lactating mothers volunteered for the study. None of them had a history of toxemia of pregnancy, renal disease or diuretic therapy. All of them had an uncomplicated vaginal delivery at term. They were on normal diet and the estimated daily sodium intake was about 100-120 mmol/day. Group I consisted of 11 mothers and their newborn infants. The mothers were taking 10 mg metoclopramide (Cerucal, GERMED, Berlin) in every 8 hours for 5 days, started at the first day after delivery. Group II included 11 mothers and their newborns without metoclopramide or any other medical treatment which could influence lactation. Clinical data of the enrolled mothers and their newborns are shown in Table I. There were no statistical differences between the two groups in parity, maternal age, gestational age, birth-weight, and Apgar score.

TABLE I

Clinical data of the enrolled mothers and their newborns

	Group I (treated)	Group II (control)
Maternal age (years)	25.2 (17-35)	24.9 (18-34)
Parity	1.6	1.5
Gestational age (weeks)	39.3 (37-41)	39.1 (37-40)
Birth weight (g)	3221 (2850-4230)	3462 (3000-4400)
Apgar score (1 minute)	8.8 (7-9)	8.9 (7-9)
n	11	11

Milk samples were obtained on the 5th day post partum at the beginning, and at the end of feedings by expressing, at least three times a day. The total amount of milk was estimated by weighing the baby before and after each feeding and/or by measuring the volume of the expressed milk. On the 5th postnatal day blood was taken from the newborn, after separating the plasma the samples were frozen and stored at -20 ^{o}C until analyzed by radioimmunoassay for PRL /2/. Sodium concentrations were determined by flame photometry. Milk PRL was measured as reported by Healy et al /10/ using SERONO-BIODATA kits. Statistical analysis was done by Student's t-test.

RESULTS

Maternal metoclopramide treatment in a dose of 30 mg/day, started after delivery, augmented significantly the daily milk production within 5 days. In group I the estimated milk expression was 276.4 $\pm$ 36.6 ml/day, meanwhile the milk production in group II was 150.9 $\pm$ 25.3 ml/day ($p<0.01$). The PRL and sodium concentrations in the milk samples of the mothers taking metoclopramide were 80.5 $\pm$ 17.7 ng/ml and 22.1 $\pm$

1.6 mmol/l, respectively, but no statistical differences were found compared to group II (90.7 ± 27.3 ng/ml and 24.3 ± 3.2 mmol/l). The neonatal plasma PRL levels were similar in both groups (29.8 ± 2.6 ng/ml in group I vs 30.7 ± 2.4 ng/ml in group II) on the 5th postnatal day. Results are listed in Table II.

TABLE II

Milk production, PRL, sodium concentrations and neonatal plasma PRL levels on the 5th postnatal day with (group I) and without (group II) metoclopramide treatment (mean ± SE).

	Group I (treated)	Group II (control)
Milk production ml/day	276.4 + 36.6	150.9 + 25.3 *
Milk PRL concentration ng/ml	80.5 + 17.7	90.7 + 27.3
Milk Na concentration mmol/l	22.1 + 1.6	24.3 + 3.2
Plasma PRL of the newborn ng/ml	29.8 + 2.6	30.7 + 2.4
n	11	11

* $p < 0.01$

DISCUSSION

Lactation results from complex interaction of hormones, although PRL appears to be the most important hormone involved in the initiation and maintenance of lactation /18, 19/. Maternal PRL levels continue to increase during gestation, consequently, the plasma PRL level is lower at preterm than at term delivery /3/. Metoclopramide - as an antagonist of dopamine - has been shown to induce lactation successfully /4, 5, 9, 11, 12, 14, 15, 16/. Guzmán et al /9/ and Kuappila et al /12/ pointed out that metoclopramide increased the basal PRL level and in a dose of 30 mg/day significantly elevated the

maternal serum PRL level and the daily milk production. Ehrenkrantz and Ackerman /5/ investigated the effect of metoclopramide therapy in women who delivered premature infants and the therapy was started at a mean of 32 days post partum. In agreement with previous results, the basal PRL level and the daily milk production increased within a few days after having started the therapy and lasted during the treatment. Besides, the same authors observed that milk expression did not produce any additional PRL response in the treated women /5/.

PRL is present in the human milk and may play some role in lactation, in the regulation of milk sodium content, and the intestinal absorptive function of the suckling neonate /6, 8, 10/. Yuen demonstrated that the foremilk contained significantly higher PRL concentrations than the hindmilk /21/. Foremilk serves mainly to provide hydration to the newborn rather than energy. Furthermore, women with galactorrhea and hyperprolactinaemia have been demonstrated to have twofold higher PRL concentration in the milk as compared to the plasma. After bromocriptine treatment both the plasma and the milk PRL concentration decreased, the concentration gradient remained, however, in favour of the breast milk /22/. Milk of lactating mothers receiving bromocriptine treatment had lower milk sodium levels compared to placebo taking controls /1/.

The pharmacokinetics and endocrinological effects of metoclopramide were investigated by Kuappila et al /13/. Metoclopramide was detected in milk samples, generally in higher concentration than in maternal plasma, but the estimated intake by the newborn was considered only 1 to 5% of the recommended therapeutic dose for children. Sulyok et al /17/ giving metoclopramide for premature babies noted an increase in urinary sodium excretion, a decrease of potassium excretion, and a decrease of plasma and urinary aldosterone concentration. The effect of metoclopramide therapy on the composition of human milk is not known. However, Kuappila et al /13/ found that 4 out of 7 breast-fed neonates sampled during maternal metoclopramide treatment had higher PRL concentration compared to the infants of untreated mothers. The plasma concentration

of thyrotropin in the newborns remained within the normal range.

In the present study mothers giving birth at term were given metoclopramide to improve milk production in a dose of 30 mg/day for 5 days, started after delivery. On the 5th postnatal day the daily milk production was significantly higher in the treated group compared to untreated mothers. No serious side effects were noted. The milk PRL and sodium concentrations were similar in both groups and the maternal metoclopramide treatment had no apparent influence on the neonatal PRL secretion indicating that the amount of metoclopramide transferred into the milk has no effect on the hypothalamo-hypophyseal axis of the neonate. The results could be partly explained by the lack of suckling-induced PRL release of metoclopramide treated mothers. Furthermore, we have to take into consideration the differences observed in the PRL concentrations of the foremilk and hindmilk. Further studies are needed to elucidate the long term effect of metoclopramide on the composition of human milk, especially after preterm delivery.

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Acta Paediatrica Hungarica 31 (4), pp 423-432 (1991)

BIOCHEMICAL AND ULTRASTRUCTURAL DIAGNOSTIC PROBLEMS IN MUCOLIPIDOSES

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Received 2 October 1990

Biochemical and ultrastructural investigations were made in 2 children suffering from mucolipidosis type III. Among the lysosomal hydrolases the activity of beta-galactosidase and alfa-fucosidase diminished in the homogenate of the peripheral leukocytes in case I. The activity of serum and leukocyte arylsulfatase was normal.

By electron microscopy typical storage organellums for mucolipidosis were detected in different biopsy materials - liver, skin, conjunctival ones - and in the cytoplasm of the peripheral lymphocytes and leukocytes.

Definitive diagnosis was given by the specific electron microscopic investigations detecting the typical storage patterns for mucolipidosis.

INTRODUCTION

Mucolipidoses (ML) are lysosomal storage disorders with recessive autosomal inheritance. Clinical symptoms are different, Hurler-like dysostosis can be observed in ML type II. (I-cell disease). The cells of patients with I-cell disease and pseudo-Hurler polydystrophy (ML III) are characterized by a deficiency of N-acetylglucosaminyl phosphotransferase. This defect results in an inability of the cells to synthesize the mannose-6-phosphate recognition marker. As a consequence newly synthesized lysosomal enzymes are unable to bind to the mannose-6-phosphate receptors. In the fibroblasts from these patients, the lysosomal enzymes are excreted into the extracellular milieu and a generalized secondary deficiency of lysosomal enzymes in many cells of the body occurs /8/.

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Fibroblasts from patients with ML-II have extremely low or undetectable phosphotransferase activity whereas fibroblasts from patients with ML-III have partial phosphorilating activity.

Lysosomal sialidase deficiency, increased ganglioside content in autopsy tissues of sialidosis patient (ML-I) were detected by Ulrich-Bott /16/. Biochemical heterogeneity in ML-II was determined with sucrose-loading test classifying two distinct subtypes /14/.

Biochemically ML – IV. is characterized by accumulation of gangliosides, phospholipids and acidic mucopolysaccharides /11/.

By electron microscopy storage organelles typical of the mucolipidosis group are seen in cells /12/. The deficiency of ganglioside sialidase was reported as a metabolic defect in ML-IV. /16, 15/.

Our aim was to summarize the biochemical and ultrastructural investigation made in 2 children suffering from ML type III.

CASE REPORTS

Case 1. Dóra K. (female) was investigated at her 7 year-old age as our patient. Hurler-like phenotype, dysostosis, mild mental retardation, without any cornea opacity, vacuolated and metachromatically granulated peripheral lymphocytes were the pathognomic signs for mucopolysaccharidosis or mucolipidoses.

Urinary glycosaminoglucans were analysed: total glycosaminoglucans: 21.66 µmol uronic acid day, macromolecular GAG fragment GAG = 0.2, hyaluronic acid 84.4, K-heparansulphat 10.5, keratansulphat 1.4, chondroitinsulphat 3.1, dermatansulphat 0.6%. - Oligosaccharid-chromatography: negative.

