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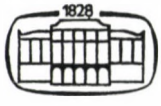

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1985

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Plasma hypoxanthine and xanthine levels in the early newborn period in problem-free preterm babies and those with idiopathic respiratory distress syndrome

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The use of hypoxanthine measurements for quantitative monitoring of intrauterine asphyxia is generally accepted. A high level in blood or in CSF is a consequence of tissue hypoxia. Hypoxanthine and xanthine were measured by selective high pressure liquid chromatography in mature newborns, in healthy, symptom-free preterm babies, and in preterm babies affected by idiopathic respiratory distress syndrome. The measurements were carried out from peripheral venous blood within three hours after birth and at the age of 48—72 hours. In mature newborns the mean hypoxanthine level was 11.10 μmol/l in the early determinations, and 8.45 μmol/l in the second set of measurements. In unaffected prematures there were significantly higher levels, and the highest values (44.22±15.13 μmol/l) were encountered in premature babies subsequently dying of severe hypoxia. Xanthine showed a similar course. In addition to establishing normal values for prematures we desired to clarify the changes in the levels of purine metabolites during idiopathic respiratory distress and their prognostic value. Hypoxanthine and xanthine levels were found to be informative in postnatal hypoxia, especially together with other parameters.

Catabolism of purine nucleotides is a complex process consisting of several steps. In man, the final metabolite is uric acid arising by oxidation of hypoxanthine and xanthine, a process catalyzed by xanthine oxidase (Fig. 1). Metabolism of nucleic acids, purine containing coenzymes and nucleotides carrying high-energy phosphate bounds is channelled to this final step. The relationship of oxygen and energy carrying purine compounds has long been known. In hypoxia, characterized by anaerobic conditions, their synthesis is impaired and their breakdown is enhanced, ATP is used up [5]. Hypoxanthine, an intermediary product of purine catabolism, is regarded as a good indicator of the severity of hypoxia at all ages [12]. In neonatal hypoxia its level may be increased by two factors. First, ATP is used up at a dramatic speed, this leads to formation of ADP, AMP and hypoxanthine. Second, there is a direct action of the lack of oxygen; xanthine oxidase, converting hypoxanthine to xanthine and xanthine to uric acid, only works in the presence of oxygen. The enzyme ceases to function in hypoxia, thus resulting in an elevation of hypoxanthine and xanthine levels in tissues and body fluids [11].

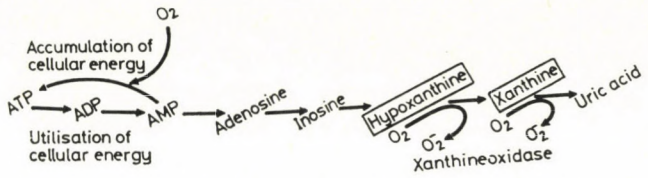

FIG 1. Breakdown of purine derivatives

Improvement inlaboratory techniques added hypoxanthine measurement to the list of indicators of hypoxia; lactate, base deficit and pH measurements.

For exact measurement of purine metabolites, enzymatic, column chromatographic and oxygen electrode methods are available [13]; the method utilizing oxygen electrodes is the most exact one but its drawback is its inability to determine the individual metabolites separately, thus to distinguish between xanthine and hypoxanthine. High pressure liquid chromatography (HPLC) is now the method of choice, being suitable for separate determination of hypoxanthine and xanthine [2, 4, 6, 9, 10, 14].

HPLC was used for measuring the plasma hypoxanthine and xanthine levels in symptom-free premature babies and those affected by idiopathic respiratory distress. Mature healthy babies born by normal delivery after an uneventful pregnancy were chosen for a control group.

MATERIAL

Hypoxanthine and xanthine determinations were performed in all babies admitted ot our intensive care centre, without any selection during the study period. Gestational age was determined on the basis of obstetrical data and the maturity score of Dubowitz. Babies exhibiting intrauterine growth retardation with clinical symptoms of injury were excluded from the study. The distribution of newborns according to gestational age and birthweight is shown in Fig. 2; birthweight

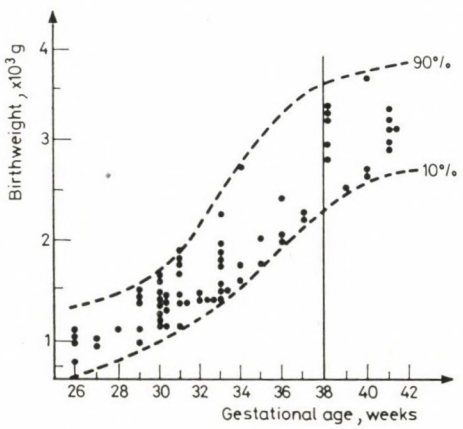

FIG 2. Distribution of newborns according to gestational age and birthweight

TABLE I

Grouping of newborns and distribution of prematures affected by idiopathic respiratory distress syndrome, according to outcome, mean gestational age and birthweight in the various groups

TABLE Ia

Newborns studied

	Age at blood sampling	
	Within 3 hours after birth	48—72 hours
Number of cases	70	60
Mature newborns		
n	15	15
gestational age, weeks	39.3±1.0	39.3±1.0
birthweight, g	2918±406	2918±406
Premature newborns		
Healthy		
n	24	24
gestational age, weeks	32.1±2.8	32.1±2.8
birthweight, g	1444±397	1444±397
IRDS		
n	31	21
gestational age, weeks	30.6±2.7	31.4±2.8
birthweight, g	1498±392	1616±399

n: number of cases
means and standard deviations
IRDS: idiopathic respiratory distress syndrome

TABLE Ib

Prematures affected by IRDS

	n	gestational age, weeks	Birthweight, g
All cases	31	30.6±2.7	1498±392
Survivors	13	31.5±2.2	1641±285
Died during the neonatal period			
within 72 hours	10	29.1±1.4	1252±243
between 4—7 days	8	31.3±3.8	1576±558
together	18	30.0±2.9	1396±432
dying of pulmonary haemorrhage	11	30.1±2.9	1406±459
Sepsis score points			
exceeding 10	14	30.0±2.3	1349±354
died within 72 hours after birth	4	29.8±2.9	1250±334
died within 7 days after birth	8	28.6±2.4	1258±450
		means and standard devations	

n: number of cases
IRDS: idiopathic respiratory distress syndrome

was between the 10th and 90th percentiles in all cases [8].

The symptom-free babies needed only care. The babies affected by idiopathic respiratory distress syndrome exhibited all clinical and radiological signs of the condition and needed respiratory treatment and correction of acid-base imbalance. In all babies who died of IRDS, necropsy revealed the presence of hyaline membrane and alveolar haemorrhages.

Since the idiopathic respiratory distress syndrome can be simulated by sepsis, a score for establishing the probability of severe infection was used; a score value exceeding 10 made sepsis highly probable [18]. Respiration itself may cause infection, therefore the above-mentioned score was applied several times during IRDS and the highest value was taken as the indication for therapy and grouping. Grouping, number of cases, their gestational age and birthweight and the outcome of IRDS are shown in Table I. Autopsy confirmed the primary pulmonary changes resulting from immaturity leading to severe hypoxia in all fatal cases.

The blood samples were drawn from a peripheral vein. Clotting was prevented by addition of one part of 3.8% sodium citrate to nine parts of blood. Hypoxanthine and xanthine were determined in plasma.

Method

Hypoxanthine and xanthine were first extracted from the plasma [14]. A double volume of ice-cold, freshly prepared 10% trichloroacetic acid (TCA) was added to each plasma sample, the precipitate was removed by centrifugation. TCA was removed from the supernatant by 1.5 volume of diethylether saturated with water. The aqueous phase was filtered through a 0.5 μm Millipore filter and the sample was stored at -20°C. This extraction procedure could be carried out from samples of 100 μl.

The measurements were carried out in a Hewlett-Packard 1084/B type HPLC device by the method of Khym and Simmonds modified by ourselves [6, 14]. The column consisted of ODS-Hypersil, length: 10 cm, mesh size: 5 nm, pressure: 86 bar, working temperature: 30°C. The detector with variable wavelength sensitivity was sensitive to ultraviolet light, a lambda_{max} of 255 nm was used for hypoxanthine determination, 270 nm for xanthine. The flow speed was 1 ml/minute. The eluent was 0.01 mol/l potassium hydrogen phosphate pH 5.3, containing 1% methanol. The 20 μl sample was injected by automatic injector. Quantitative determination of xanthine and hypoxanthine was achieved by establishing calibration curves and computer evaluation of the area below the corresponding peak in the chromatogram (Fig. 3).

For calibration, serial dilutions of plasma hypoxanthine and xanthine (Sigma) were used. From all artificial plasma samples the whole procedure including extraction, was carried out and the area below the curve was measured and taken as the relative unit. Figure 3 illustrates, in addition to the calibration curves, a chromatogram as well. The lower limit of sensitivity was 0.1 μmol/l of hypoxanthine and xanthine, respectively.

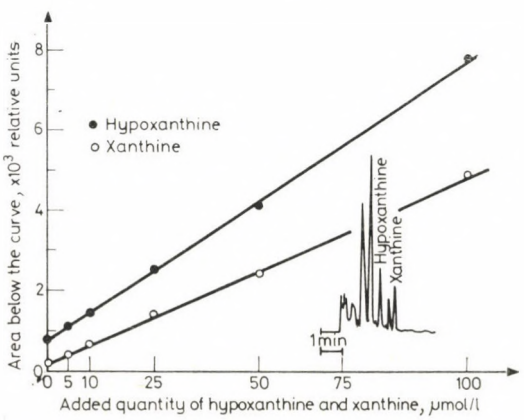

Fig 3. High pressure liquid chromatography curve of a plasma extract and calibration curves of hypoxanthine and xanthine

Since the determinations were carried out in citrated blood, real hypoxanthine and xanthine levels in plasma were calculated as follows: real concentration = measured concentration $\left\langle \frac{1}{9(1-\mathrm{PCV})} + 1 \right\rangle$ where PCV meant packed cell volume.

Results

Figure 4 shows the plasma hypoxanthine level found in the newborns. Within three hours after birth the

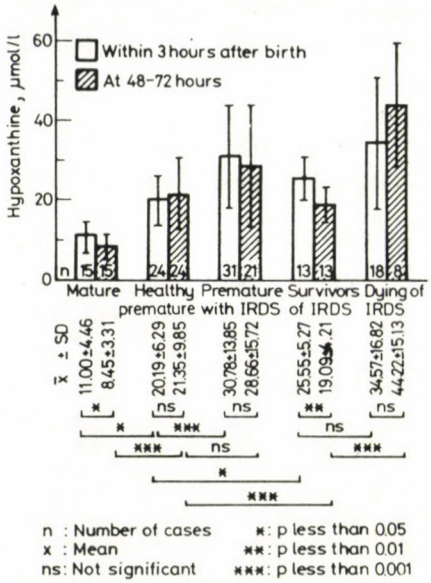

FIG 4. Plasma hypoxanthine levels of newborns within three hours after birth and between 48 and 72 hours of life

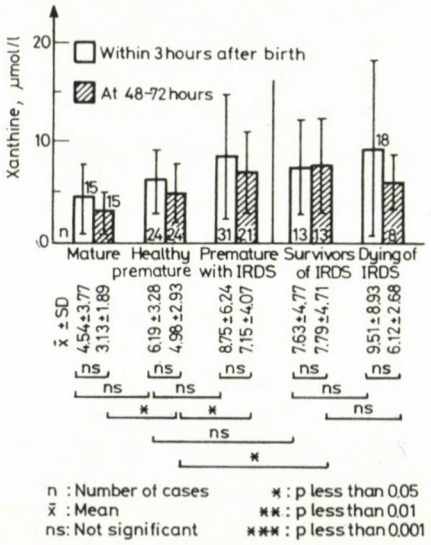

FIG 5. Plasma xanthine levels of newborns within three hours after birth and between 48 and 72 hours of life

lowest values were encountered in mature healthy babies. A mean value nearly twice as high was obtained in premature babies free of symptoms or problems, while in premature neonates affected by idiopathic respiratory distress syndrome the mean value was about three times higher than that of the mature babies. In prematures with IRDS dying subsequently of intractable hypoxia the mean was even higher, 34.57 μmol/1.

In the 15 mature newborns, the mean value observed between 48 and 72 hours after birth was significantly lower than the first mean value. In the premature babies, on the contrary, there was a slight increase instead of a decrease. An especially marked increase was seen in the 8 prematures affected by IRDS in whom the 48—72 hour measurement could still be carried out but of whom two died thereafter: the mean value amounted to 44.22 μmol/1. In some babies dying during the perinatal period, values as high as 100 μmol/1 were measured.

Similarly, both the values obtained within three hours and on the third day of life were higher in the preterm than in the term newborns. The increase was even more pronounced in the babies affected by IRDS. Again, in the newborns dying subsequently, a considerable fall occurred by the third day compared to the three-hour value. In the whole material, the lowest values were encountered in mature newborns on the third day of life while the highest values at three hours after birth in premature neonates dying during the perinatal period. The extreme values were 4.54 and 9.51 μmol/1, respectively (Fig 5).

Discussion

The key to perinatal adaptation is the baby's cardiorespiratory function. Postnatally there are more possibilities for diagnosis and treatment than immediately before birth, but estimation of the severity of hypoxia is still difficult. Measurement of the hypoxanthine level has primarily been used for judging the degree of intrauterine asphyxia; its value was compared to that of the Apgar score, blood lactate, blood pH, base deficit and individual asphyxia scores [1, 15, 17]. It was found that plasma hypoxanthine had a complementary value in this series of tests [16]. The highest hypoxanthine levels in neonates delivered after intrauterine asphyxia were usually measured 20 minutes after birth. The base deficit was highest at 30 minutes, the maximum of lactate during the first three hours. There is a strong correlation between the three parameters but none could be found between the blood pH and plasma hypoxanthine, probably because of the rapid changes in pCO_2 [15]. In intrauterine hypoxia the optimum time for plasma hypoxanthine determination is 10—20 minutes after delivery, but elevated values are still encountered three hours after birth [3]. If a single determination is carried out, the

hypoxanthine level may not be correlated with the clinical features. This is due to the fact that the plasma hypoxanthine concentration is influenced not only by hypoxia but also by other factors. Since hypoxanthine is electrically neutral, it seems very probable that it can escape from the hypoxic cells to the extracellular space by simple diffusion, hence it can easily reach the blood plasma. It appears in the plasma immediately after the hypoxic episode but this may be hindered by poor circulation. After birth there is a marked peripheral vasoconstriction in all newborns, even in mature babies, and this is especially pronounced in asphyxiated neonates. Under pathological conditions the pump function of the heart is impaired and redistribution of the circulating blood occurs: perfusion of all organs but of the brain and the heart deteriorates. Hypoxanthine appears in the circulation of the arteries or veins available for blood sampling only when the peripheral circulation has improved and the bloodstream is able to wash out hypoxanthine from the tissue that had been hypoxic.

The primary determinant of an elevated hypoxanthine level is hypoxia, but its effect may be retarded by impaired heart function and disturbed microcirculation. Furthermore, the plasma hypoxanthine level can be altered by the volume of plasma and the size of the extracellular space. From the practical point of view it is important that no difference should occur between the arterial and venous hypoxanthine levels.

Timing of the first blood sampling within three hours after birth has been chosen on the basis of the dynamics of hypoxanthine levels. In addition to clarify the severity of perinatal and very early postnatal hypoxia, we also wanted to establish the range of basal values for healthy premature babies. To see the course of hypoxanthine and xanthine levels, they were measured in mature newborns, in prematures without problems and in prematures affected by IRDS, 48—72 hours after birth. Determinations in the healthy mature babies had the purpose of obtaining normal values of our own.

The normal values for hypoxanthine and xanthine obtained in this study fell within the upper third of the range described in the literature. In some instances we compared the hypoxanthine and xanthine levels determined by an oxygen electrode or by high pressure liquid chromatography; the results are shown in Table II. It can be seen that the ranges obtained by the two methods were similar but the individual values of the two different determinations did not coincide. We think that the uniformity of the method throughout our present study has contributed to the reliability of our findings.

The lowest early values were found in healthy mature babies. The values then decreased by the third day of life. This indicates that a certain degree of hypoxia occurs even during normal delivery but is soon abolished after birth, consequently the blood level of the purine derivatives is normalized

TABLE II

Normal plasma hypoxanthine and xanthine concentrations obtained by various authors

Author	Method	Site of blood sampling	Compound	Mean ± SD μmol/l	Range
Saugastad [12]	pO_2electrode	cord	hypoxanthine	5.8 ± 3.0	0 – 11
Lipp-Zwahlen [7]	pO_2 electrode	umbilical artery	hypoxanthine	16.1 ± 5.7	≈8 – 26
	pO_2 electrode	umbilical vein	hypoxanthine	14.4 ± 4.7	≈9 – 24
Bratteby [3]	pO_2 electrode	umbilical artery	hypoxanthine	11.9 –	0 – 23.8
	pO_2 electrode	umbilical vein	hypoxanthine	9.0	1.4 – 18.9
Thiringer [16]	pO_2 electrode	cord	hypoxanthine	5.7 ± 5.8	0 – 18.3
O'Connor [11]	pO_2 electrode	cord	hypoxanthine	5.1	1 – 14.0
Swanström [15]	pO_2 electrode	umbilical artery	hypoxanthine	7.7 ± 5.9	–
Simonds [14]	HPLC	adult blood	hypoxanthine	2.04 ± 0.6	–
			xanthine	0.61 ± 0.21	–
Boulieu [2]	HPLC	adult blood	hypoxanthine	3.2	–
			xanthine	2.0	–

when circulation and respiration have become fully adapted. The significantly higher value observed in healthy prematures three hours after birth can be explained by their slightly impaired cardiorespiratory adaptation causing a somewhat protracted transitory hypoxia in the tissues. But in these babies, too, the hypoxanthine level fell by the third day of life, pointing to an improved cellular respiration. Thus, hypoxanthine estimations have proved here a useful laboratory marker of adaptation.