Case 2. Peter N. (male) was admitted to our clinic at 11 year-old-age after a tetaniform attack with carpo-pedal spasmus and GM epilepsy attack suspecting for metabolic disease according to his Hurler-like course face and mental retardation. He attends at IV. class or special school for mentally retarded children. Mild Hurler-like vertebral dysostosis has been proven by X-ray examination.

Ophthalmological investigation: visus 0.7-0.8 D, without any corneal opacity.

GAG-uria: was normal, 29.8 mg/l g kreatinine.

EEG finding was suspected for temporo-parieto-occipital

irritative focus on the right side. As treatment Stazepine, Oradexon and Calcimusc were started.

Mucolipidosis (ML III. type) was diagnosed: vacuolated and metachromatically granulated peripheral lymphocytes, no pathological glycosaminoglucanuria (GAG-uria) and according to lysosomal enzyme analysis and the ultrastructural investigation of the different biopsy materials. Liver, skin and conjunctival biopsies were carried out for histochemical and ultrastructural (EM) specific investigations.

Biochemical investigations: lysosomal enzymes were determined from leukocyte homogenate with Griffith's /7/ method. The enzyme activities have been summarized in Table I.

TABLE I

Biochemical findings in patients suffering from mucolipidosis

Enzymes	nmol/mg/protein/h		Normal values SD± n = 20
Case 1.			
In leukocyte homogenate:			
Beta-galactosidase	114.0	D	888.0 388.8
Alfa-mannosidase	39.6	N	63.0 22.8
Alfa-fucosidase	7.2	D	231.6 157.2
Neuraminidase	47.4	N	36.0 7.2
In serum:			
Arylsulfatase-A	35.4 U/l	N	8-32 umol/l/h
Case 2.			
In leukocyte homogenate:			
Alfa-mannosidase	121.4	N	
Alfa-fucosidase	79.1	N	
Arylsulfatase-A	138.0	N	40-180 nmol/mg pr./h

N = normal

D = decreased

Ultrastructural examinations were carried out in both of the two cases on the skin, liver, conjunctival biopsy materials and on the peripheral lymphocytes. The lymphocytes were isolated

with the Ficoll-Hypaque method fixed in buffered glutaraldehyde, washed in the same buffer and postfixed in OsO_4. After a dehydration process the blocks were embedded in Araldite. The ultrathin sections were examined with Tesla BS 500 and a Zeiss EM 9S2 electron microscope.

RESULTS

Among the lysosomal hydrolases the activity of beta-galactosidase and alfa-fucosidase diminished in the homogenate of peripheral leukocytes in patient 1, while the activity of serum and leukocyte arylsulfatase was normal.

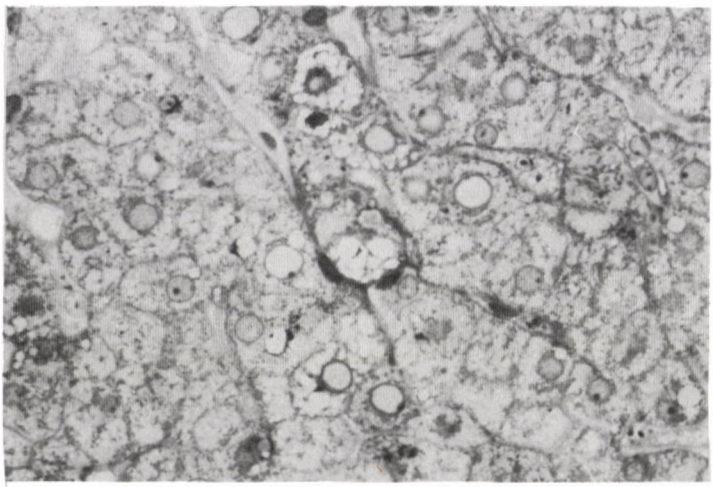
Result of the morphological investigations: liver biopsy material: light microscopy showed diffuse vacuolisation and in a small number lipid inclusions in the hepatocytes.

In the conjunctival biopsy material few epidermal cells were noted with both PAS and alciane blue positive large vacuoles in their cytoplasms. These vacuoles showed metachromasia with toluidine blue dye.

Electron microscopy revealed a great number of membrane limited inclusions in the conjunctival epidermal cells, in the endothelial cells and in the fibroblasts. These inclusions contained lamellar osmiophilic material often with a clear center. The latter component frequently contained fibrillar or fibrillogranular material. Similar inclusions were also seen in the hepatocytes (Fig. 1.a, b, Fig. 2.a, b) and in the cytoplasm of lymphocytes and polymorphonuclear leukocytes.

DISCUSSION

The different genetic types of mucolipidoses (ML) can be distinguished according to the manifestations, the onset of the



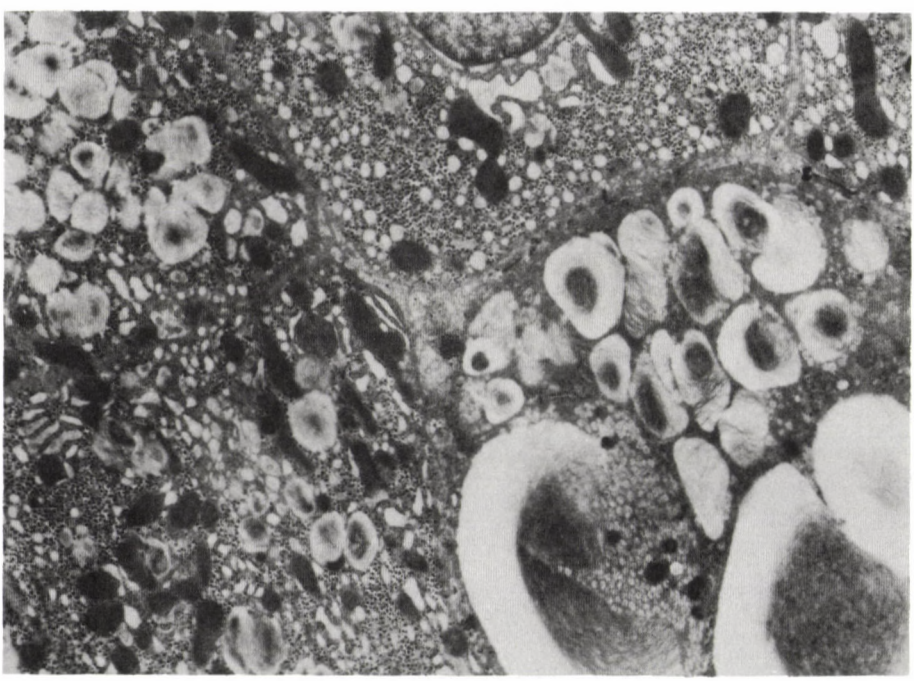


Fig. 1.a. Patient No 1.
Light micrograph of liver biopsy specimen.
Hepatocytes showed vacuolisation and a few lipid inclusions are also present x 320
Electron microscopy revealed a lot of number of membrane-limited inclusions filled with fibrillar or fibrillo-granular material x 3200

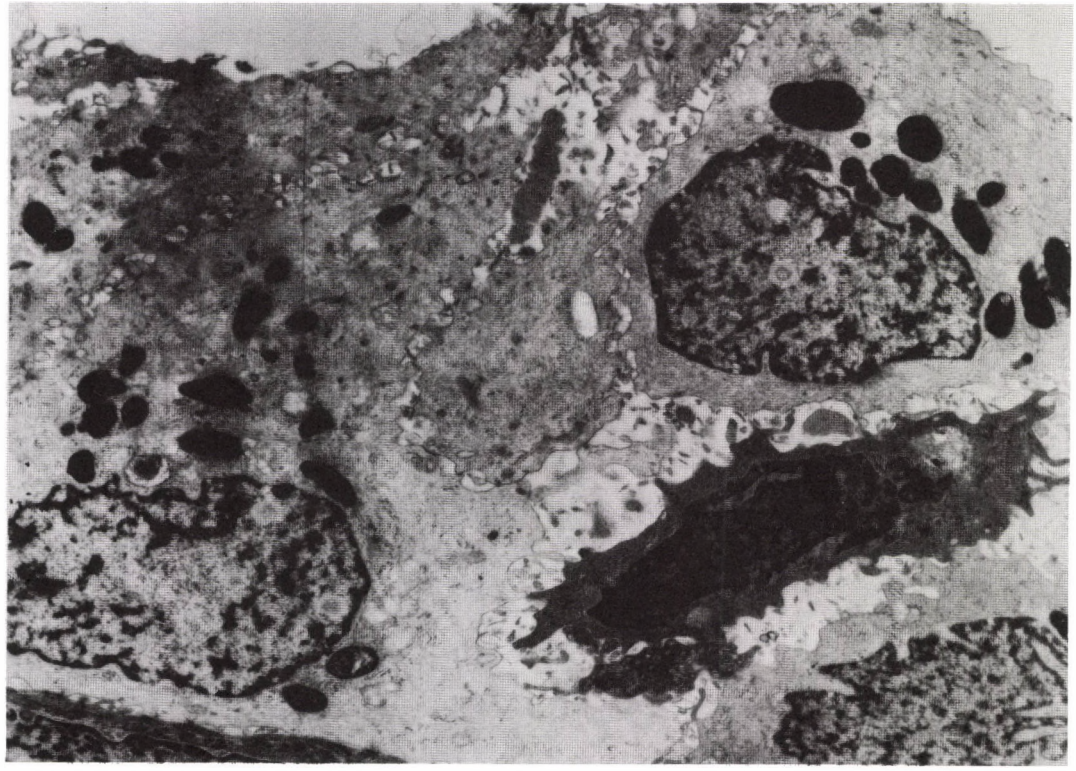


Fig. 2.a.b. Patient No 1
Conjunctival biopsy material.
Dense, lamellar material is seen in the epithelial cells, with fibroblasts in the endothel cells x 4800

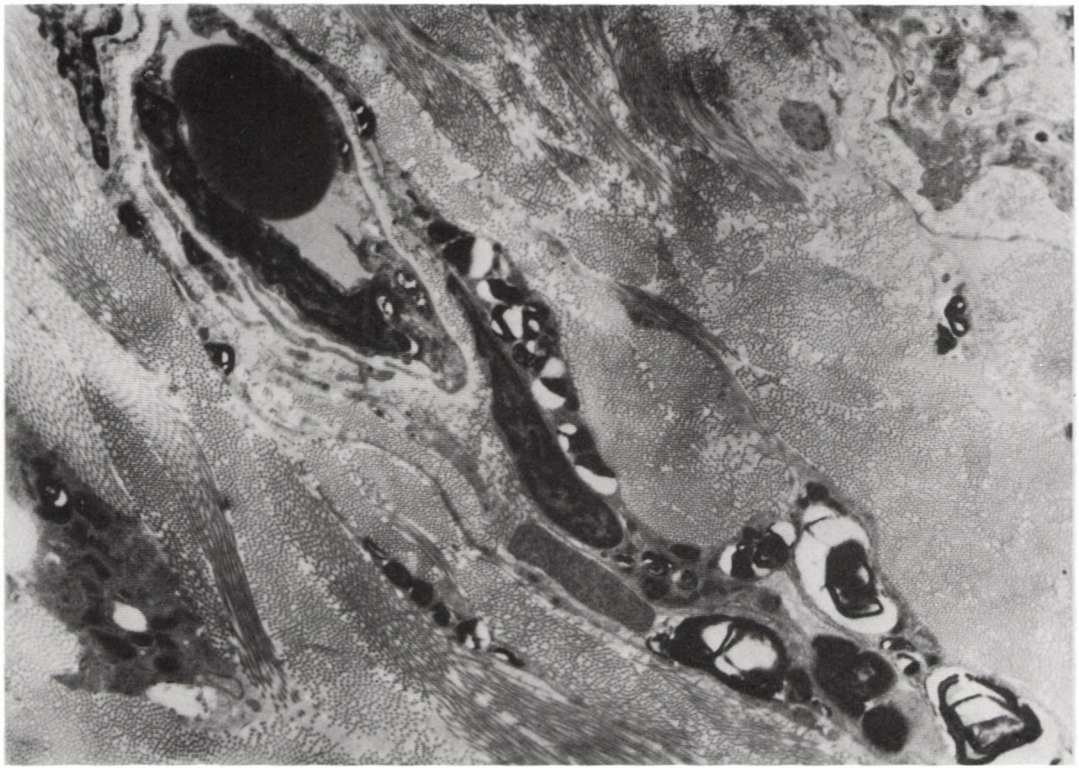


Fig. 2.a.b. Patient No 1
Conjunctival biopsy material.
Dense, lamellar material is seen in the epithelial cells, with fibroblasts in the endothel cells x 4800

disease, to the neurological deterioration, to the type of dysostosis, the lack of pathological GAG-uria, vacuolisation of lymphocytes, according to the stored substance and to the specific enzyme defects.

The type of the dysostosis is Hurler-like in ML type II, while in type III of ML there is a mild dysostosis multiplex, as we have noticed in our patients.

Cornea opacity is found only in mild degree in ML III. type but it is very frequently occurred in ML type IV. together with retinal degeneration. The split lamp finding was negative in our ML patients without any cornea opacity. The most severe mental retardation can be seen in ML type II, so-called inclusion cell disease.

Amir /1/ published the clinical spectrum and developmental features of ML-IV. analysing the data of 20 patients. The clinical manifestation of the disease - psychomotor retardation and visual impairment appeared during the first year of life.

The decreased lysosomal beta-galactosidase activity of the liver is a common biochemical feature in mucopolysaccharidosis (MPS) type I., ML type II. and III., but that is typically increased in ML type I. Multiple lysosomal enzyme deficiencies can be detected in fibroblasts and in the liver biopsy material in ML type II, so alfa-D-mannosidase, beta-D-mannosidase, beta-D-xylosidase and alfa-D-galactosidase; while beta-D-glucosidase, N-acetyl-beta-D-glucosaminidase, acid-phosphatase and alfa-D-glucosidase activities are not changed in the liver tissue.

The arylsulfatase-A activity was normal in the serum and leukocyte homogenate of our patients. The arylsulfatase-A activity is enhanced in ML type II. and contrary to the previous data it is decreased or normal in the type III. of ML. According to the clinical picture, the ultrastructural findings and the investigated lysosomal enzyme activities our patients proved to be III. type of ML manifesting in different severity of the same disease.

Specific enzyme analysis can be available for the different types of ML: sialidase for ML type I., glycoproteine-N-acetyl-

glycosamine-phosphotranferase for type II., sialoglycoprotein sialidase and sialoglangliosid sialidase for the type IV. of ML /6, 4/.

The lymphocytic vacuolisation and the storage of glycose-aminoglucans (GAG) can be detected in extreme amount in ML type I. and II., but these might be seen in type III., too.

GAG-s and glycolipids are stored in ML I. and II. types, GAG-s and neutral lipids in type III., while GAG-s, lipids and gangliosids are typical for the type IV. of ML /2, 3, 4, 5, 9/.

Ultrastructural findings of liver and conjunctival biopsy materials proved to be the most informative morphological diagnostic signs.

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Acta Paediatrica Hungarica 31 (4), pp 433-442 (1991)

ULTRASTRUCTURAL INVESTIGATIONS IN LATE INFANTILE TYPE OF CEROID LIPOFUSCINOSIS (JANSKY-BIELSCHOWSKY)

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Received 2 July 1990

Ultrastructural findings of biopsy materials of four gipsy first cousin infants suffering from late infantile type of ceroid lipofuscinosis (Jansky-Bielschowsky) were investigated.

The diagnostic significance of the conjunctival biopsy is emphasized. The pericytes and the vascular smooth muscle cells of the arterioles proved to be the main inclusion storing cells.

INTRODUCTION

The term ceroid-lipofuscinosis (CL) designates a group of inherited disorders which result in motor-sensory deterioration, mental retardation, visual impairment and early death. The disease is characterized by the accumulation of tertiary lysosomes containing an electron-dense autofluorescent storage material. Four different forms have been differentiated on the basis of age of onset, clinical course, inheritance and the ultrastructural appearance of the storage bodies, Santavuori-Haltia, Jansky-Bielschowsky, Spielmeyer-Sjögren and Kufs disease. Until recently it was generally accepted that the autofluorescent pigment probably results from non-specific peroxidation of polyunsaturated fatty acids /14/.

Diagnostic criteria are: onset of visual loss at around 5 years age, dementia occurring a few years later, extinguished electroretinograms between ages 5-12 years and typical electron microscopic findings in tissues.

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Kohlschütter /10/ analyzed the clinical variability of juvenile neuronal ceroid lipofuscinosis (JNCL) using disease specific scoring system including the patient's vision, intellect, language, motor functions and epilepsy.

The results of several authors /7, 9, 13/ suggest that ceroid-lipofuscinoses might involve a defect in the metabolism of dolichol-oligosaccharides.

In this study ultrastructural findings of 4 children suffering from late infantile type of CL (Jansky-Bielschowsky) will be presented.

PATIENTS AND METHODS

Case reports: 4 gipsy first cousin infants or children were investigated suspected for heredodegenerative disorder (Fig. 1). In the investigated family, parents are II. cousins. There are autosomal recessively transmitted genes. The affected family members who died in childhood (II/1, 2, 3 and 5) have not been investigated.

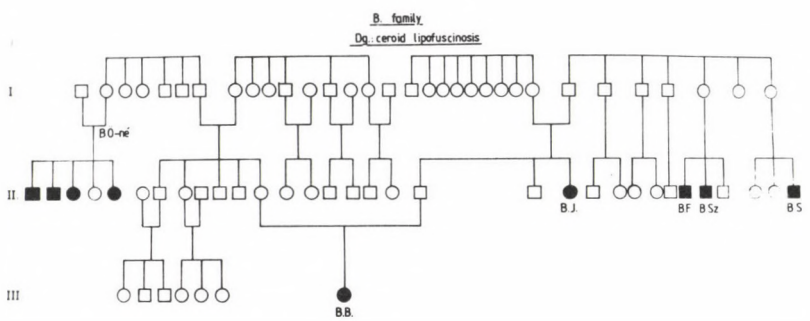

Fig. 1. Pedigree of B. family. Dg.: ceroid lipofuscinosis

Tapetoretineal degeneration, or/and optic atrophy, epilepsy (GM, or petit mal), dementia process were the main clinical symptoms. Later data are summarized in Table I.

Lysosomal hydrolases (N-acetyl-hexosaminidase-A and beta-galactosidase, arylsulfatase-A were determined by Griffith's /6/ method from the peripheral leukocytes. GM_1, GM_2 type of gangliosidosis and metachromatic leukodystrophy (MLD) had been excluded (Table II).

Light and electron microscopic examination of n. suralis, skin, conjunctival or/and liver biopsy material were carried out.