In the case of prematures suffering from IRDS, the relative role of intrauterine asphyxia, immaturity and postnatal respiratory distress in causing high hypoxanthine levels can hardly be established, but the high value itself shows that the persisting respiratory failure maintains a hypoxia. Successful respiratory treatment, restoration of the circulation and correction of the imbalance of acid-base equilibrium result in a fall in the plasma hypoxanthine level, as it has been observed in out survivors of IRDS, and the contrary could be seen in the group of prematures who subsequently have died. If attempts to restore cardiorespiratory functions fail, the hypoxanthine level inexorably increases, showing the persistence of severe energetical disturbances at the cellular level. In this group the xanthine level was comparatively low. It appears that as a consequence of hypoxia, the failure of xanthine oxidase function prevents normal catalysis of the conversion of hypoxanthine to xanthine and thus the xanthine level falls in spite of extremely high hypoxanthine concentrations.

Our results suggest that plasma hypoxanthine and xanthine are good indicators not only of intrauterine asphyxia but also of postnatal adap-

tation and that they even have a prognostic value. These data show that the pathogenesis of IRDS is complex and its therapy must be aimed at improving cardiorespiratory function, microcirculation and cell metabolism.

Acknowledgment

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Factor VIII related antigen in term and preterm newborns with severe neonatal haemorrhage

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Plasma factor VIII related antigen (VIIIR:Ag) concentrations were studied in term and preterm newborn infants. The control population was a group of normal children 2 to 12 years of age without any manifest disorder. The aim of the present investigation was to follow the change of VIIIR:Ag in the newborn and to study its level in sick preterm infants with severe bleeding disease. The VIIIR:Ag level in the control children was $86.2 \pm 20.5\%$. The lowest concentration was measured in term infants between 48—72 hours of life. The highest VIIIR:Ag level ($129.1 \pm 8.1\%$) was found in those preterm newborns who died of pulmonary and intracranial bleeding in the early neonatal period. VIIIR:Ag is a useful marker of endothelial cell damage in the perinatal period.

Haemorrhage, both localized and generalized, is a significant cause of mortality and morbidity in the neonatal period [11, 13, 17, 20]. Numerous studies have demonstrated deficiencies of various blood coagulation factors in the normal newborn and more severe ones in preterm infants [5, 8, 9, 18]. The endothelial cells and platelets have an important role in the maintenance of vascular integrity and their factors, besides ensuring the normal balance, are useful markers of pathological conditions. Endothelial cell injury is probably a primary event in the pathology of a number of diseases affecting blood vessels. Plasma level of factor VIII related antigen (VIIIR:Ag) may be a sign of intimal damage [1, 4, 7].

Factor VIII/von Willebrand factor complex (FVIII/vWF), being a glycoprotein of high molecular weight, has distinct constituents, i.e.: factor VIII procoagulant activity protein (VIII:C), the antigenic expression of VIII:C (VIII:CAg), von Willebrand factor protein, the antigenic expression of von Willebrand factor (VIIIR:Ag), and the ristocetin cofactor (VIIIR:RCo) [22]. VIIIR:Ag and VIIIR:RCo are closely related. Both are synthesized by vascular endothelial cells and possibly by megakaryocytes and platelets [14, 16, 19).

Estimation of VIIIR:Ag in plasma by precipitation with rabbit antiserum is a simple and useful parameter of endothelial cell injury.

The aetiological factors that have been suggested as the basis of endothelial cell damage in the perinatal period are the respiratory distress syndrome, asphyxia, acidosis, infec-

tions, hypoglycaemia, hypercapnia, icterus, congenital heart disease, fetal distress and the mode of delivery [10]. In these disorders, oxygen radicals are produced in large quantity in hypoxia and hyperoxia, especially during the rapid change from hypoxia to hyperoxia [2]. This state may lead to severe damage of cells and tissues, mainly in preterm infants whose endogenous antioxidant activity is weak [3]. The endothelial cell injury then affects the microcirculation, leading to generalized neonatal bleeding syndrome with massive pulmonary and intracranial haemorrhage.

The aim of the present investigation was to study the plasma VIIIR:Ag level in normal children, term and preterm infants and in those sick preterm infants who died in consequence of pulmonary and intracranial haemorrhage.

Material and Methods

The VIIIR:Ag level was estimated in children hospitalized at our Department. Blood samplings were carried out only when blood was needed for other purposes, too. A total of 67 children were examined; their grouping was as follows.

Group 1. Thirty control children 2 to 12 years of age, without any manifest disorder.

Group 2. Twelve term newborn infants of 37 to 41 (mean 39.3 ± 1.1) weeks gestational age and 3203 ± 378 g mean birthweight, without any adaptational problems.

Group 3. Eleven preterm newborn infants of 26 to 36 (mean 31.2 ± 2.7) weeks gestational age and 1457 ± 354 g mean birthweight, without any risk factor considered to be important in the development of endothelial cell damage.

Group 4. Fourteen preterm newborn infants of 26 to 36 (mean 30.5 ± 2.6) weeks gestational age and 1358 ± 389 g mean birthweight who then died at 1 to 8 (mean 3.6 ± 2.5) days of age with severe pulmonary and/or intracranial haemorrhage confirmed at necropsy.

The first blood sample was drawn of these infants within the first four hours, the second between the 48—72 hours of life. In the fourth group, in 6 infants we could not measure the second VIIIR:Ag level, because they died earlier.

VIIIR:Ag was determined in citrated venous plasma. Blood was mixed 9:1 with 3.8% trisodium citrate solution and plasma was prepared by centrifuging at 3000 *g* at 4°C for 15 minutes and stored at −30°C. VIIIR:Ag was measured by the Laurell rocket immunoelectrophoretic technique using rabbit antiserum (Behring Diagnostics, Germany). Serial dilutions of the pooled and test plasma were prepared for each plate and run simultaneously; the amount of VIIIR:Ag in the patients' plasma was compared to that of the pooled plasma and the result was expressed in percents [21].

Statistical analysis was performed by Student's *t* -test.

Results

The VIIIR:Ag level of the control children in Group 1 was 86.2 ± 20.5% of the normal adult level (Fig. 1). In Group 2 the protein concentration was higher (106.7 ± 15.8) in the first hours of life than in Group 1, but on the third day the level fell significantly by 23.7% and reached the normal level. Group 3 showed shortly after birth a level (89.6 ± 12.2%) lower than in Group 2, but it increased 3 days later. The highest VIIIR:Ag concentration was measured in Group 4, where in the first hours the VIIIR:Ag concentration (119.8 ± 18.0%) was significantly higher than in Group 3, and in those 8 children who survived the first three days, the level ($128.1 \pm \pm 8.1$%) was the highest between 48 and 72 hours of age.

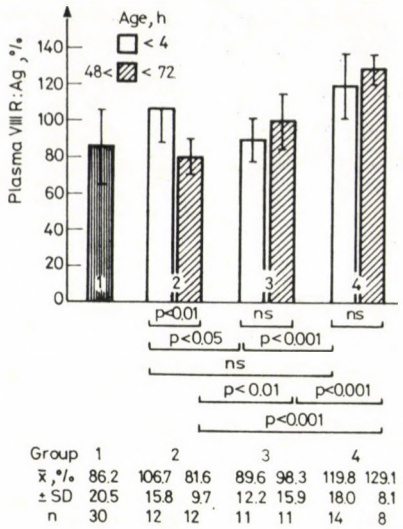

FIG 1. Plasma VIIIR:Ag levels in the four groups. Group 1: control children; Group 2: term newborns; Group 3: preterm newborns; Group 4: preterm newborns with bleeding disorder. Blood samples were obtained within the first four hours, and between the 48 — 72 hours of life

DISCUSSION

The aim of the present study was to follow the circulating VIIIR:Ag level in the neonatal period and especially in preterm infants. The VIIIR:Ag value of control children 2—12 years of age was found to be lower than the normal adult level. The reason for this might be a decreased synthesis of VIIIR:Ag, or an increased resistance of the endothelial cells of children against damaging effects. The only fact supporting the latter assumption is that thromboembolism and angiopathies are less frequent in children than in adults.

The stress during delivery might be the reason for the high concentration of VIIIR:Ag in term newborn infants. The placenta can also be the source of VIIIR:Ag, because obliterations may occur during delivery. In term infants the decrease of VIIIR:Ag concentration three days after birth points to improvement in the microcirculation and in the condition of endothelial cells.

The initial low value of VIIIR:Ag in preterm babies increases with time, indicating the sensitivity of endothelial cells. The cardiovascular system is also immature and the stress situations impair the repair mechanism. The phenomenon is reflected in the VIIIR:Ag concentration measured in preterm infants suffering from generalized bleeding syndrome; the highest values were observed in that population. Sometimes, the damage to endothelial cells is further increased by medical treatment; one of such factors is the toxicity of oxygen. Thus, VIIIR:Ag is a good marker of endo-

thelial cell damage in the perinatal period. We found the highest concentrations in that population where generalized bleeding had developed in the early neonatal period.

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Intracranial sonography in infancy

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Neonates and older infants were subjected to sonography through the anterior fontanelle with grey-scale compound scanner. The patients were referred to us from neurosurgery or showed clinical symptoms of perinatal CNS damage (ventricular or intracerebral haemorrhage, subdural effusion, etc.) or malformations (meningomyelocele, encephalocele, cystic brain, cerebral dysgenesis, etc.).

Sonography is the simplest non-invasive diagnostic method to exclude suspected hydrocephalus in cases of macrocephalus, or when the head of premature infants grows more rapidly than the rest of the body. When screening showed alterations, sonography was performed weekly or fortnightly according to the extent of deviation from the normal. In cases of progressive hydrocephalus, when shunt surgery was indicated, ultrasound was used to monitor the operation of the shunt. In early age, ultrasound is a tool equal in value to CT, but is far less expensive and the examination can safely be repeated at any time.

During the 24 years of its existence, ulrasound examination has developed into one of the most important tools of preventive medicine. Before that the intracranial cavity could only be examined by invasive procedures such as pneumo-encephalography or iodine ventriculography. CT scanning is a method with comparatively low radiation dosage but the exposure to radiation limits its serial repetition.

The ultrasound technique is a non-invasive diagnostic procedure which is completely harmless, painless and which can be repeated at will. In neonates and infants it gives reliable information on the cerebral ventricles until the anterior fontanelle remains large enough i.e. until the 8—10 postnatal month.

Initially, ultrasound as an examination technique was applied principally for echo-encephalography. This was based on the A-echo technique but this required extensive experience and there was a large margin of error [18].

Two-dimensional ultrasound examinations were first used extensively in obstetrics to visualize the fetal skull. This then gave rise to the idea of trying to examine the intracranial region. The possibility of examining the cerebral ventricles and brain structures of neonates and young infants through the anterior fontanelle seemed self-evident [2, 3, 4, 13, 16].

Through this acoustic window it was generally possible to get a look into the intracranial cavity until the

fontanelle was open. At the beginning, the size of the transducers limited accurate depiction. Depending on the angle of penetration (60—120°) the mechanical real-time heads generally gave a suitable picture in the plane in question.

Of the two ways used for visualizing the intracranial structures, the one which spread more rapidly in the beginning was the real-time technique and this was then followed by the advanced grey scale method. The latter gave structural distinctions using the black and white colour scale, thus creating two-dimensional pictures. A cross-sectional picture with far more detail is obtained from the multifocal compound scanning method which also gives gross morphological cross-sectional depiction [3, 4, 8, 16].

There is no real difference in quality between real-time and compound scanning installations with up-to-date digital ancilliaries but the linear arrays depict only part of the important regions [10]. The breakdown of detail on modern digital real-time scanners coincides with that of compound scanning. With computer-aided link-up of several transducer heads it is possible to examine the intracranial cavity of children older than two years but since the skull bone refracts the majority of acoustic energy, the pictures contain less detail even with use of a water bag [5, 6].

Intraoperative real-time ultrasonic sector scanning was performed through the unincised dura mater of the intact brain surface during craniotomy, to define the location, configuration and consistency of the tumour mass [12, 15, 16].

As far as the cerebral ventricles and cerebral structures are concerned, the accuracy of echotomographic examination shows a good coincidence with the results of CT examination [3, 6, 7, 8].

Method

A Brüel—Kjaer type 3402 real-time sector scanner was used for screening and a Brüel—Kjaer type 3401 one for compound scanning. The nominal frequency of the transducer heads was 4 and 5 MHz and the transducer diameters were 12 and 5 mm, respectively. (These are short-focussed transducers). The sole requirement of the examination is the presence of an open anterior fontanelle of at least 1 cm^2 area. No sedation was required. A restless infant could always be placated by feeding during the examination. Satisfactory temperature conditions were provided for premature and small-for-dates infants. Average examination time was ten to fifteen minutes, the maximum was twenty minutes. For documentation a video-recorder or photography was used.

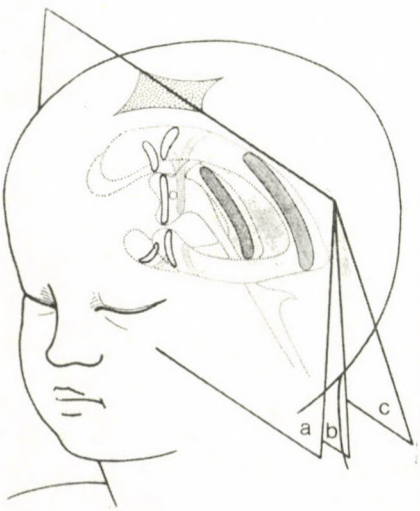
Planes of Section and Normal Anatomy

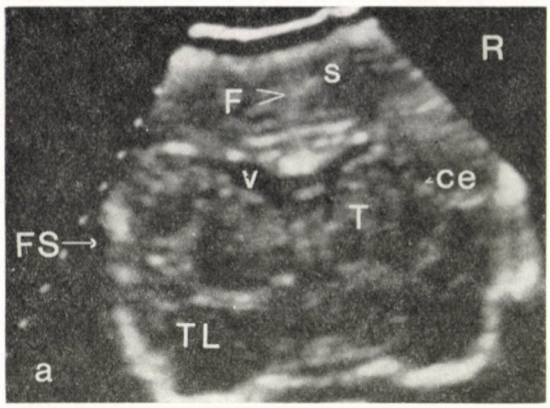
Ready given specific planes of section should be used to standardize documentation and facilitate repetition. We used serial frontal and sagittal planes (Figs 1 and 2).

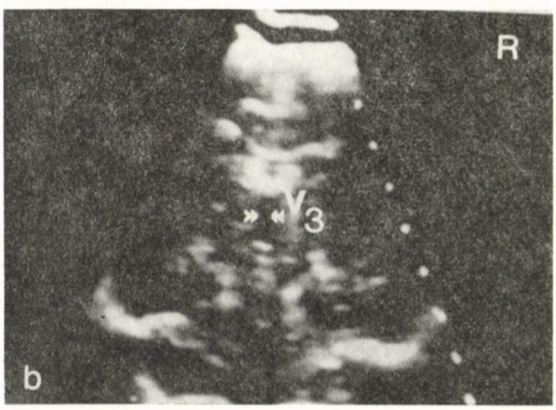
Clinical indications for intracranial examination

Premature Babies

Low birth weight premature infants require special attention since intracranial haemorrhage is more frequent among them than in full-term infants.