TABLE II

Lysosomal hydrolase activities of leukocyte homogenate

Name	N-acetyl-hexosaminidase total nmol/mg protein/h	N-acetyl-hexosaminidase A% nmol/mg protein/h	Beta-galactosidase nmol/mg protein/h
Szabolcs B.	745	92	81.8
Judit B.	894	80	70.4
Simon B.	609	67	85.2

	Arylsulfatase- A U/l
Szabolcs B.	76
Judit B.	51
Simon B.	48

RESULTS

Electron microscopic examination of the biopsy materials revealed inclusion bodies only in the conjunctival biopsies. The characteristic curvilinear bodies (Fig. 2) were found mainly in the pericytes or in the smooth muscle cells of small arterioles (Fig. 3) but some endothelial cells also contained such inclusions (Fig. 4). We could not demonstrate any inclusions in other cell types or organ. In the arterioles not all cells stored the specific inclusions and not all arterioles

TABLE I

Clinical and morphological findings

Case, name born	Sex	Ophthalmological visus	VEP	ERG	EEG	Onset of epilepsy	Therapy	Biopsy material EM
1. Sz.B. 5.Apr.1981 6 y. at. dg.	M	optic atrophy	extinguished		GM	18 m	Sertan B_6	skin: negative conj. +
2. J.B. 21.Nov.1982 5 y. at. dg.	F	optic atrophy	extinguished diminished amplitudes, stretched latency (l.d.) no responses (l.s.)		GM	2,5 y	Sertan B_6	skin: negative conj: +
3. S.B. 22.July 1983 5 y. at. dg.	M	optic atrophy	extinguished		PM	3 y	Sertan B_6	skin: negative conj: +
4. B.B. 12.July 1985 4 y. at. dg.	F	subnormal hardly detectable optic and retineal function	intact P_1 component	positive P_2 component subnormal nearly extinguished	slow electric activities, BNS irritative like signs	15 m	Sertan Lipoic acid B_1, B_6	liver: negative skin: negative conj.: +

M = male

F = female

P_1 = early VEP component

P_2 = late VEP component

conj. + = curvilinear bodies in endothel cells and in the pericytes of vessels.

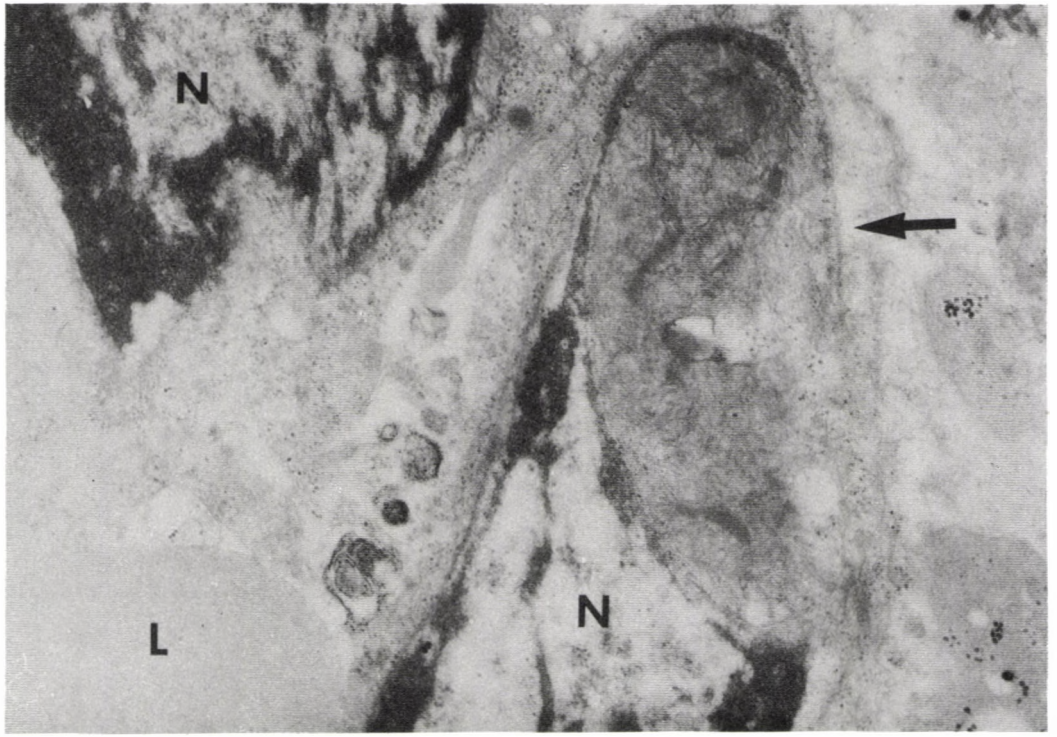

Fig. 2. Conjunctival biopsy (1461/89 K.Sz. 14.000 x EM) Intracytoplasmatic characteristic curvilinear body (arrow) in an endothelial cell compressing the nucleus (N)

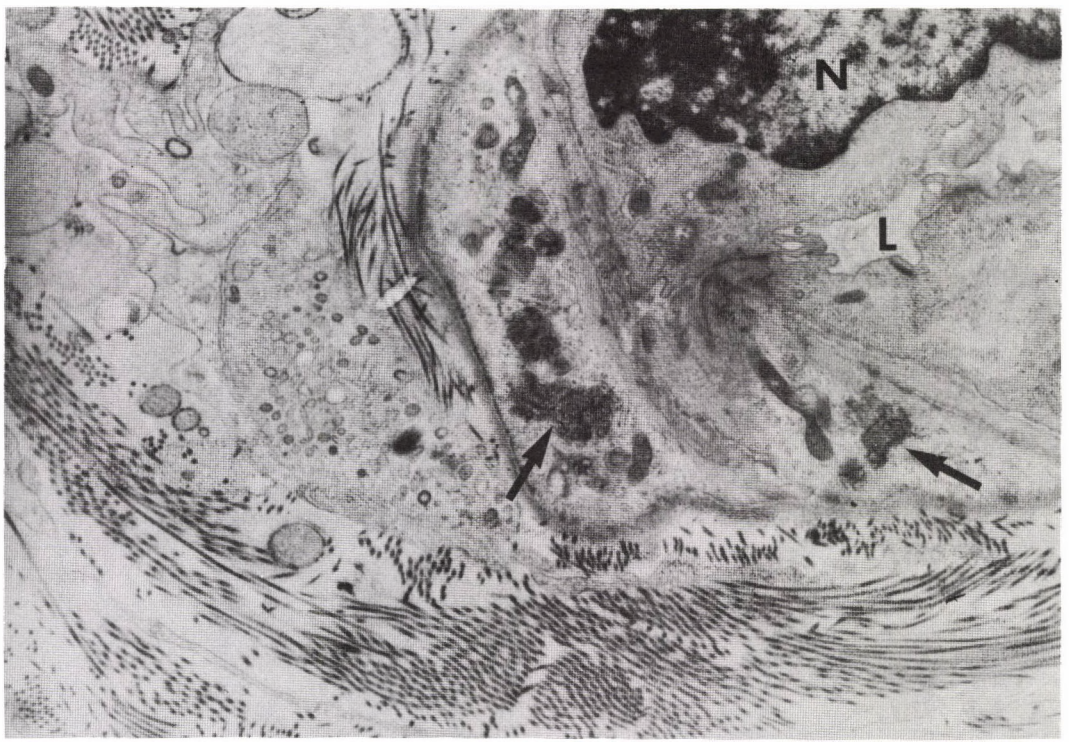

Fig. 3. Conjunctival biopsy (291/88 K.Sz. 6.000 x EM) curvilinear bodies (arrow) in the smooth muscle cells or in pericytes of arteriole (L=lumen of the arteriole)

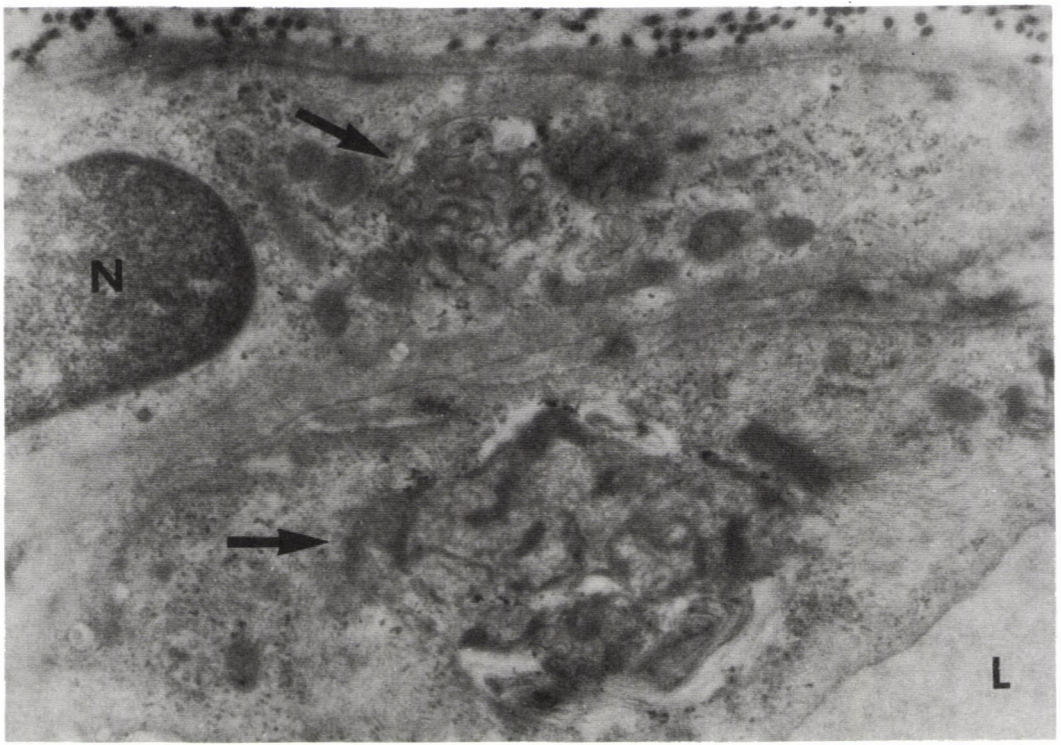

Fig. 4. Conjunctival biopsy (2722/88 K.SZ. 14.000 x EM) curvilinear bodies in an endothelial cell and a smooth muscle cell of an arteriole

contained much storing cells. The rare endothelial inclusions could be detected only after careful examination.