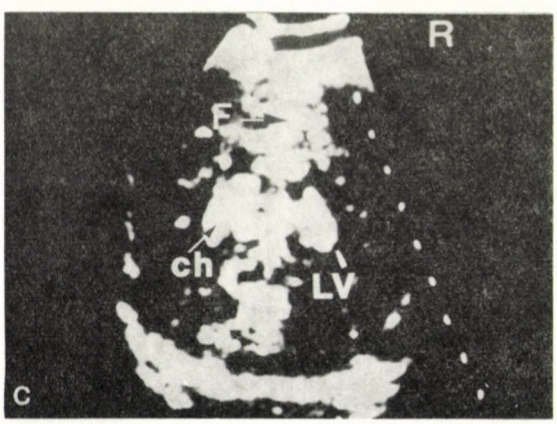


FIG 1. A: Standard frontal planes. — Scheme and pictures: a) The frontal section is the plane of the coronal suture (the plane of the sutura coronaria toward the base following the line of the external auditory canal) showing normal lateral ventricles (V) of echo-free, butterfly-shaped form; the anterior longitudinal cerebral fissure with the echogenic dura; the falx cerebri (F); the cingulate sulcus (s), the thalamic area (T), the Silvian fissure (FS), the external capsule (ce), and the temporal lobe (TL). R: right side. b) The second frontal plane aimed backwards and downwards from the coronary suture. The third ventricle usually gives a narrow echo-free zone (V3). c) The third frontal section where the plane is even further backwards and downwards, behind the foramen occipitale magnum. It shows the posterior areas of the lateral ventricles, the transition of the temporal horns and the choroid plexus in homogeneous, echogenic symmetric form (ch), surrounding a narrow echo-free space, i.e. the lateral ventricle (LV)

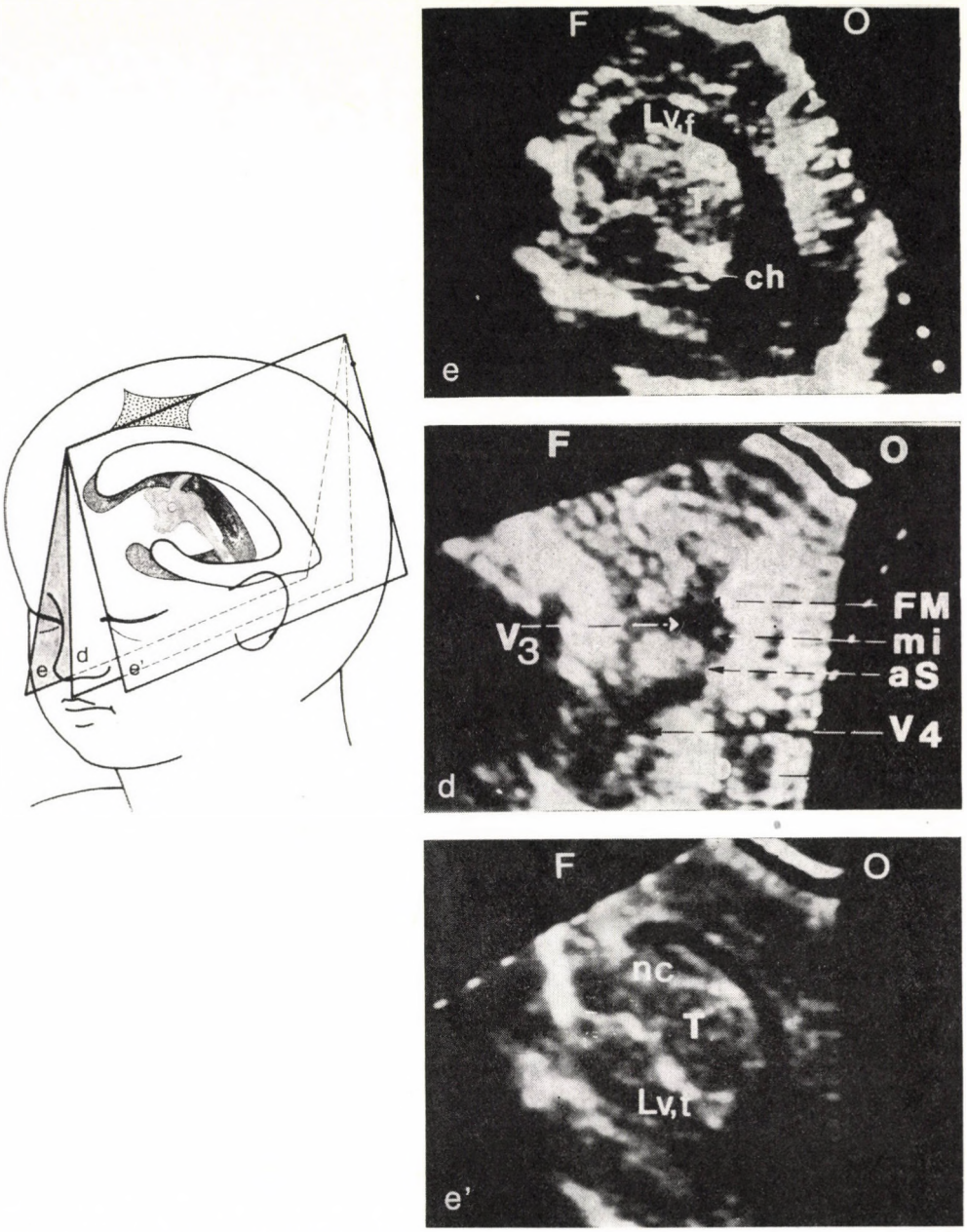


FIG 2. B: Standard median and parasagittal sections. Scheme and pictures: d) The median sagittal section shows the echo-free third ventricle (V3) with the echogenic massa intermedia (mi), above this the foramen of Monro (FM) and the connected Sylvian aqueduct (aS), the fourth ventricle (V4) and the mixed echogenic cerebellum (Cb). F: frontal, O: occipital direction. e-e') In the parasagittal sections the planes are shifted on both sides, displaying the full echo-free arc of the lateral ventricle, its frontal (Lv, f) and temporal (Lv, t) parts, the thalamic area (T), the caudate nucleus (nc), and the periventricular germinal matrix which is a common site of intracranial haemorrhage in preterm infants. The most echogenic structure is the choroid plexus (ch)

TABLE I

Clinical Indications for intracranial sonography

I	Pre-term, full term and small-for-dates babies under 1500 g in all cases.
II	Over 1500 g birth weight
	Complications (asphyxia or other) during delivery
	Respiratory distress, hypoxia and post-hypoxic conditions, artificial respiration, CPAP, PEEP
	Any type of bleeding
	Small for-dates
	High-risk neonates
	Monosymptomatic or multiple developmental anomalies (myelo-meningocele, congenital hydrocephalus, etc.)
	Macrocephalus (suspect cerebral malformation)
	Perinatal infection
III	Older infants
	Rapidly increasing head circumference
	Inflammatory conditions of CNS
	Subdural effusion
	Ventriculitis
	Parenchymal changes (abscess, cyst or others)
	Traumatized or asphyxiated infant, "battered child" syndrome, follow-up after surgery (shunt placement, etc.)
	Suspicion of intracranial space occupying process, hypertension (tumour, cyst)
IV	Older children (over two years)
	Intra-operative monitoring or guide through a bone flap
	Shunt placement
	Biopsy (through bone flap or directly)

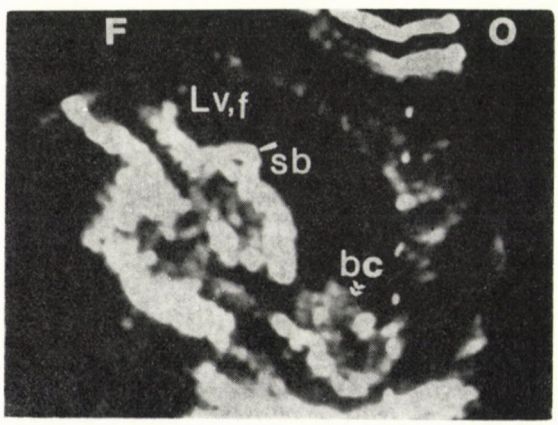


FIG 3. A three weeks old preterm infant of 2000 g birthweight had required CPAP for distress syndrome. He had intracranial hypertension, and the lumbar puncture showed sanguinolent CSF. Right parasagittal section. Enlarged ventricle. In the frontal horn of the lateral ventricle (Lv, f) the place of the germinal matrix, above the caudate nucleus. An echogenic area representing subependymal bleeding (sb) and in the temporal horn a blood clot are visible (bc). Subependymal and intraventricular haemorrhage, dilated ventricles

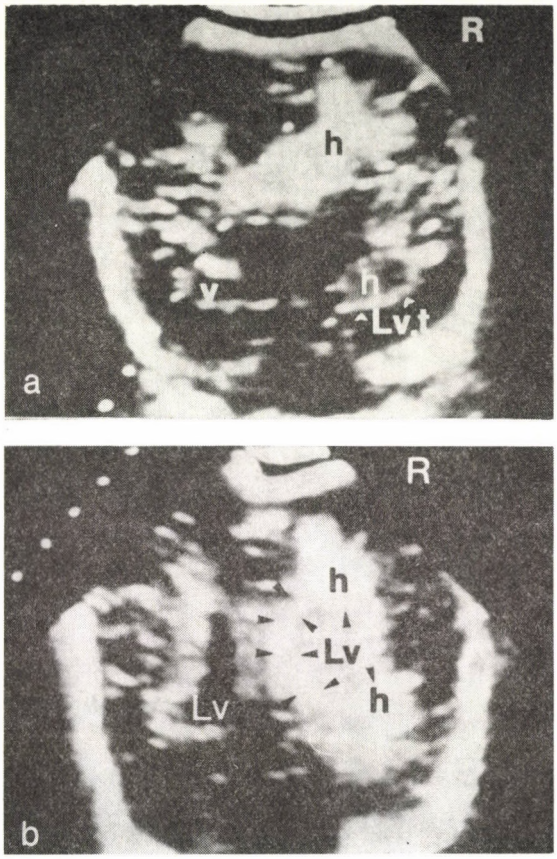


FIG 4. 5 day old full term infant after complicated delivery had had various neurological symptoms such as repeated apnoeic periods, convulsions. He required intubation, artificial respiration and became anaemic. a) The first frontal plane shows expressed intra and periventricular echogenicity (h-black) and in the temporal pole of the right lateral ventricle (Lv, t) a moderately increasing echodensity (hwhite). b) Intraventricular haemorrhage on right side. The expressed asymmetry of the ventricles in the third frontal plane is evident from the comparison of the echo-free left ventricle (LV-white) with the echodense right ventricle (LV-black). The full arc is filled with blood (h) (arrows). R: right side

To increase the proportion of successful interventions it is necessary to conduct regular and broad-scale examinations as early as possible. Haemorrhage is most likely to occur in the weight group under 1500 g born before the 32nd week of gestation. The frequency of intracranial haemorrhage in such infants is estimated at 40—45% [8, 10, 11]. In low birth weight prematures requiring artificial respiration the percentage is even higher, near 70% (Fig. 3).

In infants with a birthweight under 1500 g born before the 32nd week of gestation, sonographic examination should be done within 48 hours after birth and should be repeated at 7 to 10 days, this being the time when changes are likely to occur. Most

haemorrhages occur at this time and may or may not progress.

Haemorrhage usually begins with subependymal bleeding in the area of the periventricular germinal matrix and may later break into the ventricles (Fig. 4). In survivors, hydrocephalus may often develop due to intermeningeal adhesion or to occlusion of the aqueduct [1, 10, 11, 15, 19].

Preliminary detection of intracerebral haemorrhage including most subependymal and intraventricular haemorrhages in the neonate should be verified by real-time sonography in the newborn ward. If a haemorrhage is detected, the infant should be monitored by sonography daily or every second day, and it is essential to follow it up for at least 12 months, in view of the danger of hydrocephalus and various necrotic processes caused by the bleeding [17].

Term neonates and infants

All neonates should be examined following complicated delivery, asphyxia or hypoxia, or any disorder involving resuscitation. Abnormal cranial ultrasound findings have been reported in infants with severe asphyxia [2].

Infants with suspected intracranial defects equally requires sonographic examination. Congenital hydrocephalus, intracranial cyst, porencephalis, arachnoid cyst, Dandy—Walker cyst, septum pellucidum cyst, etc., may be detected. Sonography may help to determine whether or not neurosurgical intervention is possible or necessary (Figs 5, 6).

Moderate ventricular dilatation in neonates and the dilatation almost always observable in premature infants as well as an asymmetric ventricular system should also be monitored. They may indicate idiopathic changes such as acute transitional dilatation, or may show the result of an earlier subependymal bleeding (Fig. 7).

Subdural effusion is a special type of infantile intracranial disorder. It is often a complication of meningoencephalitis or subdural haemorrhage, and may also be a concomitant of hydrocephalus. Since the subdural effusion usually takes a mantle-like form, its ultrasound diagnosis is reliable only if there is a large amount of fluid (Fig. 8).

Neonates with meningomyelocele

Only 29% of such patients show clinical symptoms of hydrocephalus while 97% actually have ventricular dilatation, smaller or larger in size.

It is necessary to clarify the neurological status of the patient as well as to determine the initial size of the ventricle prior to reconstruction surgery. In the postoperative period, sonography repeated every two weeks is in most cases sufficient to monitor even a rapid progression of the hydrocephalus but in some cases progression is extremely rapid and the examination must be repeated weekly. In these cases shunt surgery should be considered. If progression is not rapid, then monthly controls are sufficient to determine whether surgery is indicated, as in some cases a balance

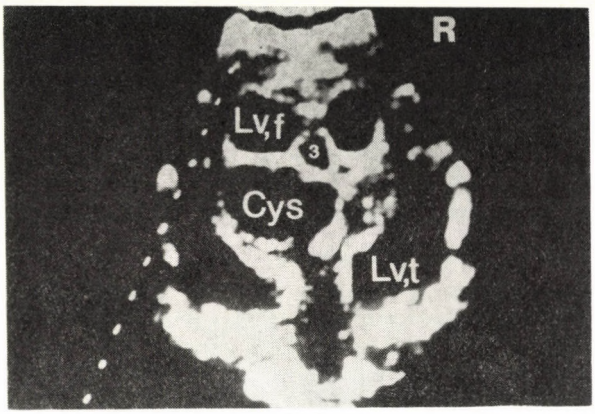


FIG 5. Arachnoid cyst (Cys) with hydrocephalus. Four months old infant with multiple defects (cleft lip, palatoschisis, internal hydrocephalus). On the coronal section enlarged frontal (Lv, f) and temporal (Lv, t) horns, moderately echo-free third ventricle (V3) with separate anechoic lesion pressing the third ventricle to close the CSF passage

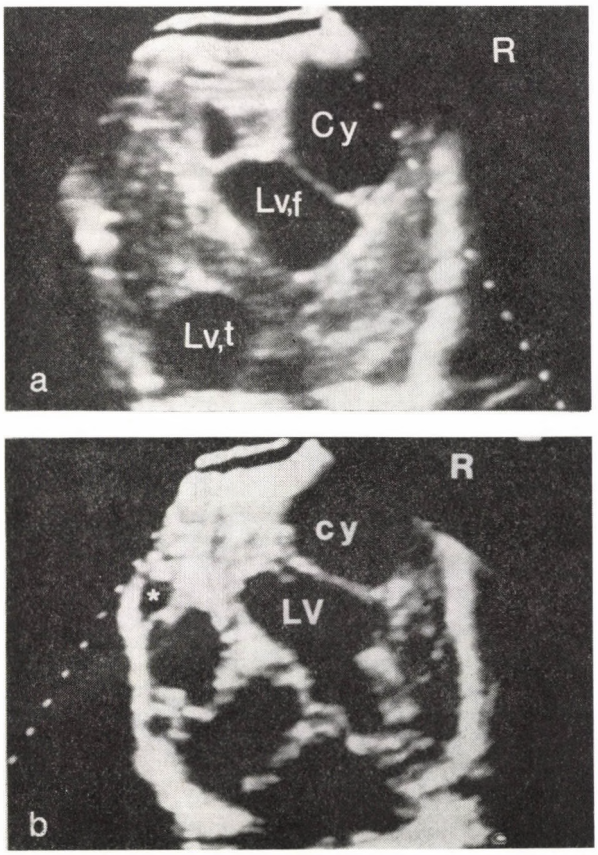


FIG 6. Three and half month old boy with neurological symptoms i.e. positive transillumination on right side and hypertensive macrocephalus. Cystic porencephaly complicated with internal hydrocephalus. a, b) The first and third frontal planes showe a moderately dilated ventricular system with asymmetric cyst (Cy) on right side and other cystic echo-free spots on left side (*) on the third section. Frontal (L, v, f) and temporal (Lv, t) horns of the lateral ventricles (LV)

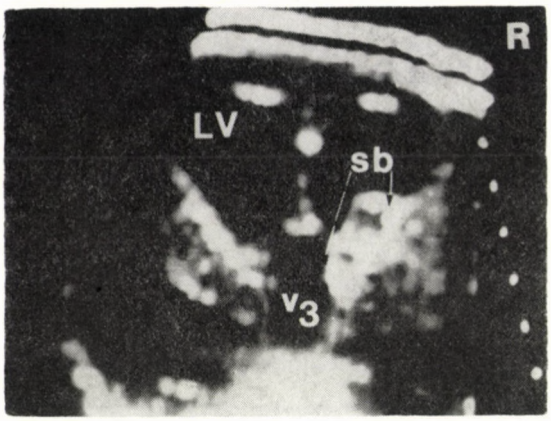


FIG 7. Subependymal haemorrhage and posthaemorrhagic hydrocephalus. Second frontal, plane of preterm baby with 2100 g birthweight, organic heart defect. After heart surgery ventricular haemorrhage and posthaemorrhagic hydrocephalus developed. Subependymal bleeding (sb) with arrows in the typical area. Lateral ventricle (LV), third ventricle (V3)

develops between fluid formation and absorption (Fig. 9).

All infants suspect of primary or secondary hydrocephalus should be screened since dilated cerebral ventricles due to delayed CSF outflow, prolonged elevated intracranial pressure, compression of the brain mantle, ischaemia and thinning precede the rapid growth of head circumference which earlier was the main sign of hydrocephalus (Fig. 10/a.) Early detection of the impending ventricular dilatation may help the surgeon to choose the time suitable for shunt surgery.

The degree of ventricular dilatation can also be monitored by sonography. Two weeks are sufficient to show even a moderate dilatation of the ventricles (Fig. 10/b). In cases of progressive hydrocephalus a ventriculo-atrial or ventriculo-peritoneal shunt should be implanted (Fig. 10/c). Then the ventricular dilatation declines during 18 to 36 days while the head circumference remains unchanged, due to expansion of the brain tissue (Fig. 10/d).

Sonography during the postoperative phase helps the detection of favourable changes in ventricle size and the recognition of eventual problems with the implanted shunt. Complete obstruction of the shunt produces symptoms of increased intracranial pressure, but diagnosis of a partial obstruction in the shunt system is more complicated. This is one of the reasons why long-term follow-up is required.

In cases of bacterial or viral CNS infections (meningitis, meningoencephalitis) ultrasound detection is possible if intermeningeal adhesion, secondary hydrocephalus or parenchymal change (cystic disorders or abscess) are present. Ultrasound results are somewhat better in the detection of moderate hydrocephalus and post-

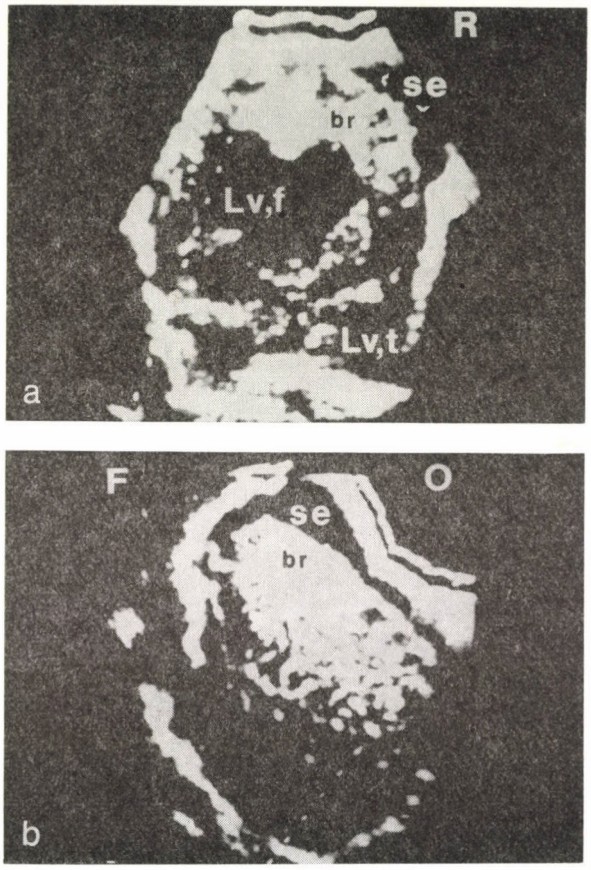


FIG 8. Large subdural effusion on right side with dislocated, enlarged ventricles in seven-month-old boy after reconstruction surgery because of myelodysplasia. a, b) Slight ventricular dilatation and expressed asymmetric subdural effusion (se) on right side (R) on the frontal section (a) and the right parasagittal section (b) above the brain mantle (br) as an echo-free space. Frontal horn of the lateral ventricle (Lv, f) and temporal horn of the lateral ventricle (Lv, t) with brain mantle on right side about 2 cm wide. The subdural fluid has dislocated the brain to the left side

operative ventriculitis. In some cases a change in echodensity indicates the subependymal reaction (Fig. 11).

Primary brain tumours are a rarity in infants. It is possible to detect them with ultrasound if there is a change in the normal echogenity of the brain tissue (an increase caused possibly by calcium deposition or a decrease due to cavity formation, etc.).

Emergency ultrasound examination should be considered in every case when there is a sudden deterioration in the neurological status of the infant, or convulsions or repeated apnoeic episodes occur, since ultrasound-guided ventricular punction or drainage may be necessary. Antibiotic treatment administered directly into the ventricle under ultrasound guidance

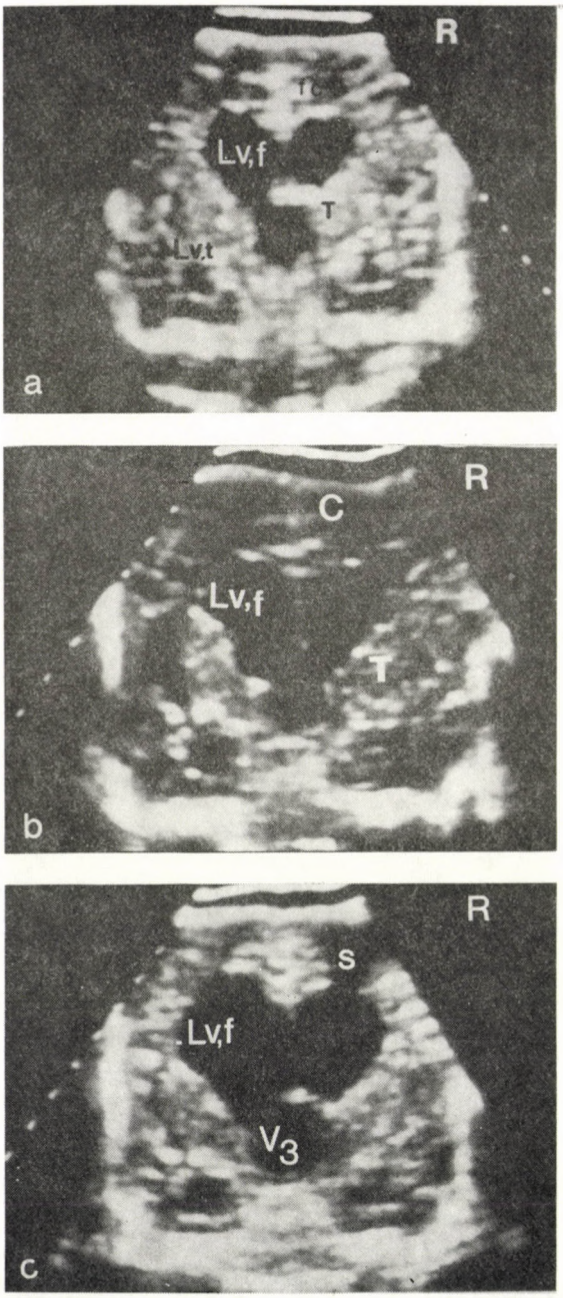


FIG 9. Progressive hydrocephalus. 19 days old boy after reconstruction surgery for lumbosacral myelodysplasia. a) The second frontal section shows slight ventricular dilatation. Frontal horn of lateral ventricle (Lv, f) and temporal horn near normal in size (Lv, t). Falx cerebri (fc) and thalamic area (T). b) Same section two weeks later, moderate hydrocephalus. c: brain mantle. c) four weeks later, at two months of age. The same section shows progression of hydrocephalus. Subdural effusion (s) is seen as an echo-free area on the right side (R). V3: third ventricle

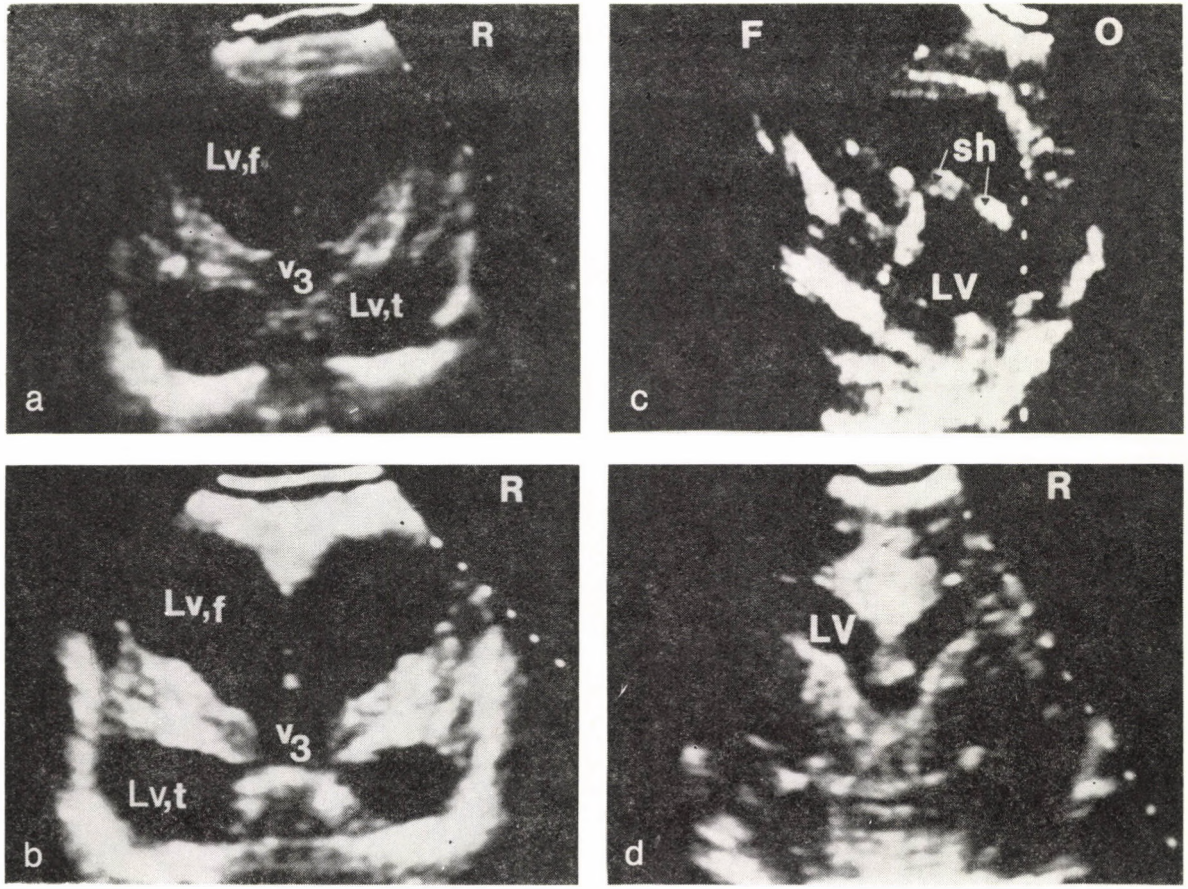


FIG 10. Hydrocephalus regression after shunt surgery. Three months old infant with macrocephalus and moderate ventricular dilatation. a) In the second frontal plane, dilated frontal (Lv, f) and temporal (Lv, t) horns of the lateral ventricles and the third ventricle (V3). b) The same section two weeks later. Large ventricular dilatation. After progression of hydrocephalus shunt surgery was performed. c) Three weeks after implantation of the ventriculo-atrial shunt. The shunt tube is visible as a double-walled echodense line (sh) in the right parasagittal section. Lateral ventricle (LV). d) Six weeks following shunt surgery. The intraventricular pressure has decreased. The coronal section shows near normal lateral ventricles (LV) and the brain mantle has expanded

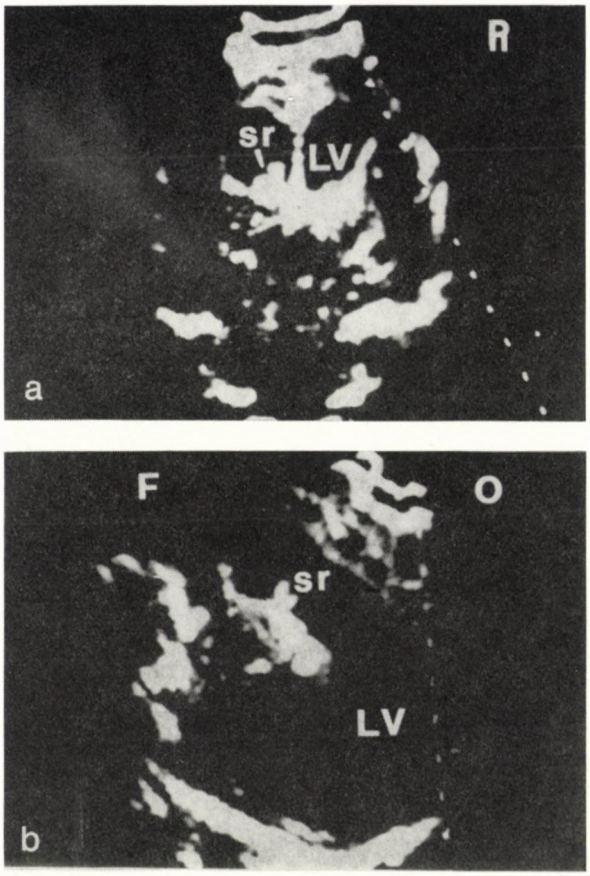


FIG 11. Ventriculitis and internal hydrocephalus in one month old infant with myelodysplasia. a, b) Diffuse low echogenicity in both lateral ventricles. On the ependymal surface of the left ventricle a fibrin clot is seen in both the coronal and parasagittal sections. Ependymal-subependymal fibrin reaction (sr)

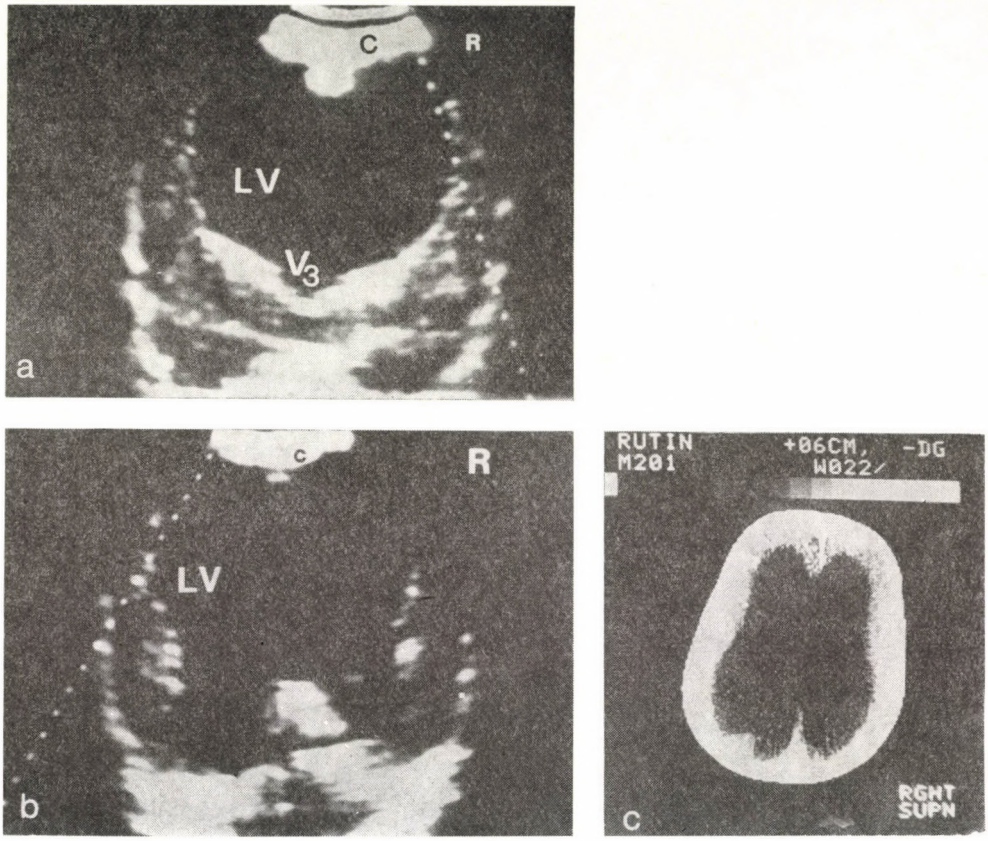


FIG 12. Large internal hydrocephalus in two years old boy with macrocephalus with consequential occlusion of aqueduct. a, b) The frontal first and third sections (lateral ventricle) (LV), C: brain mantle, c) Comparison with CT shows excellent agreement. R: right direction

is also possible if the ventricles are near-normal in size [14].

Sonography is the most harmless method for examining the intracranial space of an infant suspected of having hydrocephalus (Fig. 12).

Often one single examination at the outpatient clinic will allow to arrive at a final negative diagnosis and thus to save the costs of hospital admission.

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Acute monosymptomatic aseptic meningitis caused by Toxoplasma gondii

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Acute monosymptomatic aseptic meningitis was observed in a 4 year old male patient. Toxoplasma gondii tachyzoites were detected in Giemsa-stained smears prepared from the CSF. Inoculation of mice gave the same result. The patient was cured after the application of pyrimethamine and sulpha drugs. On basis of the smears, the serological results and data in the literature, a direct infection through the nasal cavity has been assumed.

Toxoplasma gondii (T. g.), this obligate intracellular protozoon, is widespread throughout the world and can be found both in humans and in animals. According to surveys conducted in different countries, a large portion of the world's population proved to be infected by T.g. at one time or another [14, 17]. In Hungary the frequency of symptomless toxoplasma infection is between 30 and 50%. The first infection is symptomless in two thirds of the cases, while mild, non-specific manifestation is observed in the rest. There is a great difference in the number of T.g. infections and the number of diseases attributed to T.g., due to the difficulty of diagnosis.

Acquired toxoplasmosis manifests itself with lymphadenitic, cerebrospinal, exanthematous, ocular and myocardial forms [18]. Meningitis caused by T.g. infection is rarely mentioned; even special monographs hardly devote more than a sentence to it [4, 17]. Some papers occasionally mention cases which, considering the changes in antibody titre, could be regarded as T.g. meningitis, although the pathogenic agent was not found in the smears nor was the infection proved by inoculation of mice [1, 7, 8, 9, 10, 11, 13, 15, 19]. Reporting the case of our patient, we aim at providing clinical data to acute, acquired T.g. induced, extremely rare monosymptomatic aseptic meningitis successfully diagnosed, proved and treated.