DISCUSSION

Kohlschütter /10/ detected typical inclusions in the rectal biopsy material of juvenile neuronal CL patients. The above-mentioned authors's score system allows definition of relatively mild and severe courses representing the variability of JBCL. Our patients must be regarded as the severe form of the disease, they are not juvenile but late infantile type.

Goebel /4/ proposed muscle biopsy examination in neural CL and also ultrastructural analysis of the peripheral nerve biopsy tissues /5/. Haynes /8/ considered electron microscopic analysis of a skin biopsy sample and of the lymphocytes to be informative in the above disease. Fingerprint-like ultrastructural formations of the lymphocytes were reported /2/ to be pathognostic in juvenile NCL. Investigating the chronic neurological diseases of childhood in conjunctival and skin biopsies the CL-s were the largest group in the patient material of Arsenio-Nunes /1/b/. In the latter material three conventional cytosome types were demonstrated with a predominance of granular inclusions in the early infantile form, of curvilinear bodies in the late infantile form and of fingerprint bodies in the juvenile form /1/a, 3, 12/. Electron microscopic study of skin and conjunctival biopsy specimens is an important diagnostic tool in chronic progressive encephalopathies /11/. Ultrastructural abnormalities are not entirely specific, as the finding of multilamellar bodies may be seen in a variety of different conditions. The biochemical techniques are available for the diagnosis of many chronic neurological diseases and these are more specific than morphological techniques, but the electron microscopic investigation may confirm and document diagnoses already suspected on the basis of the history, clinical examination and

other laboratory tests, even in the absence of a specific or available biochemical methods. According to our results the conjunctival biopsy seems to be not only a suitable but sufficient biopsy sampling. The main inclusion storing cells in the late infantile type of the CL are the pericytes, the vascular smooth muscle cells and the endothelial cells. During the ultrastructural investigation a more careful examination should be taken of the arterioles.

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Acta Paediatrica Hungarica 31 (4), pp 443-456 (1991)

SURGICAL MANAGEMENT OF PULMONARY ATRESIA WITH INTACT VENTRICULAR SEPTUM. RIGHT VENTRICULAR SIZE AS A GUIDELINE FOR SURGICAL INTERVENTION

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Received 17 April 1991

61 infants with critical valvular pulmonary stenosis (21 cases) or pulmonary atresia with intact ventricular septum (40 cases) were operated on between 1975-1989 in the Semmelweis University Medical School. Right ventricular volume, area, outflow tract dimension and tricuspid anulus diameter was measured by angiocardiography and echocardiography to evaluate right ventricular size, and to predict the operative outcome using these data. Our results suggested, that if the normalized right ventricular volume was less than 3 ml/m^2, the normalized right ventricular area was less than 2.5 cm^2/m^2, and the normalized right ventricular area was less than 2.5 cm^2/m^2 and the normalized tricuspid anulus diameter was less than 1.2 cm^2/m2/3 only a systemopulmonary shunt procedure is needed. In all other cases pulmonary valvotomy is necessary to decompress the right ventric and to help increase the right ventricular size. Where a different part of the right ventricle is hypoplastic a systemopulmonary shunt procedure is needed too. In the follow up period the right ventricular dimension and tricuspid valve diameter was measured by echocardiography. This noninvasive assessment can predict the preoperative diagnosis, postoperative outcome and demonstrates an adequate growth of the right ventricle after pulmonary valvotomy.

INTRODUCTION

Pulmonary atresia with intact ventricular septum (PAIVS) and critical valvular pulmonary stenosis (IS) are uncommon congenital cardiac anomalies accounting for less than 1% of all congenital heart diseases. The surgical intervention is

essential for survival, nonetheless, a high mortality rate is associated with this lesion. The purpose of this report is to determine: 1.) is there any satisfactory repair to provide adequate pulmonary blood flow and to maximize the development of the right side of the heart, 2.) is the right ventricle able to increase after pulmonary valvotomy, 3.) are 2-dimensional echocardiographic measurements useful predictive indexes of late outcome?

METHODS

Between 1975 and 1989 61 babies were operated on in the Semmelweis University Medical School Second Paediatric Department (40 cases with PAIVS, 21 cases with PS). The age at the time of operation ranged from 1 day to 42 days (mean: 10 days), most of them were younger than 7 days. 3 babies were older than 28 days. Their weight was between 1.9 and 4.2 kg (mean: 3.4 kg).

In 40 cases between 1975 and 1984 the diagnosis was based on angiocardiography. At cardiac catheterization the right ventricular pressure was suprasystemic and routine balloon atrial septostomy was carried out in all cases.

Between 1985 and 1989 21 babies were operated on the basis of echocardiographic diagnosis. Right ventricular and atrial measurements were performed in the apical four chamber view. The dimensions of the right ventricle were measured at the end of diastole. Right ventricular outflow tract was measured as the shortest distance between the aorta and the anterior wall of the right ventricle (Fig. 1). We used direct computer analysis of the echocardiographic image for right ventricular volume analysis. Right ventricular volume calculated by echocardiography and angiography presents reasonable correlation /13/.

RESULTS

The choice of surgical approach was based on the preoperative angio or echocardiographic determination of right size, as described in recently published reports /1, 6, 7, 10, 12, 13, 14/. Our cases were divided into 3 groups: 1.) the normal size or large right ventricle, 2.) the hypoplastic right ventricle, 3.) the intermediate-size right ventricle, where a

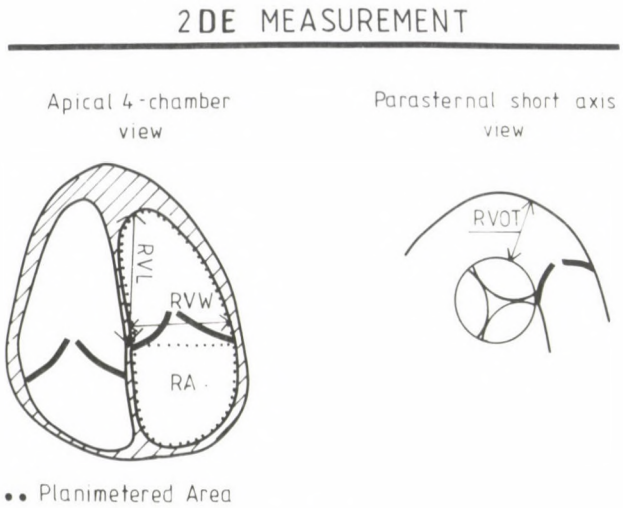

Fig. 1.

different portion of the right ventricle is hypoplastic. Since 1984 every baby was initially stabilized routinly with an intravenous infusion of Prostaglandin E2 and followed during the critical postoperative period.

Group 1. 32 babies with normal size or enlarged right ventricles underwent only pulmonary valvotomy (Table I). A transventricular pulmonary valvotomy was performed, on the first 4 babies but all cases died. Since then we have only done transpulmonary valvotomy: in 4 babies with extracorporeal circulation (2 cases died), in 11 babies under inflow occlusion (3 cases died). Through left thoracotomy transpulmonary valvotomy was carried out on 13 babies, 5 of which died. We have followed up 18 cases from 3 months to 14 years with 1 late death. All 17 survivors are showing excellent results no patient needed a second operation, and the right ventricle size is normal now.

Group 2. In the case of an extremely small right ventricle a Waterston-Cooley systemopulmonary artery shunt was constructed in 17 cases (Table I). The early result was satisfactory with only 4 deaths. 3 of these babies had myocardial sinusoidal - coronary artery communications. In the follow up period we lost

TABLE I

	mortality			
	cases	early	late	follow up
1. NORMAL RIGHT VENTRICULAR SIZE	32	14	1	17
Transventricular valvotomy	4	4	-	-
Transpulmonary valvotomy				
extracorporeal circulation	4	2	-	2
inflow occlusion	11	3	1	7
through left thorecotomy	13	5	-	8
2. HYPOPLASTIC RIGHT VENTRICLE	17	4	9	4
Waterston-Cooley shunt				
3. INTERMEDIATE-SIZE RIGHT VENTRICLE	12	8	-	4
Transpulmonary valvotomy - shunt				

9 patients; 2 of them had myocardial sinusoids. In 4 patients the criteria for a Fontan circulation were not fulfilled and are being considered for definitive palliation. The right ventricle was still hypoplastic in all cases, and now 4 cases are awaiting Fontan procedure.

Group 3. In those cases where with a different portion of the right ventricle is hypoplastic, we have created a left-sided modified Blalock-Taussig shunt to improve the pulmonary circulation, and then a transpulmonary valvotomy to decompress the right ventricle in the hope that this may enhance the long-term prospect of right ventricular growth. 12 patients were operated on, 8 babies died (Table I). 2 autopsied cases had long-segment infundibular atresia, 1 baby had myocardial sinusoids and another had an interruption of the left anterior descending coronary artery. In the follow up period no patients were lost. The right ventricular size became normal in 2 cases and we had to reconstruct the right ventricular outflow tract in 2 other cases.