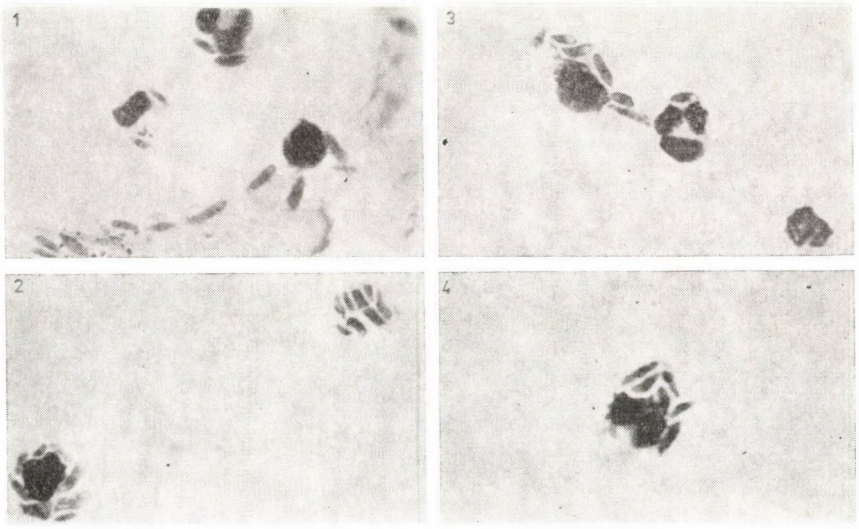
Report of a Case

The history of F.S., a 4 year old boy, did not contain data worth mentioning. On 1st October, 1982, he awoke with a headache, then projectile vomiting and a temperature of 38°C were noted. The doctor found occipital stiffness and positive Kernig sign and sent the child to our hospital with the diagnosis of meningitis. Upon admission the temperature was 38.5°C and the patient complained of mild headache. Physical examination showed no pathological change apart from the mild occipital stiffness. On lumbar puncture water-clear CSF of slightly increased pressure was obtained. It contained 0.24 g/l

TABLE I

Verification of Toxoplasma gondii infection

Examination	Date					
	01.10.	02.10.	04.10.	07.10.	28.10.	03.02.
Demonstration of tachyzoites in smears	positive			positive		
Isolation of the organism				positive		
IgM, IgG/IF		negative				
Complement fixation test			negative		negative	negative
Indirect haemagglutination test					negative	



FIGS 1—4

protein, 3.14 mmol/l glucose and the cell count was 30 n/μl, with 50% granulocytes and 40% lymphocytes. The Giemsa stained smear prepared from the sediment revealed a large number of T.g. tachyzoites extracellularly and a few intracellularly, indicating an acute infection (Figs 1, 2, 3 and 4).

Treatment with pyrimethamine, trimethoprime-sulfamethoxazole and folic acid was administered for a month. In two days the temperature became normal, the complaints and symptoms ceased completely. A second lumbar punture gave a CSF with slightly increased pressure, but this time the cell count was normal and a single T.g. was only discovered.

CFLP strain mice weighing 15—20 g were inoculated with 0.02 ml CSF intracranially and 0.3 ml intraperitoneally; ten days later a large number of T.g. tachyzoites was found in the abdominal exudate. Results and verification of the T.g. infection are summarized in Table I. Further examinations, including X-rays of the head and chest, ECG, EEG, ophthalmologic examination, biochemical tests and quantitative immune electrophoresis did not reveal any pathological change.

DISCUSSION

The positivity of the smear and the inoculation result were regarded as a proof of fresh T.g. infection. On due

treatment and possibly by the protective mechanism of the host immune system the tachyzoites could not invade the tissues and encyst there. This is the explanation why no demonstrable quantity of antibody was produced (Table I).

The ability of the host immune system to protect against T.g. infection has been confirmed by the studies of Hauser and Remington [6] who found that some of the anti-T.g. immune globulins act as opsonin against tachyzoites, most probably by activating the complement system and through the C_{3b} linked to the immune complex. This was a significant observation because phagocytosis of the tachyzoite may occur without opsonization, as indicated by other data, too [20], in that from blood of healthy subjects formerly not infected by T.g. more than 80% of the freshly isolated monocytes and 50% of the polymorphonuclears rapidly destroyed the intracellular T.g.

In our case the linkage of extracellular T.g. tachyzoites to lymphocytes and granulocytes indicated the presence of humoral antibodies (Figs 1, 2, 3 and 4) although cellular immunity to T.g. infection develops only some months after infection [12]. It was remarkable that although the ratio of granulocytes and lymphocytes was nearly equal in the smears, the tachyzoites adhered principally to lymphocytes. A possible explanation of this may have been that while lymphocytes possess mainly IgG_2 receptors, granulocytes and macrophages primarily have IgG_1 and IgG_3 receptors, to which antibodies have the greatest affinity and the anti-T.g. immune globulins belong primarily to the IgG_2 subclass and to a lesser extent to the IgG_3 subclass [5]. In CH_2 and CH_3 domaines the Fc receptors of lymphocytes bind only molecules in the immune complex, therefore the preference of tachyzoites to bind to lymphocytes may be considered an indirect sign of the presence of specific immune globulins. In the smears we have observed several times tachyzoites that formed a bridge-like connection between a granulocyte and a lymphocyte (Fig. 3). An interesting characteristic of tachyzoites is their sporadical clustering around a pole of the cell, thereby capping it (Fig. 4). The same phenomenon was observed in 5—20% of the T.g. tachyzoites by Dzbenski et al [3] who assumed that cap formation and later detachment of the T.g. surface antigen and corresponding antibody from the host cell is an indication of the tachyzoite trying to escape from the host immune system.

The distribution of T.g. in our case pointed to the presence of specific immune globulins. Why was then the IgM IF result negative? We suppose that the infection was discovered in its early stage when only a small quantity of IgM was present and could not be demonstrated by the rather insensitive IF method. Naot and Remington [16] compared the IF methodology with the more sensitive double-sandwich IgM-Elisa test and found that in 25% of the acute cases the IgM IF was negative. Neverthe-

less, in 93% of the examined sera the double-sandwich IgM-Elisa test was definitely positive while the IgM IF result was negative. Recently, McCabe and Remington have concluded that the absence of IgM type antibodies by IF test does not necessarily mean that the infection is not acute [14].

It is conceivable that the pathogenic agent had entered the host organism through the nasal cavity and from here, just like Naegleria and Acantamoeba [2], it passed through the cribriform lamina, reached the meninx along the olfactory nerve and induced meningitis. Infection was possible, for the child often played in the neighbouring sandy playground frequented by cats, and he had a habit of picking his nose.

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Breast feeding as prophylaxis for atopic eczema: a controlled study of 368 cases

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The present study was undertaken in an attempt to draw data whether breast-feeding is beneficial in prevention of atopic eczema. Three-hundred and sixty-eight babies given different feeding modalities were examined for the presence of atopic eczema at the age of three and six months. Seven percent of breast-fed infants developed eczema compared to 10% of formulae-fed and 6% of mixed breast and formulae-fed infants. No difference in the severity of atopic eczema was recorded in the three study groups. Our experience demonstrates the absence of a protective effect of breast-feeding against the development and severity of atopic eczema.

Atopic eczema during infancy is an annoying condition with disappointing treatment results. The role of breast-feeding and dietary elimination of foreign antigens in the prevention of atopic diseases has long been controversial. Some authors indicated that prolonged breast-feeding is a protective measure for infants prone to atopic diseases by hereditary factors [6]. Matthew et al [10] and Saarinen et al [13] reported that breast-feeding prevented atopic eczema up to one to three years of age, respectively. Other studies, however, failed to demonstrate any prophylactic benefit of breast-fed in comparison to bottle-fed babies in prevention of atopic eczema [1, 11]. Conducting a detailed study of 636 children referred with atopic eczema, Kramer and Moroz [8] have concluded that breast-feeding and delay in introduction of solid artificial food did not protect against atopic eczema.

The present prospective controlled study including 368 babies was designed in an attempt to draw data whether breast-feeding could be recommended for prevention of atopic eczema.

Material and Methods

The study included 394 healthy babies at the age of three to six months who were examined during 1980. Only 368 babies fulfilled the study criteria. Sex, ethnic group, family history, duration and severity of atopy were recorded. The babies were examined for the presence of atopic eczema at the age of three and six months. Infants with contact dermatitis were excluded because of the known inverse relation between this condition and atopy [7].

Mothers were interviewed by a qualified nurse prior to their babies' examination and were requested not to give any information regarding their babies' feeding. Diagnosis of atopic eczema was established by a skilled paediatrician and only babies

with definite eczema were included in the study. During the study period all babies were examined by the same paediatrician and nurse in order to ensure comparability of cases.

In order to qualify as having breast-fed, an infant must have been breast-fed for at least six months with no extra bottle feeding (Group A). Partial breast-feeding included all babies in whom breast-feeding was discontinued or only partially given before the age of six months (Group B). The third group of patients included babies who were bottle-fed immediately after birth with no breast-feeding (Group C).

Criteria used in the diagnosis of atopic eczema were derived from Hanifin and Lobitz [4]. Determination of the severity of the condition was classified in three grades. Severe eczema was called definite when a chronic course and extensive distribution with papules, vesicles, oozing, crusting, erythema and scaling were encountered. Mild eczema had an episodic course and localized distribution with erythema, pigment changes and mild scarring. Moderate eczema had intermediate features between the mild and severe forms.

Results

Age, race and ethnic origin in the three groups were found to be confounding. The family history of atopy was found to be of no significant difference between the three groups. Four out of 13 babies with family history of atopy in Group A, three out of 10 in Group B, and three out of 15 in Group C developed eczema. More babies from atopic families developed eczema compared to those from non-atopic families. Babies with a family history of atopy suffered from more severe and prolonged eczema (Table I).

Twenty-six out of 128 (20%) breast-fed babies developed atopic eczema at the age of three months and nine (7%) at the age of six months. In group B which included 99 breast and bottle feeding babies there were 14 (14%) cases of atopic eczema at the age of three months and six (6%) at the age of six months. Thirty out of 141 bottle fed babies had atopic eczema at the age of three months and 14 (10%) at the age of six months.

Table II indicates that breast-feeding did not change the incidence of atopic eczema compared to bottle-fed or mixed-fed babies. The severity of atopic eczema in the different feeding groups was not significantly different (Table III).

Discussion

Mothers should be encouraged to breast-feed which has many physiologic and psychologic advantages. The development of aritificial formulae was followed by a decline in the prevalence of breast-feeding in many parts of the Western world. Human milk contains bacterial and viral antibodies, including relatively high concentrations of secretory IgA antibodies [10]. It has been shown that growth of different types of viruses can be inhibited by substances in human milk [12]. Macrophages are present in human milk and colostrum which may have the ability to synthesize complement, lysozyme and lactoferrin [9]. Stevenson et al [14] noted a higher incidence of respiratory infections during the second six months of life of formulae fed infants. Allergy and intolerance to cow's milk including diarrhoea, intestinal bleeding and colic are less common in infants receiv-

TABLE I

Atopic eczema in babies with a family history of atopy

	Total	Atopic eczema (6 Mon.)	Family history of atopy	Atopy in babies with family history			
				Total	Mild	Modarate	Severe
Breast-feeding	128	9	13	4	1	2	1
Bottle-feeding	141	14	15	4	1	2	1
Mixed breast and bottle feeding	99	6	10	3	1	1	1

TABLE II

Severity of atopic eczema in relation to breast-feeding

	Mild		Moderate		Severe	
	3 Months	6 Months	3 Months	6 Months	3 Months	6 Months
Breast-feeding	8	5	13	2	5	2
Bottle-feeding	14	8	10	5	6	1
Mixed breast and bottle feeding	9	4	3	1	2	1
Total	31	17	26	8	13	4

TABLE III

Incidence of atopic eczema in different groups of babies

	Atopic eczema		Asymptomatic		Total
	3 Months	6 Months	3 Months	6 Months	
Breast-feeding	26 (20%)	9 (7%)	102	119	128
Bottle-feeding	30 (21%)	14 (10%)	111	127	141
Mixed breast and bottle feeding	14 (14%)	6 (6%)	85	93	99
Total	70	29	298	339	368

ing human milk [9]. Cow's milk is the basis for most formulas and a reduction of morbidity and mortality from gastrointestinal infections resulted from sterilization and refrigeration of the formulae.

A possible benefit for the dietary effect of human milk is that special vulnerability to sensitization is transient as in IgA deficiency which commonly precedes allergy [15]. It was also suggested that sensitization requires antigen entry with E. coli endotoxin which is greatly restricted by human milk formulae [5]. A child lacking these protective mechanisms may have general sensitization, and atopic eczema is probably one of them.

The protective effect of breast-feeding against the development of atopic eczema was found by several authors [10, 13] whereas other investigators concluded that breast-feeding did not provide such prophylaxis [1, 11]. Halpern et al [3] reported allergy in 128 out of 803 children with a family history of allergy, either being breast-fed, cow's milk fed or soya milk fed. Glaser et al [2] found that only 15% of children from allergic families fed soya bean milk from birth to six months developed allergic diseases by the age of six years, compared with 52% of retrospective non-related controls fed cows' milk formulae.

Babies' feeding was a self selected maneuver and could not be assigned in our study. It is likely that there is some difference between breast-feeding mothers and those choosing bottle-feeding. It is probable that some babies were specially breast-fed in order to prevent allergic disease. In our study we could not control the mothers' diets and, as a result, breast-fed babies could have been exposed to foreign antigens.

A possible explanation of the large difference in the incidence of atopic eczema at the age of three and six months is probably the inclusion in the diagnosis of seborrhoeic dermatitis which is not necessarily an allergic disease.

We thus concluded that breast-feeding regardless of its many important advantages does not protect against the subsequent development of atopic eczema. Onset, duration and severity of atopic eczema did not change significantly between the three groups. Babies with a family history of atopy had higher prevalence and more severe form of atopic eczema which was not altered by the source of food.

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The effect of fenoterol on fetal metabolism: cord blood studies

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Albumin, total protein, total bilirubin, glucose, cholesterol, triglycerides, blood urea nitrogen, alkaline phosphatase and glutamic oxaloacetic transaminase levels were determined in the serum of cord blood of neonates born to mothers previously treated with the betamimetic drug fenoterol. The results were compared with those of untreated controls. More deviations from the control were found if the interval between termination of treatment and delivery was shorter than 48 hours. A longer drug-free interval seems more favourable for the metabolic balance of the newborns. Newborns whose mother has received beta-sympathomimetic tocolytic treatment need careful supervision.

The side-effects of beta-sympathomimetic treatment of imminent premature delivery on the maternal circulation and metabolism are well-known [13, 14]. Less is known about the effects on the fetus or on the neonate born following such treatment. A few papers have already pointed out that newborn babies born during betamimetic treatment, i.e. after unsuccessful tocolysis or following betamimetic therapy applied during labour are prone to metabolic acidosis and hypoglycaemia [1, 5]. These effects may be secondary to maternal effects but also direct effects of the drugs on the fetus may occur, since their passage through the placenta has been confirmed [2, 9, 10, 17].

Thus, it seemed justified to study the fetal effects of betamimetic drugs. We have attempted to clarify whether changes in cord blood composition indicating drug effects on fetal metabolism can be demonstrated following betamimetic tocolysis. We also compared newborns born immediately after tocolysis and those delivered after an interval following completion of tocolytic treatment. Isolated blood samples taken from the umbilical vein and the arteries have also been compared.

Methods

The following metabolic indicators were determined from cord blood: bilirubin, total protein, albumin, serum GOT, alkaline phosphatase, triglycerides, cholesterol, urea nitrogen and glucose. The blood was

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sampled immediately after cutting the cord, before birth of the placenta. Mixed cord blood was obtained after easing the ligation of the cord, paying attention to avoid admixture of maternal blood to the samples. Isolated venous and arterial blood samples were taken by cannulating the corresponding blood vessel of the ligated cord. The blood was centrifuged immediately after clotting, the serum was kept at $-20°C$ until analysis. All measurements were performed by a Technicon MT II Autoanalyser.

Material

1. *Neonates born immediately after tocolysis.* Fifteen babies were born by delivery taking place during the intravenous period of betamimetic treatment or during the oral period of sustained betamimetic therapy, within 48 hours after termination of treatment. Nineteen newborns born by uncomplicated delivery during the same period and after a gestation similar in duration served as controls. The two groups were comparable on the basis of gestational age, birthweight, Apgar-score, and duration of delivery, as tested by the two-sample *t*-test. The mean duration of tocolysis was 25.29 ± 13.42 days, the mean interval between termination of therapy and birth was 30.07 ± 17.40 hours. In these groups isolated venous and arterial blood samples were also collected.

2. *Neonates born later after completion of tocolytic treatment.* Fourteen newborns born after sustained betamimetic treatment and 26 untreated newborns were examined. The mean duration of tocolysis was 24.14 ± 15.92 days and the mean interval elapsing between termination of therapy and birth was 21.00 ± 8.59 days. The treated and control groups were again comparable in respect to the above obstetrical criteria.

Betamimetic treatment with fenoterol was administered in all cases for imminent premature labour. The treatment was introduced by a saturating dose of 2 μg/min given in intravenous infusion, this was followed by a maintenance dose of 1 μg/min over 12—16 hours. Thereafter oral therapy was given with one 5 mg tablet administered every 6—8 hours. In each case the treatment was supplemented with verapamil in an intravenous dose of 40—80 μg/min during intravenous tocolysis, and one 40 mg tablet for each tablet fenoterol. None of the mothers had received glucocorticoid treatment during pregnancy.

Statistical analysis of the data was carried out by the two-tailed *t*-test (Student and Welch).

Results

It has to be stressed in advance that neither clinical observations nor the examinations revealed any symptom ascribable to deranged fetal metabolism. In the group treated with betamimetics, one newborn died, he was severely malformed and died of peritonitis when three days old. All the other newborns were healthy during their six-day stay in hospital and at discharge.

Results are summarized in Table I. In addition to cord blood results, the maternal venous values obtained in Group 2 are also indicated for information. Evaluation of these values or searching for an eventual relationship between the maternal and fetal values was not intended.

Several parameters obtained in the group born immediately after tocolysis, Group I in Table I, were found to be significantly different from the values measured in the control group. In the treated group serum cholesterol ($p < 0.01$) and blood urea nitrogen ($p < 0.05$) were higher, while total protein ($p < 0.001$) and serum albumin ($p < 0.01$) were lower than in the controls. There was a trend for higher blood glucose and lower SGOT in the treated newborns than in the controls ($p \approx 0.05$).

The values of Group 2, the newborns born later after termination of betamimetic therapy, differed less from the control values. The albumin and triglyceride levels of the treated newborns were slightly but significantly lower and the urea nitrogen

TABLE I

Cord blood values of newborns born to mothers treated with fenoterol resp. to untreated mothers

	Group 1		Group 2		
n	treated 15	controls 19	maternal blood 14	treated 14	controls 26
albumin, g/l	36.3±5.3**	41.1±3.0	40.1±3.2	36.8±2.3*	39.1±3.3
total protein g/l	55.7±4.7***	63.5±5.8	71.3±5.2	55.5±4.4	58.3±5.6
bilirubin, μmol/l	29.4±10.1	28.3±9.6	8.6±3.6	32.8±9.2	29.5±6.5
glucose, mmol/l	4.99±0.83~*	4.35±1.18	5.52±1.20	3.94±1.46	4.91±1.45
cholesterol, mmol/l	2.94±0.64**	2.26±0.51	6.11±1.19	2.27±0.55	2.31±0.37
triglyceride, mmol/l	0.35±0.14	0.37±0.24	3.02±1.02	0.28±0.11*	0.38±0.17
urea nitrogen, mmol/l	3.57±0.69*	3.09±0.70	3.95±1.04	3.23±0.97*	2.44±0.80
alkaline phosphatase U/l	171.2±46.4	201.5±65.1	183.8±73.6	162.0±41.7	186.0±62.2
SGOT, U/l	45.6±15.4~*	57.4±19.3	36.5±17.0	42.8±11.3	47.2±17.7

Group 1: newborns born within 48 hours after discontinuation of tocolysis
Group 2: newborns born beyond 48 hours after discontinuation of tocolysis plus maternal values (venous blood)
The data are means±SD
***: $p < 0.001$
**: $p < 0.01$
*: $p < 0.05$
~+: $0.05 < p < 0.07$
no sign: not significant

TABLE II

Results in venous and arterial blood of newborns of mothers treated with fenoterol and of untreated mothers
Means and standard deviations

	Treated		Controls	
n	Artery 15	Vein 15	Artery 19	Vein 19
albumin, g/l	35.5±5.0	36.8±5.5	40.7±2.9	41.6±3.2
total protein, g/l	56.2±8.3	57.6±6.9	64.5±6.0	64.5±5.7
bilirubin, μmol/l	30.6±10.0	28.4±10.3	26.8±10.0	29.7±9.2
glucose, mmol/l	4.85±0.69	5.08±0.92	4.34±0.84	4.27±1.33
cholesterol, mmol/l	3.26±1.02	2.79±0.77	2.28±0.55	2.23±0.47
triglyceride, mmol/l	0.33±0.11	0.39±0.14	0.36±0.22	0.36±0.22
urea nitrogen, mmol/l	3.71±0.99	3.45±0.86	3.11±0.70	3.06±0.71
alkaline phosphatase, U/l	169.2±48.8	179.2±53.1	204.1±63.5	203.0±68.4
SGOT, U/l	55.2±33.6	44.5±23.2	55.1±22.7	59.1±17.1

For none of the parameters was there a significant difference between venous and arterial values

values slightly but significantly higher than the control values ($p < 0.05$). Blood glucose appeared lower in the treated group but the difference from the control values did not attain the 5% level of significance.

None of the parameters differed between the isolated venous and arterial samples (Table II).

Evaluation of the results was partly hampered by the scarcity of normal cord blood values in the literature. On the basis of several sources, the normal mean value for glucose is 3.8 mmol/1 with a range of 2.36—5.05 mmol/1 [5, 7], for cholesterol 1.75 (1.38—2.49) mmol/1 [1, 8]. We found a single set of normal data for triglycerides [1]: 0.30 (0.24—0.47) mmol/1; for total protein [7]: 61.0 (43.0—73.0) g/1, and for SGOT [7]: 5—120 U/1.

Discussion

Brazy and Pupkin [4] observed a significantly higher incidence of hypotension, hypoglycaemia, hypocalcaemia and intestinal paralysis and an increased mortality rate in newborns born to mothers treated with isoxsuprine as compared to babies of untreated mothers. The betamimetics have been demonstrated in the blood of neonates born after tocolytic therapy by several investigators. Lierde and Thomas [10] administered ritodrine to mothers prior to elective Caesarean section and in the cord blood they found one third of the betamimetic concentration of the maternal blood. Brazy et al [2] found 0—35.6 μg/ml isoxsuprine in the cord blood of newborn babies of mothers treated with the drug in the last 24 hours prior to delivery. There was a negative correlation between the isoxsuprine concentration in the cord blood and the time elapsing between termination of maternal treatment and delivery; values exceeding 10 μg/ml were only encountered if the baby was born within 2 hours after termination of maternal betamimetic therapy. Severe complications in the newborn only occurred if that level had been exceeded. The relationship between time of betamimetic exposition and incidence and severity of complications was more marked in preterm babies. The half-time of isoxsuprine was 1.5—3 hours in term neonates while in preterm babies it was as long as 6—8 hours [2, 3].

In this study we have attempted to clarify the fetal effects of maternal betamimetic treatment by determining some chemical parameters in cord blood. The newborns in both treated groups appeared healthy in clinical terms, none of the complications described in the literature have been encountered in any of them. Blood chemistry, however, revealed clear-cut differences between the treated and the control infants. The alterations were more pronounced in babies born within 48 hours after termination of betamimetic therapy, as for four parameters out of nine the difference was statistically significant and for two other parameters there was a clear trend for a difference. In the babies born a longer time after the cessation of betamimetic therapy

only three parameters differed from the control values and the difference was slight statistically.

Cord blood glucose was higher in the babies born immediately after cessation of betamimetic treatment than in the controls. This finding was in agreement with data in the literature [5, 11, 12] and can easily be explained by the well-known effect of betamimetics on carbohydrate metabolism. Weidinger et al [15] on the other hand found diminished blood glucose levels in cord blood of neonates born immediately after tocolytic therapy, but were unable to explain the unexpected finding. Sustained fetal hyperglycaemia may set off hyperinsulinism, and this and the depletion of hepatic glycogen stores during prolonged glycogenolysis may convert hyperglycaemia to hypoglycaemia soon after birth [5]. In our material, the babies born a long time after maternal betamimetic therapy had lower blood glucose values than the controls; the difference was, however, not significant statistically. It may be speculated that the low blood glucose level encountered at birth was a consequence of forced glycogenolysis elicited by prolonged betamimetic treatment of the mother; restoration of the liver glycogen contents could, however, be expected to occur a few days after discontinuation of betamimetic therapy.

The cholesterol value was elevated in the babies born immediately after tocolysis. We found a single report in the literature on a similar finding observed after simultaneous administration of ritodrine and betamethasone [1]; these authors attributed the hypercholesterolaemia to the corticosteroid treatment and not to the betamimetic therapy. Since in our cases no corticosteroids were given, only the betamimetic therapy could have been the cause of the increased cholesterol level, and we anticipate that the same was the case in the material of Andersen and Friis-Hansen [1]. The triglyceride level, the other indicator of lipid metabolism in this study, exhibited no difference.

An eventual effect of betamimetic drugs of fetal liver function has been put forward by several authors: it may namely be anticipated that the enzymes of the fetal liver play a role in the metabolism of betamimetic drugs. Rosanelli et al [12] found increased bilirubin levels in newborns born after maternal tocolytic treatment while Weidinger et al [16] observed hyperbilirubinaemia in 28% of newborns following betamimetic treatment. Brazy et al [2] could not demonstrate any alteration in the liver function of neonates whose mother had undergone isoxsuprine treatment and supposed that either the betamimetics had no influence on liver enzymes or they may induce those metabolising the drug itself, leaving the bilirubin metabolism unaltered. In our study, fenoterol had no measurable effect on the cord blood bilirubin level. The slight decrease of alkaline phosphatase and SGOT activity encountered in both treated groups might be ascribed to an eventual effect on liver function.

Serum total protein and albumin levels were lower in both treated groups than in the controls. This finding was paradoxical, being at variance with the anabolic effect of betamimetic drugs promoting fetal growth demonstrated in several previous studies. It should also be noted that although the differences were statistically significant, all mean values fell within normal limits.

The informative value of cord blood findings may depend on the fact whether the sample was taken from the umbilical vein or from one of the arteries, e.g. in case of pH measurements. It was thought therefore that the metabolic parameters might also differ depending on the kind of blood vessel from which the sample had been drawn. Anyhow, it has turned out that none of the parameters differed in this respect significantly. Similar studies have only been published on differential values of blood glucose, the venous and arterial values were identical [6]. It can thus be concluded that isolated blood sampling is not necessary for such purposes, mixed cord blood being suitable in similar studies.

In summary, several metabolic indicators in cord blood taken from newborns delivered following betamimetic treatment differed significantly from the control values, and this points to an effect of such treatment on fetal metabolism. The changes were more pronounced in babies born immediately after betamimetic tocolysis. In spite of statistically significant differences, the deviations were of no biological importance since even the differing means fell within normal limits and the treated newborns were free of clinical symptoms.

The following conclusions have been drawn.

— In spite of demonstrable metabolic alterations, tocolysis performed according to accepted standards does not lead to considerable fetal complications;

— newborns delivered following betamimetic tocolysis need intensive observation;

— the indication of betamimetic tocolysis should carefully be weighed in cases with unfavourable prognosis, as unsuccessful treatment is not only superflous but may lead to metabolic effects unfavourably influencing the preterm baby's condition.

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The Thyroxine Screening Index in congenital hypothyroidism screening

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A modification of the RIA total thyroxine assay from a dry drop of blood has been applied in 5-day-old neonates for the screening of congenital hypothyroidism. For first classification, the thyroxine screening index was used in 13 500 newborns; it proved to be simple, rapid and economically acceptable. In borderline cases, more detailed examinations were carried out, viz. estimation by RIA of thyroid stimulating hormone, thyroxine and thyroxine-binding globulin from venous blood. The incidence of permanent and transient impairment of thyroid function is shown in a special graph.

Two methods are usually employed for mass screening of congenital hypothyroidism, radioimmunoassay (RIA) and enzyme immunoassay (ELISA) for determining thyroid hormones in the blood serum of neonates. It is possible to determine the levels of thyroxine (T_4) and of thyroid-stimulating hormone (TSH) or their combination; the latter may be regarded as the best solution [4, 6]. TSH has hitherto been considered a more reliable indicator of thyroid function than T_4, especially in the case of ectopic thyroid glands [6]. It has, however, been shown recently that primary T_4 screening may have a similar sensitivity as TSH for mass screening programs for congenital hypothyroidism [1].

To introduce the method in Czechoslovakia, we elaborated and tested clinically a modification of the total thyroxine assay from a dry drop of blood in neonates by the so-called single step T_4 RIA [5].

For first classification, we adopted a procedure which employed the thyroxine screening index (TSI). This was used for assessing the borderline and pathological values.

Material and Methods

For single step T_4 RIA, a drop of blood is withdrawn from the heel. On Schleicher and Schüll 2992 filter paper the spot measures 12 mm in diameter. Spots 3 mm in diameter containing about 3.0 μl of blood (1.5 μl of serum with an assumed haematocrit value of 50%) were cut out and analysed. The method is based on single step addition of the reagents. One ml of a stock solution containing specific serum, a buffer, labelled antigen, immunoglobulin G, polyethylene glycol and 8-amino-1--naphthalene sulphuric acid were pipetted into test-tubes, then the dried blood spot was added.

For determining TSI, the original determination of total T_4 was modified by eliminating the calibration curve and modifying the calculations. Instead of employing several samples for constructing

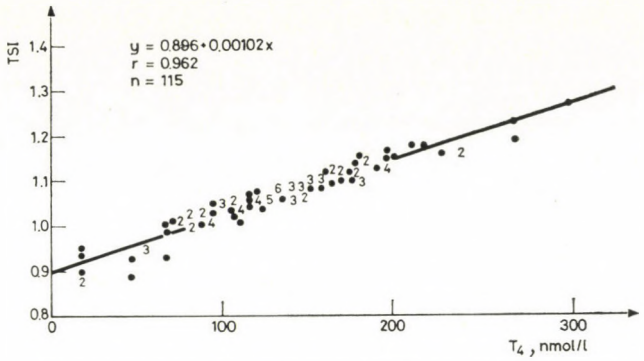

FIG. 1. Correlation between total T_4 and TSI in 115 blood samples collected on the 5th day of life. Numerals indicate the number of estimations for a given value

the calibration curve, we used a pooled sample from normal donors as the reference standard. Samples for estimation were processed in series. Each series contained estimated and reference sera for the calculation of TSI. The reference sample was represented by the pooled T_4 values of blood donors. For checking the error of analysis, we used serum from a patient with confirmed hypothyroidism. Results were evaluated by calculating TSI from the ratio of the reference and tested samples, viz.

$$\text{TSI} = \frac{\text{cpm reference sample}}{\text{cpm unknown sample}}.$$

In a normal newborn, the TSI value was 1.0 ± 0.1. Values between 0.90—0.99 indicated that the analysis must be repeated to eliminate some laboratory error. A recall was essential when TSI was below 0.90 (1.0 sigma). The validity of the analysis was controlled by the value of non-specific binding, the dispersion of values of the reference samples, and the value of the control sample. The long-term stability of the above-mentioned parameters was followed in profile diagrams.

The relation of TSI values and T_4 levels was studied in 115 neonatal blood samples collected on the 5th day after birth. Total T_4 concentrations were determined in the same samples by T_4 RIA kit (DRG) from a dry blood spot (Fig. 1). It is evident that there was a significant correlation between these values of $r = 0.962$ at the 0.0005 level of significance. So far we have examined 13 500 5-day newborns employing the TSI parameter.

RESULTS AND DISCUSSION

TSI was found to be a simple, rapid and economically acceptable test which proved useful for providing first information about the functional state of the neonatal thyroid gland. The results were classified into three groups (Fig. 2).

In Group A, pathological values are shown which require an immediate detailed assay from venous blood.

Group B includes borderline values which require repeated examination from original samples of dry blood on filter paper.

Entirely normal values were found in Group C.

From this scheme it is evident that, if necessary, this first examination can be specified in more detail by further tests such as RIA T_4, TSH and/or TBG. TSI is in accordance with the instructions of the European Thyroid Association for introducing SKH and the procedure which was recommended by Fischer et al [2] for use in the USA.