We measured the data of the right ventricular volume adjusted for body surface area, and tricuspid anulus dimension adjusted for the cube root of the body surface area (Fig. 2a). In those 21 babies whose diagnosis was based on preoperative echocardiography, 3 babies required only shunt procedures (black points). The dotted lines indicate the region below which a shunt is unquestionably needed, and the range above which a shunt appears unnecessary if adequate relief of right ventricular outflow tract obstruction is provided. Between these two dotted lines the valvotomy and the shunt procedure are necessary at the same time. In patients with either a normalized right ventricular volume of less than 3 ml/m^3 or a normalized tricuspid anulus of less than 1.2 $cm/m^3/3$ only a shunt was necessary.

We got a more demonstrable correlation between the right ventricular area adjusted for body surface area and the tricuspid anulus dimension (Fig. 2b). In patients with a normalized right ventricular area of less than 2.5 cm/m3, a shunt was needed.

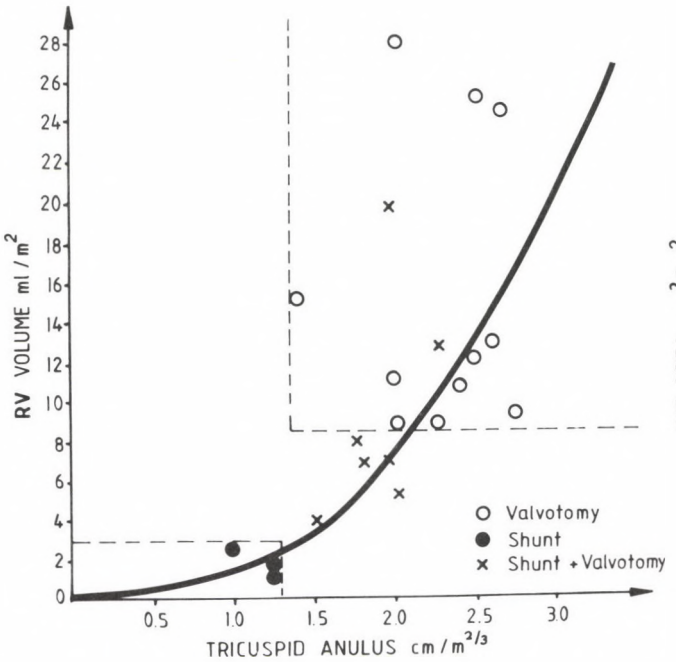
PREOP 2DE MEASUREMENTS
(21 PTS)
PREDICTION OF SURGICAL OUTCOME
RV VOLUME ml/m²
TRICUSPID ANULUS cm/m^2/3
Valvotomy
Shunt
Shunt + Valvotomy

Fig. 2a.

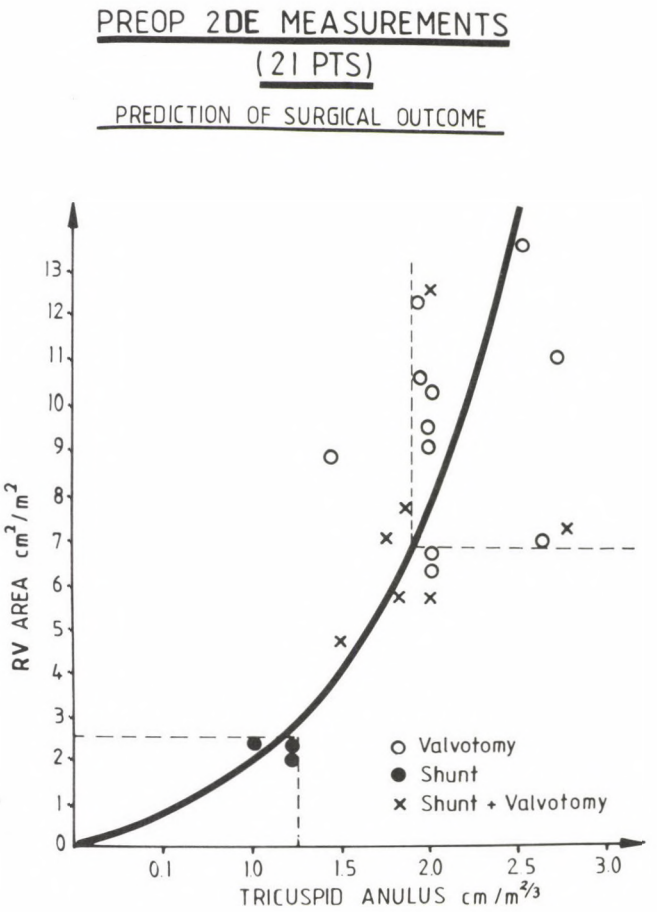
PREOP 2DE MEASUREMENTS
(21 PTS)
PREDICTION OF SURGICAL OUTCOME
RV AREA cm²/m²
TRICUSPID ANULUS cm/m^2/3
Valvotomy
Shunt
Shunt + Valvotomy

Fig.2b.

Our findings indicate that echocardiographic measurement of right ventricular volume or area and tricuspid anulus diameter is useful in predicting operative outcome.

The normalized right ventricular volume and area in the pre- and postoperative period was measured in 10 surviving patients from this group (Fig. 3a). The follow-up period ranged between 3 months and 4 years (mean: 2 years). In group 1. preoperatively the right ventricular volume was in the normal range and there was no significant change. In group 3. preoperatively the right ventricular volume was below the normal values. After the first operation in 2 case there was a significant improvement but in the other 2 cases (because of the unsatisfactory result) we had to perform a right ventricular outflow tract reconstruction as well. Measuring the right ventricular area (Fig. 3b) we got the same result.

In 1989 25 of the 61 operated cases, were alive. The follow-up period ranged between 3 months and 14 years (mean: 7 years). In all cases we measured the right ventricular area, tricuspid anulus dimension and right ventricular outflow size by echocardiography and compared this data with normal population /9/. The right ventricular area in all cases with pulmonary valvotomy was within the normal range, but in 4 cases with only shunt procedure, the right ventricular size is still small (Fig. 4a). We can find the same result in the measurement of the tricuspid anulus dimension, in those 4 cases the tricuspid valve is also very small (Fig. 4b). It was impossible to measure the right ventricular outflow tract in 3 cases, and was very small in 1 case in group 2., but in the other cases it was within the normal range (Fig. 4c).

DISCUSSION

Pulmonary atresia with intact ventricular septum is not a simple malformation, its presence generally leads to cavitary hypoplasia of the right ventricle, associated with varying degrees of hypoplasia, insufficiency and stenosis of tricuspid valve, the presence of long segment infundibular hypoplasia,

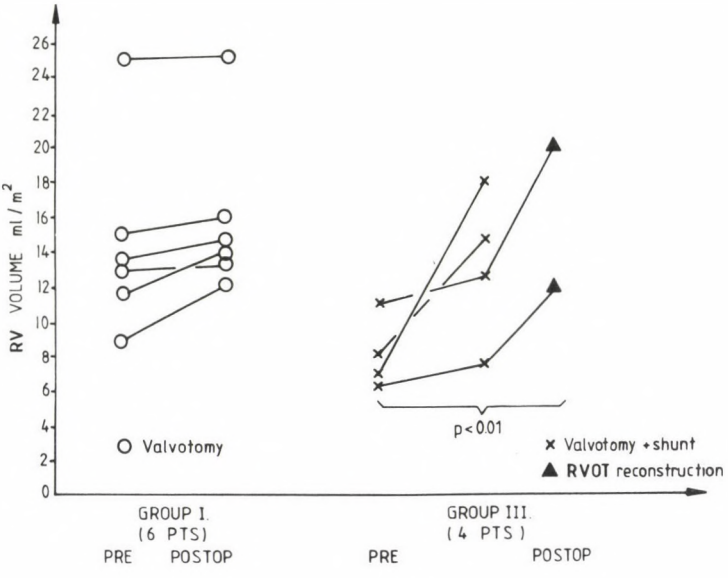
RV VOLUME BEFORE AND FOLLOWING SURGERY
RV VOLUME ml/m²
26
24
22
20
18
16
14
12
10
8
6
4
2
0
p<0.01
O Valvotomy
× Valvotomy +shunt
▲ RVOT reconstruction
GROUP I.
(6 PTS)
PRE POSTOP
GROUP III.
(4 PTS)
PRE POSTOP

Fig. 3a.

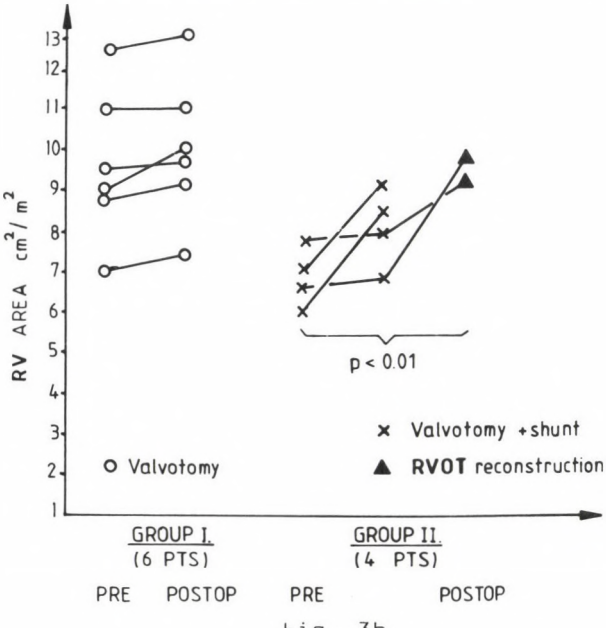
RV AREA BEFORE AND FOLLOWING SURGERY
RV AREA cm²/m²
13
12
11
10
9
8
7
6
5
4
3
2
1
p< 0.01
O Valvotomy
× Valvotomy +shunt
▲ RVOT reconstruction
GROUP I.
(6 PTS)
PRE POSTOP
GROUP II.
(4 PTS)
PRE POSTOP

Fig. 3b.

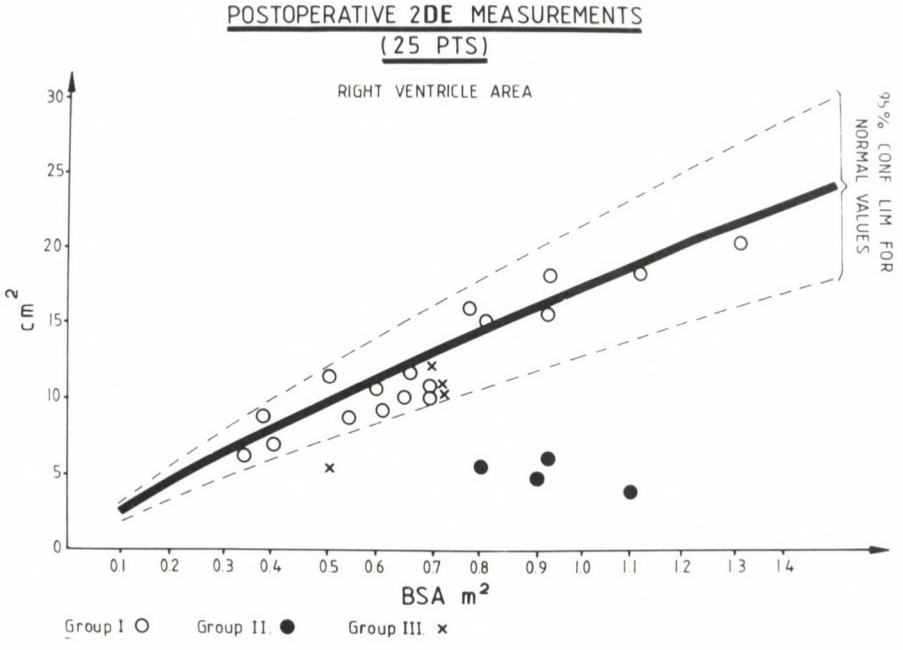
POSTOPERATIVE 2DE MEASUREMENTS
(25 PTS)
RIGHT VENTRICLE AREA
95% CONF LIM FOR NORMAL VALUES
cm²
BSA m²
Group I ○ Group II ● Group III ×

Fig. 4a.

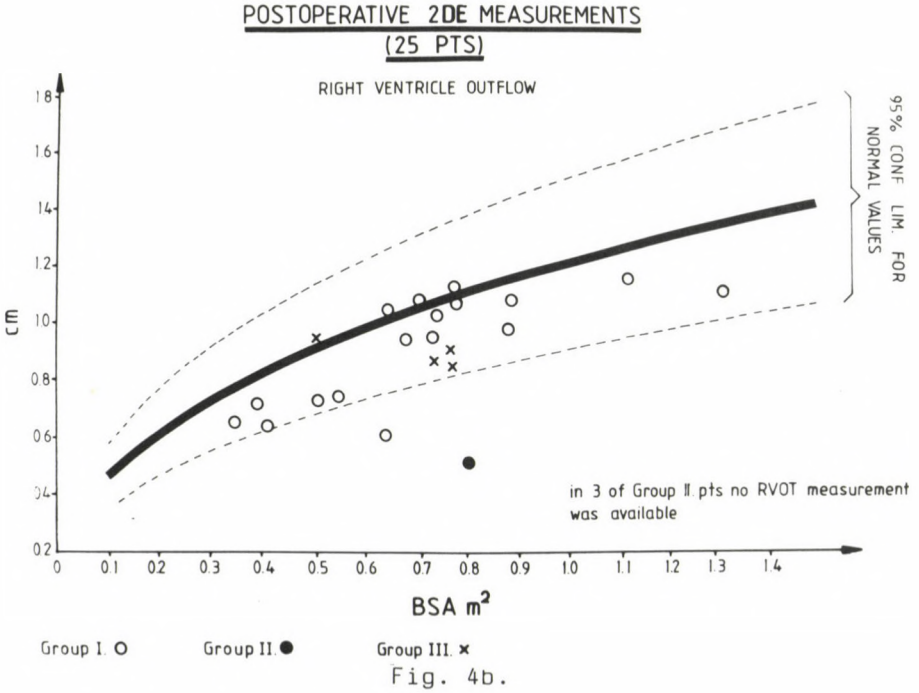
POSTOPERATIVE 2DE MEASUREMENTS
(25 PTS)
RIGHT VENTRICLE OUTFLOW
95% CONF LIM. FOR NORMAL VALUES
cm
in 3 of Group II pts no RVOT measurement was available
BSA m²
Group I ○ Group II ● Group III ×

Fig. 4b.

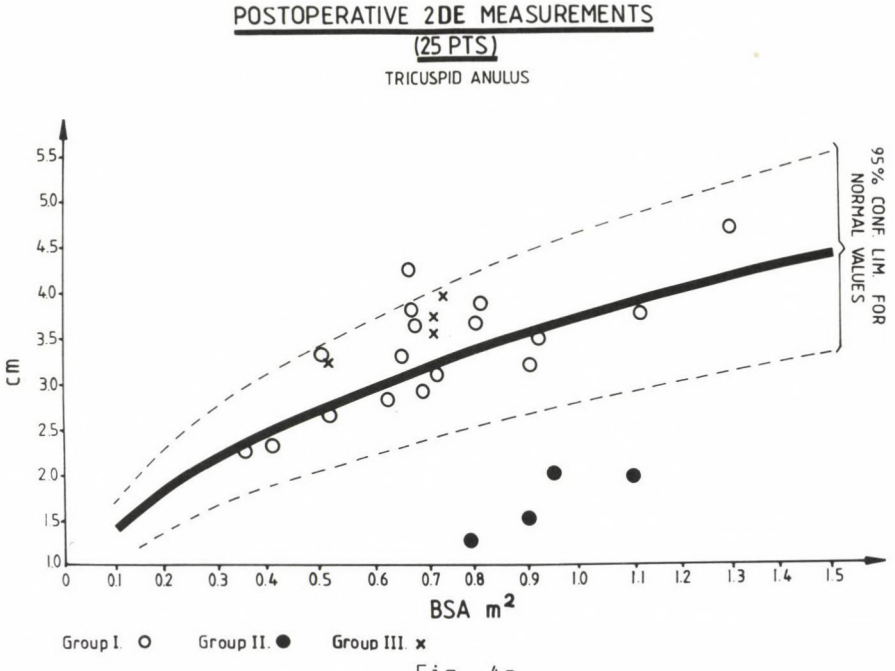

Fig. 4c.

myocardial sinusoids, or the recently described interruption of the left anterior descending coronary artery /12/. The operative and postoperative management of these infants remains controversial and the surgical results unsatisfactory. The importance of this procedure may lie in the implications regarding the ability of the right ventricle to grow. Pulmonary valvotomy is recommended by most authors to relieve the right ventricular obstruction. It is clear, however, that pulmonary blood flow after valvotomy is often unsatisfactory and the combination of valvotomy and a systemic-pulmonary shunt appears to be the most commonly used initial approach /2,16/. Furthermore, valvotomy often does not relieve the suprasystemic right ventricular pressures initially and all children require an outflow patch eventually. There have been several isolated reports of survival in neonates and infants with this lesion after reconstruction of the right ventricular outflow tract by a transanular patch /4,16/, but the mortality has been thought to be unacceptably high /2,4,5/.

The introduction of Prostaglandin E (PGE2) therapy has solved he initial problem of ductus-dependent pulmonary blood flow. The ability of PGE2 to open the ductus, maintain ductal patency, and stabilization of these infants is now widely recognized. PGE2 infusion has improved the preoperative condition of these infants and has eliminated the need for emergency operation. All of the patients received and responded favorably to a preoperative infusion of PGE2. With careful wearing of the PGE2 infusion the results have been excellent. After isolated pulmonary valvotomy a shunt can be placed if the right ventricular compliance and pulmonary blood flow remain unsatisfactory after several days or longer. The use of PGE2 in the immediate postoperative period can preserve adequate pulmonary blood flow while right ventricular compliance improves with better ventricular relaxation, resolution of operative edema, and possibly recession of hypertrophy.

An important point is that the atrial septum is allowed to remain open in these infants with hypoplastic right ventricle after only systemic-pulmonary shunt procedure, but in other cases with pulmonary valvotomy, the atrial septum defect is not necessary.