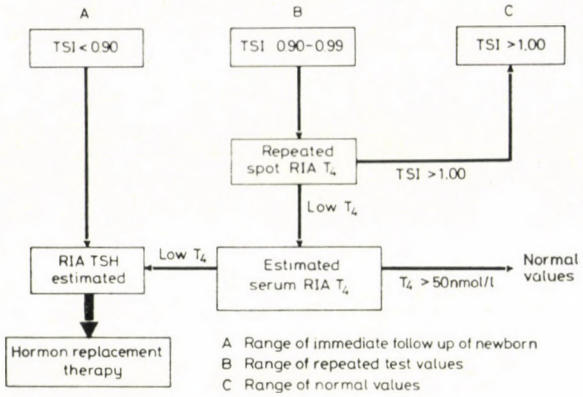

FIG. 2. Evaluation of TSI results of screening for congenital hypthyroidism. Group A: pathological values which require immediate detailed assay from venous blood. Group B: includes borderline values which require repeated examination of the original sample of dry blood. Values in Group C correspond to normal values

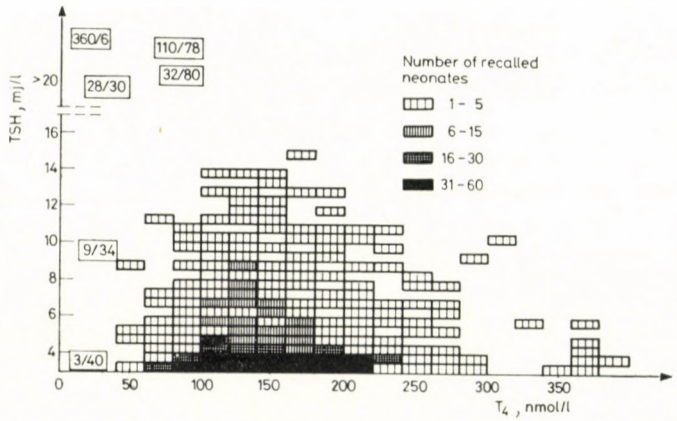

FIG. 3. Statistical distribution of total T_4 and TSH values in neonatal venous blood serum. The black area represents the highest frequency attained during a given period:
360/6: 360 I.U./l TSH, 6 nmol/l T_4 — primary hypothyroidism (athyreosis)
110/78:110 I.U./l TSH, 78 nmol/l T_4 — primary hypothyroidism (rudimentary thyroid gland)
32/80:32 I.U./l TSH, 80 nmol/l T_4 — transient hypothyroidism
28/30:28 I.U./l TSH, 30 nmol/l T_4 — transient hypothyroidism
9/34:9 I.U./l TSH, 34 nmol/l T_4 — defect in TBG production
3/40:3 I.U./l TSH, 40 nmol/l T_4 — defect in TBG production

In our material we encountered 3 cases of primary hypothyroidism, 2 cases of TBG defect and 3 cases of transient hypothyroxinaemia.

The distribution of the results of venous serum samples is shown in Fig. 3. The radioimmunological values of total T_4 obtained by the method of single step analysis are on the abscissa, while the TSH values on the ordinate. The TSH values were obtained by RIA using the Calbiochem

semikit. The black area represents the highest incidence of neonates recalled during the given period. In the upper part of the map are cases of primary hypothyroidism, such as athyreosis and ectopic thyroid gland, and below are the cases of transient hypothyroidism. In the bottom part are cases of secondary hypothyroidism and those with a defect in TBG production.

After having introduced a national screening program for congenital hypothyroidism, computer evaluation is being planned for processing the results of the above model.

Our investigations are part of a pilot study which is being employed in five regions of Czechoslovakia, where 60 252 newborns were examined on the fifth day after birth by the end of 1982 [3]. The incidence was so far 1:5 000 newborn infants. It is expected that in the near future this screening program for hypothyroidism will cover the whole of Czechoslovakia.

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Complete recovery from paraquat poisoning causing severe unilateral pulmonary lesion

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Poisoning by paraquat, a plant-protecting agent, its clinical manifestations and treatment are discussed. The case of a 5-year-old boy who had ingested an unknown quantity of paraquat is described. Peritoneal dialysis proved to be effective in overcoming renal and hepatic failure. Subsequently, a pulmonary lesion with unilateral preponderance developed; this showed marked radiological regression and in a year nearly complete functional recovery ensued.

Paraquat was synthesized during the last century and has been used as an oxidoreduction indicator in chemistry [4]. Since the sixties of this century it has been utilised as a plant-protective agent, in granulated form, or 20 % solution in aerosol. Its aqueous solution is reddish-brown in colour, and may be mistaken for beer or Coca-cola. Paraquat is a quaternary bipiridyl cation, it inhibits the conversion of NADP to NADPH in the cells, and induces damage to the cell membrane by polymerising the unsaturated lipids.

Since 1966, several hundred papers reported on accidental or suicidal paraquat poisoning. Most frequently, the agent enters the organism orally; its aerosol may penetrate the skin at plant spraying [13, 23] or directly invade the airways [8]. Absorption of the orally ingested agent is poor, only 1—5 % is absorbed, the rest is excreted with the stools [4, 11, 15]. The major part of the absorbed paraquat is excreted by the kidneys, a small proportion appears in the bile; the pathogenesis of renal and hepatic damage is thus obvious. A third target organ is the lung [24], the paraquat concentration in the pulmonary tissue exceeds about fifty times that of the plasma. This explains the occurrence of severe pulmonary complications.

Paraquat can be demonstrated in the urine and this test may be of use in judging the effect of treatment. Knowledge of the blood level may also be useful. It can be measured by spectrophotometry, ion exchange, gas chromatography and RIA [4, 10, 14, 21, 22]. A blood level exceeding 0.1—0.2 μg/ml is usually lethal within 24—48 hours, but exceptional survivors displaying higher levels have also been described [10, 15, 21]. The mortality rate is very high, amounting to 33—60 % [3, 4, 24].

Ingestion of 6—10 g paraquat leads to convulsions, pulmonary oedema, shock and death within several hours or a few days. Smaller doses cause burning sensation in the mouth, oesophageal erosions and sometimes perforation [1], abdominal pain and hepatic and renal failure followed by adult respiratory distress syndrome ending in death. Cellular damage may occur in the adrenal glands manifesting with cortical necrosis [20]. If smaller doses are ingested, the pulmonary changes ensue only after 2 or 3 weeks. They consist of oedema, damage to the alveolar epithelium, haemorrhage, atelectasis, infiltrates, pleural effusions, bullous changes and ultimately alveolar and interstitial fibrosis [2, 4, 7, 11, 24, 26]. These changes can be followed radiologically and by pulmonary function tests [8, 17]. Time is the most important factor in therapy: the mortality rate of cases whose treatment was started beyond the first five hours after ingestion of the agent was as high as 64% as observed in a material of 68 cases [12]. First aid comprises binding of the agent to prevent its absorption application of an emetic, cautious gastric lavage (danger of perforation!), administration of Fuller's earth and purgation. The second step is elimination of the poison by forced diuresis [10, 21], haemoperfusion [19, 21, 23, 27], haemodialysis, plasmapheresis [5] or peritoneal dialysis [15]. Administration of ascorbic acid may be of benefit [9]; steroids or even immunosuppressive drugs have been attempted for prevention of pulmonary damage [4, 15]. In addition, administration of air with a low oxygen concentration not exceeding 20% even in hypoxia has been recommended; breathing of nitrogen gas may be useful in preventing or slowing down the oxidative process initiated by paraquat [6, 21].

Report of a Case

P. K., a five-year-old boy, was admitted on 18 May, 1982. Three days earlier he had drunk an unknown quantity of 20% paraquat solution. On the next day he had vomited but the general practitioner did not find any abnormality. On the following day he had had haematemesis and was admitted to a country hospital. There the oral erosions and haematemesis suggested some poisoning and then the parents remembered that the child may have drunk of the plant-protecting agent. Gastric lavage was performed, Fuller's earth was administered and the child was admitted to our department.

At admission the child was azotaemic, with a blood urea nitrogen level of 39.8 mmol/l and a serum creatinine of 465 μmol/l. Chest X-rays revealed no abnormality and all other laboratory findings were normal. Only traces of paraquat could be shown in blood and urine taken at the time of admission. In view of the renal failure peritoneal dialysis was instituted; it was terminated after five days when the blood urea nitrogen and creatinine levels had returned to normal values. On the fifth day of treatment slight jaundice appeared, laboratory findings revealed mild hepatic damage (serum GOT, 82 U/l; GPT, 52 U/l; serum bilirubin, 13 μmol/l). On the seventh day, tachypnoea of 70—80 per minute set in and repeated chest X-rays showed a large infiltration in the whole right lung, with a minor infiltrate in the left lung (Fig 1). A high dose of methylprednisolone (250 mg per day), later daily 20 mg dexamethasone, antibiotics and 20% oxygen were administered; lower oxygen concentrations were not applied as the pO_2 value was low.

The patient's condition improved gradually, the respiration rate diminished. Chest X-rays on the 46th day showed mediastinal dislocation to the right, rarefaction within the right lung and several

bullae could be demonstrated by tomography (Figs 2, 3). Increased transparence of the left lung accompanied by a fascicular pattern in the basal parts was then found. Treatment was complemented by administration of atomised mucosolvents and steroids, the dose of oral corticosteroids was gradually diminished.

Pulmonary scintigraphy, carried out after the acute phase, showed markedly decreased perfusion in the right lung, with complete absence of activity in the apical region. The effective capillary perfusion of the right lung was 25—30% of that of the left lung (the normal proportion is right: left = 60—55%:40—45%).

Repeated lung function tests pointed to the possibility of pulmonary fibrosis: forced vital capacity (FVC) was markedly depressed, intrathoracal gas volume (IGV) and tidal volume (TV) showed low values. All lung function findings are shown in Table I.

After termination of corticosteroid treatment, aerosol therapy was complemented with respiratory exercises. The patient was discharged with normal respiratory rate and normal renal and hepatic function tests on the 84th day after admission.

At home respiratory exercise was prescribed and the patient was regularly followed at the clinic. Blood gas analysis and bicycle ergometry revealed normal values. The parents reported on normal activities of the boy at home, lung function tests demonstrated gradual improvement. By the end of the first year all values reached or exceeded the lower limit of normal. A second lung scintigraphy demonstrated reduced perfusion but the improvement was striking (Fig. 4). The chest X-rays also revealed gradual improvement and on 15 August, 1983, they showed the mediastinum in nearly normal position and a nearly normal translucency of the right lung (Fig. 5).

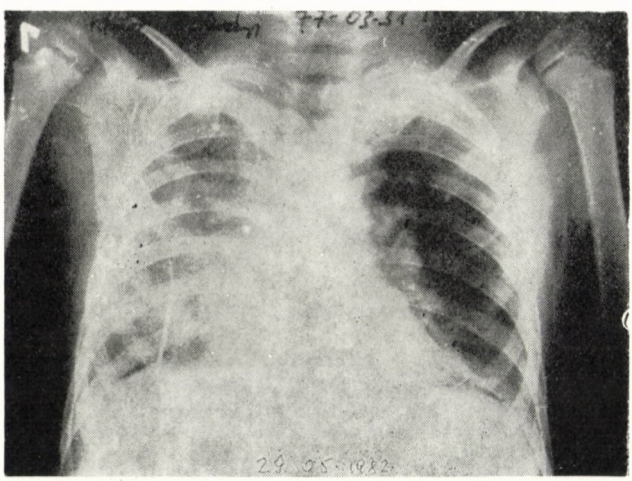


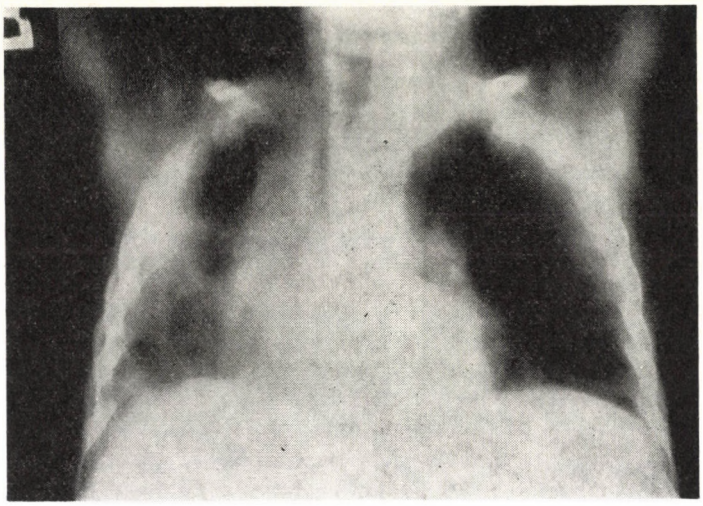
FIG. 1. Chest X-rays 10 days after ingestion of paraquat: massive infiltration of whole right lung, moderate infiltrate on left side

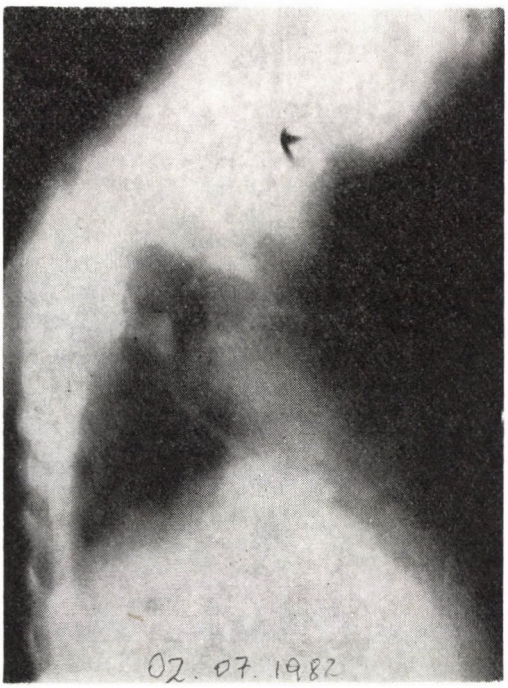
TABLE I

Results of lung function tests

	FVC		FEV		PEFR		R		IGV	
	ml	percent	ml	percent	l/sec	percent	mbar/l/sec	percent	ml	percent
29 June, 1982	662	(45)	424	(35)	1.44	(49)	—	—	—	—
9 August, 1982	896	(61)	666	(55.5)	1.86	(63.4)	17.25	(297)	315	(39)
12 April, 1983	1125	(77.5)	923	(77)	3.09	(105)	5.8	(100)	932	(116)
15 August, 1983	1120	(77)	980	(79)	3.02	(104)	5.7	(99)	926	(115)

Figures in brackets: percentage of the corresponding normal value





FIGS 2. 3. Tomography on 46th day: bullous changes in right lung, the mediastinum is dislocated to the right

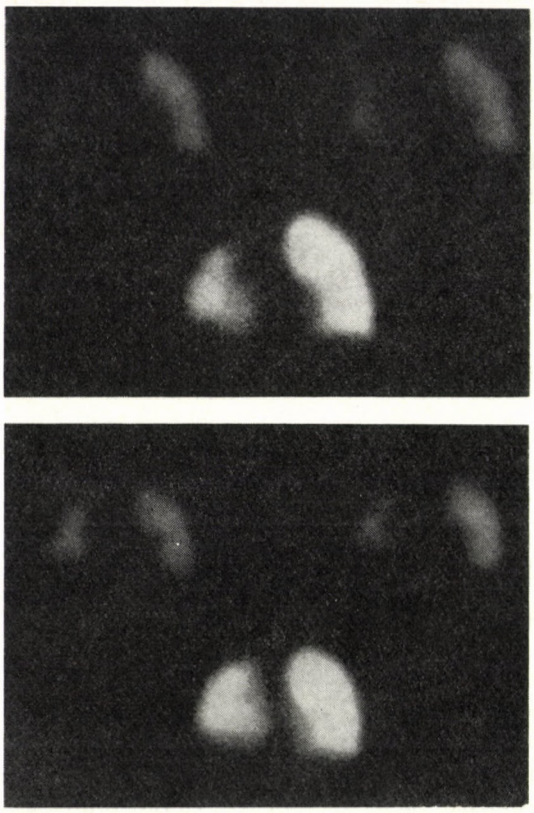


FIG. 4. Scintigraphy, immediately after the acute phase: very marked diminution on right side. After half a year: distinct improvement

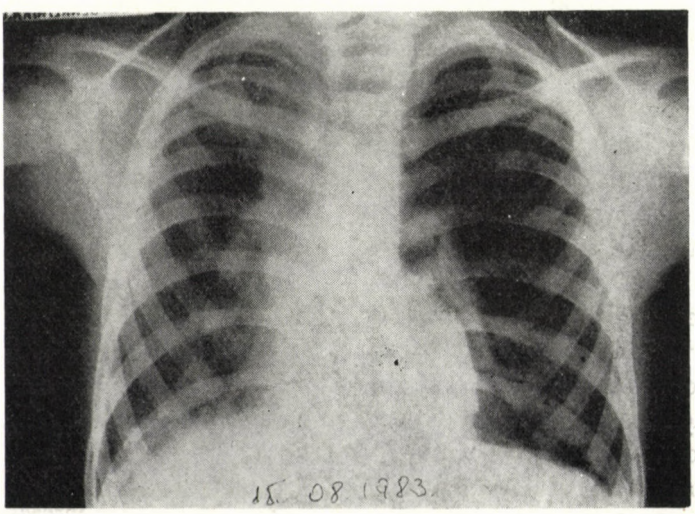


FIG. 5. X-rays on 15 August, 1983: nearly normal finding, the mediastinal dislocation has almost completely disappeared, increased transparency of right lung

Discussion

In spite of improvement of the therapeutic methods applied in paraquat poisoning, the quantity ingested and the time of introduction of treatment are the factors determining the outcome. In our case the ingested quantity of paraquat remained unknown, it may however, be anticipated, that it had not been within the lethal range. The symptoms showed a comparatively late onset, the administration of Fuller's earth could not have been effective [12].

In this situation treatment of renal failure and the liver damage seemed of primary importance; haemoperfusion was not done because only traces of paraquat could be found in the patient's blood and urine. Steroid treatment introduced at admission did not prevent the development of pulmonary damage although it has been recommended for this purpose [4, 8, 21]. Mahieu et al [15] succeeded in preventing pulmonary damage by bleomycin, forced diuresis and peritoneal dialysis. In our patient the pulmonary complication was nearly completely restricted to the right lung, as confirmed by X-rays and scintigraphy; bullous changes developed in addition.