Some authors have found a close correlation between tricuspid valve diameter and right ventricular size, this more accurately measured variable was thought to provide a better estimation of the real right ventricular size /3,6,8,11/. Our finding based on 21 patients pre- and postoperative echocardiographic measurements, indicate that echocardiographic measurement of right ventricular volume, area and tricuspid anulus diameter are useful in predicting postoperative outcome. If the ventricular volume is normal, only pulmonary valvotomy is necessary. However, if patients have a normalized right ventricular volume of less than 3 ml/m^2, a normalized right ventricular area of less than 2.5 cm/m^2, or a normalized tricuspid anulus of less than 1.2 cm/m^2/3 a shunt procedure is required. Correct management in these patients with intermediate values remains problematic. It is possible to distinguish those who required a shunt procedure from those in

whom only repair of pulmonary stenosis is needed by combined utilization of right ventricular volume, area and tricuspid valve anulus diameter. Thus, in this intermediate group a shunt procedure and pulmonary valvotomy are necessary if the normalized right ventricular volume is higher than 3 ml/m^2, area higher than 2.5 cm/m^2 and the tricuspid valve diameter higher than 1.2 $cm/m^2/3$.

CONCLUSION

1. Echocardiographic determination of right volume, area, outflow tract dimension and tricuspid anulus diameter proved to be a satisfactory index for the selection of initial palliative procedures and also for later definitive operations.

2. With a normalized right ventricular volume of less than 3 ml/m^2 or a normalized tricuspid anulus diameter of less than 1.2 $cm/m^2/3$ a shunt procedure is needed.

3. This method provides a useful means of predicting the postoperative outcome in patients with reduced ventricular size.

4. Pulmonary valvotomy should be attempted in all patients in whom an outflow tract is identified, so as to maximize the potential for right ventricular growth.

5./ Our study demonstrated inequivocal growth of the right ventricle after pulmonary valvotomy.

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Acta Paediatrica Hungarica 31 (4), pp 457-462 (1991)

ACUTE HEAD INJURIES IN CHILDREN - A REVIEW OF 100 CONSECUTIVE PATIENTS

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Received 15 Oct 1990

Head injuries of children between 4-16 years in the first hour after the trauma have been studied. The neurological examination was completed by CT-scan. Glasgow Coma Score (GCS) as well as Glasgow Outcome Scale (GOS) were also applied and proved to be very helpful. Our findings show a significantly frequent occurrence of epidural haematomas. Their treatment in patients with GCS above 8 was in 90% successful.

INTRODUCTION

Patient's age is one of the most important factors in the prediction of outcome after head injury /1, 2, 3, 9, 10/. It is generally accepted, that children suffer a lower risk of mortality from head injury than adults /1, 2, 3, 10/ and also have a lower rate of occurrence of intracranial haematomas /10, 11, 12/. Some authors reported a better outcome in children younger than 10 years /3/ compared to adolescents.

In this study we analysed 100 consecutive children admitted to our Department directly after head trauma. All patients with concomitant extracranial injury were excluded from the study due to the important influence of this injury on outcome, especially in cases with chest trauma /8/ or lower extremities fractures /7/. It must be pointed out, that as a rule we do not admit patients younger than 4 years.

Akadémiai Kiadó, Budapest

MATERIAL AND METHOD

The files of 100 consecutive patients younger than 16 years admitted to Department of Neurosurgery of Medical Academy of Łódź within the first hour after head trauma were respectively analysed. On admission the patients were examined neurologically and CT-scans performed. The level of consciousness was evaluated using Glasgow Coma Scale /13/.

59 children were younger than 10 years, but only 2 were below 4 years. Eighty-four percent of head injuries were traffic accident-related. Table I shows the mechanism of injury related to patient's age. Twenty-one children were admitted with GCS 3-5 points, 23 with 6-8 points and 56 had 9-12 points. There were no patients with GCS above 12.

TABLE I

The mechanism of head injury related to the age of the patients

Age (y)	Mechanism of injury			
	Traffic accidents	Falls	Assault	Total
0 - 3	1	1	0	2
4 - 7	16	2	0	18
8 - 10	33	3	3	39
11 - 15	34	4	3	41
Total	84	10	6	100

Clinical examination and CT-scans led to the diagnosis of concussion in 34 cases and to brain contusion in 37. In 6 cases from the last group CT findings showed diffuse axonal injury - in 9 of the remaining 31 cases CT findings showed diffuse brain swelling. 13 children had mass lesions - 8 epidural, 2 subdural and 3 intracerebral haematomas. In 15 cases CT showed skull fractures without brain damage, but in 10 of them there were opened fractures. All children with epidural haematomas had posttraumatic fractures. Table II shows the comparison between GCS on admission and clinical diagnosis.

13 patients with expanding mass lesion and opened skull fractures were treated surgically. All children with GCS below 6 points were managed with artificial ventilation to maintain the arterial pCO_2 between 25 and 30 mmHg. In all cases with symptoms on intracranial hypertension mannitol and diuretics were used. Steroids were never used.

TABLE II

Glasgow Coma Score on admission related to CT and clinical diagnosis

Diagnosis	GCS			
	3 - 5	6 - 8	9 - 12	Total
Concussion	0	6	28	34
Brain contusion	7 (6)	9 (4)	15	31 (10)
Diffuse axonal injury	6	0	0	6
Skull fracture:				
- closed	0	0	5	5
- opened	0	2	8	10
Haematoma:				
- epidural	3	5	0	8
- subdural	2	0	0	2
- intracerebral	3	0	0	3
SAH	0	1	0	1
Total	21	23	56	100

( ) = diffuse brain swelling

Outcome status was evaluated 3 months after injury according to Glasgow Outcome Scale (GOS) /6/. The overall mortality was 14% ; all the children who died were admitted with a GCS of 3-5 points. All the patients (6 cases) with diffuse axonal injury or subdural haematoma (2 cases) died. Of the 31 patients with brain contusion, 5 who showed diffuse brain swelling on CT - died. Only 1 of the 8 children with epidural haematoma died. In 73 cases the result of treatment was good.

Table III shows the relationship between GOS and GCS on admission, and Table IV shows the results of treatment related to clinical diagnosis.

TABLE III

Glasgow Coma Score on admission related to outcome (Glasgow Outcome Scale)

GOS	GCS			
	3 - 5	6 - 8	9 - 12	Total
I	1	9	39	49
II	4	7	13	24
III	2	7	4	13
IV	0	0	0	0
V	14	0	0	14
Total	21	23	56	100

DISCUSSION

Luerssen /10/ found that mortality declined with increasing age in the pediatric age group. In our analysis there was no difference among different age groups, but it can be due to the fact, that we had almost no patients younger than 4 years.

Surgical mass lesions occurred in 13% of the patients. It confirms the findings of Alberico /1/ that in pediatric population there is a trend toward a lower incidence of surgical lesions than in adults. We found statistically significant ($p < 0.001$) difference between the frequency of epidural and subdural hematomas, epidural being 4 times more frequent than subdural ones, which is opposite to the findings of the other authors /10/. However, subdural hematomas have a bad prognosis, both children died, while in patients with epidural lesions the result of treatment was good in 7 of the 8 patients. We must also point out that all children with mass lesions were operated within two hours after the trauma.

In 10% of patients there was an opened skull fracture. Such high frequency of this lesion can suggest that the skull of the child is much less resistant to high velocity trauma than in adults.

TABLE IV

Outcome (GOS) related to CT and clinical diagnosis

Diagnosis	GOS					
	I	II	III	IV	V	Total
Concussion	32	2	0	0	0	34
Brain contusion	4	13	9	0	5	31
Diffuse axonal injury	0	0	0	0	6	6
Skull fracture:						
- closed	3	2	0	0	0	5
- opened	5	3	2	0	0	10
Haematoma:						
- epidural	5	2	0	0	1	8
- subdural	0	0	0	0	2	2
- intracerebral	0	1	2	0	0	3
SAH	0	1	0	0	0	1
Total	49	24	13	0	14	100

GCS on admission proved to be the most important prognostic factor. Deaths only occurred in children admitted with GCS below 6 points, and in 90% of patients with GCS above 8 we achieved a good result of treatment.

Diffuse brain swelling found in CT scans /14/ led to 50% mortality, thus we do not agree with the opinions of the others, that it usually takes a benign clinical course /4/. When the patient presented symptoms of diffuse axonal injury, the prognosis was always bad, and all children died.

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Acta Paediatrica Hungarica 31 (4), pp 463 (1991)

BOOK REVIEW

Christian Deindl, Gert Pistol: Atlas Kinderchirurgischer Sonographie. Hippocrates Verlag, Stuttgart, 1991. 298 pages.

The Ultrasonographic Atlas of Pediatric Surgery was published in 1991, by Hippocrates Verlag.

It is divided into three main chapters:

I. Ultrasonographic differential diagnosis of characteristic pediatric surgery symptoms.

II. Ultrasonographic signs of complications after surgery.

III. Further possibilities of ultrasonography.

In the first chapter there are sonographic pictures of skull-injuries, tumors and swelling of the head and neck areas, vomiting, icterus, recurrent abdominal pain, abdominal trauma, urinary tract infection, congenital malformation of the urinary tract. The second chapter deals with the complications of thoraco-abdominal renal and urinary tract, and central nervous system surgery. In the third chapter there are pictures of intra-operative, interventional and anorectal sonography. On one page there is the ultrasonogram of one or two diseases, and beside them are found very briefly and clearly the diagnosis, anamnesis, symptoms, ultrasonography, differential diagnosis and pathography of the patient. At the end of the chapters there is a short discussion and literature. In some cases the quality of the pictures are very bad, in the foreword the authors point out this problem, because the investigations were often made in emergency situations. The atlas is well edited, the text is short, sticks to the essentials, illustrations are explicit but sometimes the suboptimal quality of the images renders difficulty in recognition.

The atlas is recommended to specialists in ultrasonography, pediatric surgeons and pediatricians.

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PRINTED IN HUNGARY

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INSTRUCTIONS TO AUTHORS

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