We have been unable to find any report on recovery from a pulmonary complication as severe as seen in our case. In the case described by George and Hedworth-Whitty [8] pain in the chest, dyspnoea and abnormal auscultation findings ensued after inhalation of paraquat aerosol but as the X-rays revealed no abnormalities in spite of diminished FEV_1, VC and PEFR values, they thought of interstitial damage (fibrosis?). In the literature there are descriptions on 8 paediatric cases of paraquat intoxication; two of them died, in the other six patients there were no pulmonary complications demonstrable by X-rays or lung function tests [18, 23, 25]. The toxic effect of oxygen in paraquat poisoning has long been known; in an environment rich in oxygen the free paraquat radicals induce cellular damage, primarily to the pneumocytes. In addition, the drug radicals initiate superoxide formation as well [3, 4, 6]. On the basis of these findings, administration of low (10—20%) oxygen concentrations has been recommended for treatment of hypoxaemia. In our case, we applied 20% oxygen for a short time.

In our opinion the unilateral complication itself and its nearly complete healing, as demonstrated by X-rays, scintigraphy and lung function tests carried out repeatedly during the one-year follow-up, were unique features of the case presented. The explanation of this rare event may lie in the better regenerative power of children.

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Wolman disease in twins

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In newborn twins at three hours of age adrenal calcification has been detected. In addition to hepatomegaly, vomiting and diarrhoea, characteristic radiological findings confirmed the diagnosis of the rare heritable lipidosis, Wolman's disease.

Bilateral adrenal calcification may be caused by several pathological conditions [2]. Primary familial xanthomatosis or Wolman's disease [8] has to be considered in the first days of life. The characteristic radiological finding accompanying the clinical symptoms may be the first sign confirming the diagnosis [4]. As a consequence of lacking lysosomal acid esterase [5, 6], cholesteryl ester and triglycerides are stored in the liver, adrenals and bone marrow. The presence of typical foamy histiocytes in the bone marrow is pathognomonic [7].

Case reports

Of the female twins originating from a first cousin marriage of a Libyan Arab couple (Fig 1), the first-born had a birthweight of 2100 g, the second-born member of the pair weighed 1700 g. Both were admitted at three hours of age. Twin A had anaemia with 101 g/l haemoglobin and 0.34 l/l packed cell volume. She was therefore transfused with blood of her own blood group. The twins were monoplacental, monoamnial and monochorial. Their blood groups were identical within the ABO, Rh. Lutheran, Kell and Duffy systems. Physical examination revealed a prominent abdomen with hepatomegaly in both babies. Abdominal X-rays taken at three hours after birth showed bilateral calcification of the adrenal glands of both children (Figs 2 and 3). These changes were even more conspicuous in twin B two weeks later (Fig 4); in the same baby adrenal calcification accompanied by severe hepatomegaly was seen at four months of age (Fig 5). Feeding difficulties appeared as early as three days of age in both newborns, soon joined by profuse vomiting and watery diarrhoea. The patients needed glucose-saline infusions. The condition of twin A was more severe in spite of her higher birthweight. In addition to anaemia the leukocyte count was 3 G/l, platelet count 120 G/l, reticulocytes 1.10^{-3}. In the blood film 35 nucleated erythrocytes per 100 leukocytes were found, the distribution of leukocytes was normal. Twin A also had a congenital heart defect, and septic symptoms appeared accompanied by heart failure. In spite of broad spectrum antibiotics and mechanical ventilation she died at two weeks of age. Necropsy could not be performed, being prohibited by Libyan laws.

Vomiting of twin B ceased during intravenous fluid therapy but her stools continued to be of watery consistence. She failed to thrive in spite of adequate caloric intake. The patient had to be given blood transfusion several times. At three months of age

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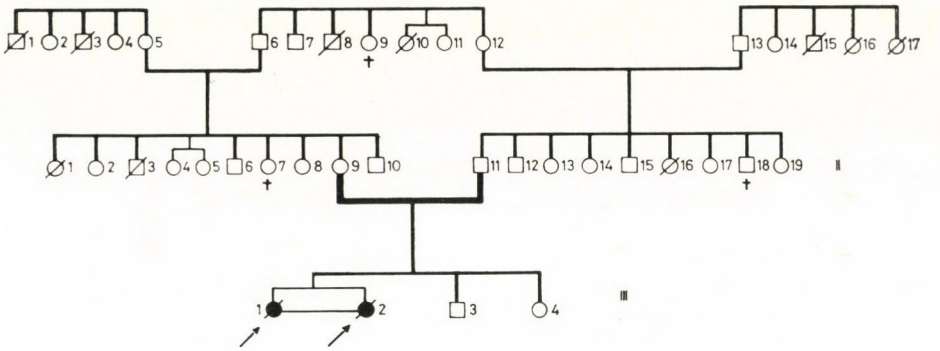


FIG. 1. Pedigree of the patients

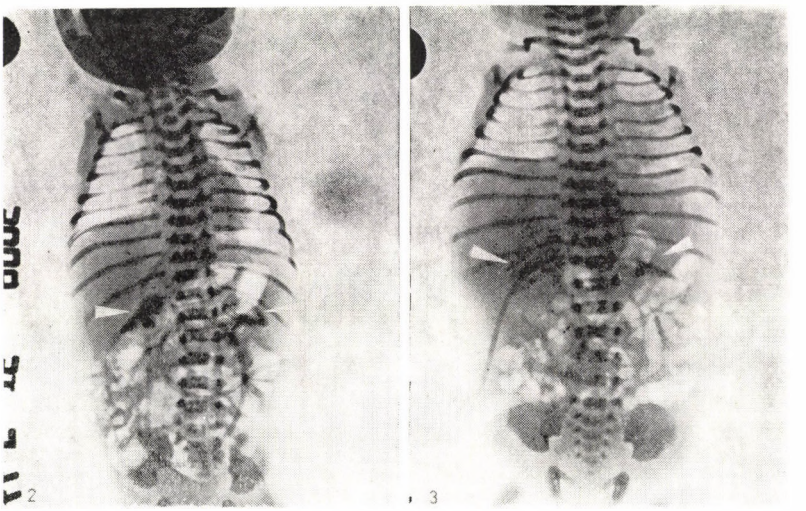
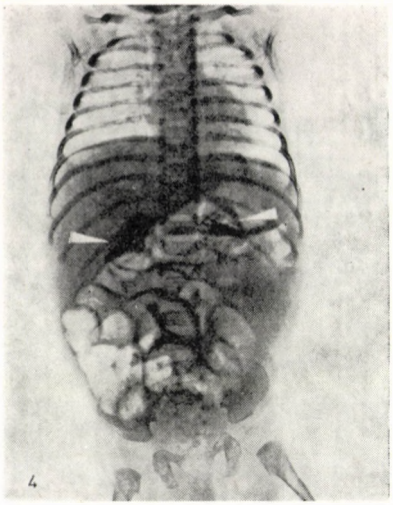


FIG. 2. Plain abdominal X-ray of twin B at three hours of age. The cap-shaped calcifications in both adrenal regions are indicated by arrows

FIG. 3. Plain abdominal X-ray of twin A. Adrenal calcification of the right side is partly covered by the umbilical catheter, intestinal gases cover the calcification on the left, the lesions are less pronounced than in twin B

she weighed 2420 g; at this time her parents took her home. At discharge from hospital her liver was palpable 2 cm below the costal margin, the spleen could not be felt.

One month later the infant was readmitted because of fever and diarrhoea. At that time she weighed 2850 g. An enlarged, soft liver was palpated 3 cm below the costal margin, only the tip of the spleen could be felt. There were no demonstrable neurological signs. Her laboratory findings were: haemoglobin: 82 g/l, PCV: 0.36 l/l, leukocyte count: 5.3 G/l, platelets: 80 G/l, differential leukocyte count: striking vacuolisation of lymphocytes. Bone marrow: diminished erythropoiesis, foamy histiocytes. Liver function tests: direct bilirubin: 3.4 μmol/l, indirect bilirubin: 6.8 μmol/l, SGOT: 17 IU/l, SGPT: 10 IU/l, LDH: 416 IU/l, alpha-HBDH: 292 IU/l, gamma-GT: 61 IU/l, alkaline phosphatase: 254 IU/l, ESR: 28 mm/hour. Urine analysis revealed no abnormality. Stool bacteriology and parasitology: negative. BUN: 3.0 μmol/l, serum sodium: 136 mmol/l, potassium: 3.1 mmol/l, chloride: 102 mmol/l. In view of the vomitings, diarrhoea and the underlying disease, glucose, saline, amino-



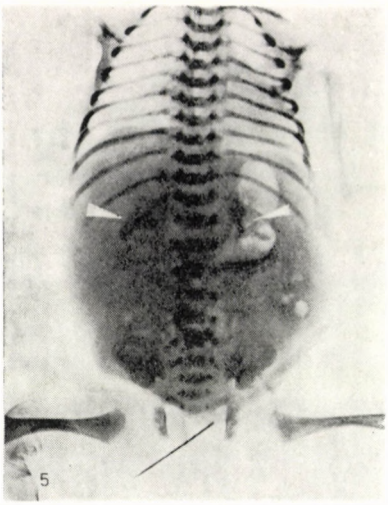


FIG. 4. Plain abdominal X-ray of twin B at two weeks of age. Pronounced calcification in the right adrenal region, on the left side it is covered by intestinal shadows. Hepatomegaly and bronchopneumonia can be observed

FIG. 5. Plain abdominal X-ray of twin B at four months of age. Adrenal calcification unchanged, marked hepatomegaly

acid solutions and protein were given. The patient died of apnoea and cardiac arrest 10 days after admission.

DISCUSSION

In type I of Wolman's disease the characteristic features are hepatosplenomegaly, diarrhoea, adrenal calcification, vacuolized lymphocytes in the blood smear, foamy histiocytes in the bone marrow and neurological symptoms like clonus, hyperreflexia and opisthotonus [1]. In the second type, neurological signs are missing. Our cases appeared to belong to type II although the patients died at an early age.

Wolman disease as a lipid storage disturbance has to be differentiated from Gaucher disease and Niemann—Pick disease. Adrenal calcification may occur in neuroblastoma when the abnormality is unilateral. In case of adrenal haemorrhage the changes may be bilateral but the adrenals usually decrease in size in such cases [3]. Demonstration of lacking or markedly depressed activity of lysosomal acid esterase in liver biopsy material or leukocytes confirms the diagnosis.

The enzyme deficiency can be diagnosed at 3 or 4 months gestational age from fibroblast cultures obtained by amniocentesis. In our cases the clinical features, the radiological findings and demonstration of foamy histiocytes in the bone marrow have confirmed the diagnosis of Wolman disease.

Therapeutic attempts with cholestyramine, d-thyroxine, clofibrate or a medium chain triglyceride diet con-

taining much protein and rich in calories have hitherto failed. Corticosteroid treatment may be attempted.

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Neonatal pulmonary haemorrhage, birthweight, gestational age and intrauterine growth

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The relation between neonatal pulmonary haemorrhage and its underlying cause was studied by reviewing the clinical data and necropsy records of 315 newborn babies who had died between day 0 and 31 of life. Necropsy revealed massive and focal pulmonary haemorrhage in 6.9% and 19.3% of the infants, respectively. It has been concluded that pulmonary haemorrhage is a complication of various neonatal diseases related to hypoxia and/or infection, which occur in preterm infants with a much higher frequency than in term babies. Among patients with pulmonary haemorrhage, males and low birthweight babies predominated.

Neonatal pulmonary haemorrhage continues to be an enigma. The lack of uniform definition may well be one of the causes of the widely scaterring frequency of pulmonary haemorrhage reported in the literature. Nonetheless, technical thoroughness and the diagnostic criteria applied at postmortem examination, just like the morbidity characteristics of the newborn population studied may also influence the reported frequencies in the different populations. The question, whether pulmonary haemorrhage was a clinical entity or merely a preterminal syndrome has not been decided, nor is it known whether it is justified to separate either clinically or pathologically the massive haemorrhages from focal ones. Furthermore, apart from anecdotical reports, we have no solid knowledge about direct or indirect relationship between gestational age, birthweight and intrauterine growth, and the frequency of pulmonary haemorrhage in the newborn infant.

The present study was undertaken as an attempt to shed more light on the above detailed problems.

Patients and Methods

During the period from 1/1/1980 to 31/12/1982, in our Intensive Care Unit 315 babies had died before the age of 1 month. Detailed postmortem examination was performed in every case, histology of the lungs included. All babies with histologically proven pulmonary haemorrhage of any location — alveolar, interstitial and mixed forms — and extension were selected for further study. Pulmonary haemorrhage was considered to be massive when at least one lobe seemed to be involved at gross examination. Less extensive haemorrhages were described as focal. For histology a tissue specimen was excised from each lobe of both lungs and after fixation and embedding it was stained with haematoxylin-eosin and PAS. By microscopic examination pulmonary haem-

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orrhage was diagnosed if a considerable amount of erythrocytes were seen in the alveolar spaces and/or the perivascular and peribronchial tissues and also in the dilated lymph vessels.

On admission all babies were weighed and their length measured to an accuracy of 10.0 g and 1.0 cm, respectively. Gestational age was calculated according to the date of the last menstrual period of the mother and expressed in completed weeks. Intrauterine growth relative to gestational age was judged by using a locally constructed intrauterine growth chart. Those with birthweight equal or less than the 10th centile were considered small for dates. In addition, the ponderal index deseribed by Rohrer [22] of each infant was also calculated, which truly reflects body mass relative to body length, in other words the degree of leanness.

The data and clinical course of all infants with pulmonary haemorrhage were then thoroughly reviewed and evaluated. Hyaline membrane disease and the various disorders of cardiorespiratory adaptation due to or related with perinatal hypoxia were termed RDS-I and RDS-II, respectively. Survival time was calculated in hours. For statistical analysis, standard mathematical methods were used.

Results

Pulmonary haemorrhage and birthweight. In 83 of the 315 succumbed infants (26.3%) pulmonary haemorrhage was found at post mortem, but the frequency of massive pulmonary haemorrhage was considerably less, i.e. 6.9% (22/315). When looking for a relationship between birthweight and pulmonary haemorrhage, a decreasing incidence could be observed in heavier babies, especially in those with birthweight $\geq$ 2000 g. In patients with birthweight $>$ 2500 g, the frequency of focal haemorrhage rose again remarkably (18.0%) in contrast with that of massive haemorrhage which had a frequency of 2.1% (Table I). Therefore, the frequency of massive haemorrhage seemed to be more weight-related than the occurrence of focal haemorrhages. Figure 1 shows that in babies of birthweight $\leq$ 1500 g and $>$ 2500 g, massive pulmonary haemorrhage occurred in 8.7% and 2.1% of the patients, respectively.

Pulmonary haemorrhage and gestational age. As expected, similar trends were observed when the relationship between gestational age and pulmonary haemorrhage was studied. Massive and focal haemorrhage was found in 9.5% and 23.8% of the infants with gestational age $\leq$ 30 weeks. After that, the frequency of massive haemorrhage decreased with advancing maturity, but there was again a sharp rise in the incidence (21.8%—28.8%) of focal haemorrhage in full term infants (gestational age $\geq$ 37 weeks), whilst the frequency of mas-

Table I

Frequency of pulmonary haemorrhage in newborn infants with different birthweight (weight-specific percent frequency in parentheses)

Birthweight (g)	≤1000	1001—1500	1501—2000	2001—2500	>2500	Total
All deaths	47(100	79(100)	57(100)	38(100)	94(100)	315(100)
Pulmonary haemorrhage	18(38.2)	24(30.3)	17(29.8)	5.(13.1)	19(20.2)	83(26.3)
Massive haemorrhage	5(10.6)	6(7.5)	7(12.2)	2(5.2)	2(2.1)	22(6.9)
Focal haemorrhage	13(27.6)	18(22.7)	10(17.5)	3(7.8)	17(18.0)	61(19.3)

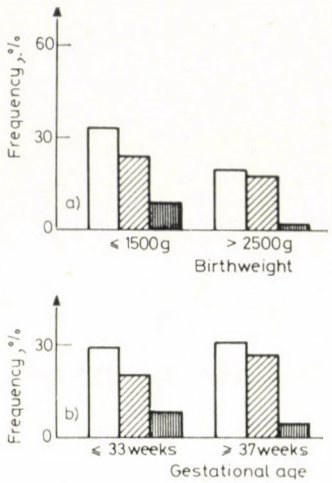

FIG. 1. Frequency of pulmonary haemorrhage in newborn infants with birthweight $\leq$ 1500 g and $>$ 2500 g (part A) — and with gestational age $\leq$ 33 weeks and $\geq$ 37 weeks (part B) · □ = pulmonary haemorrhage; ▨ = massive pulmonary haemorrhage; ▥ = focal pulmonary haemorrhage

sive haemorrhage decreased further on (Table II). It is seen in Fig. 1 that in infants of gestational age $\leq$ 33 weeks, massive pulmonary haemorrhage occurred twice as frequently than in the term babies (8.4% vs 3.8%). In contrast, no difference was found between the two groups in the frequency of focal haemorrhage; what is more, it was slightly higher in full term infants (20.7% vs 25.9%).

Pulmonary haemorrhage and intrauterine growth. Since the gestational age of 14 of the 83 study patients was $\leq$ 27 weeks, intrauterine growth of only 69 babies could be quantified with the growth chart used. Table III shows that one-third of preterm and term infants with pulmonary haemorrhage were growth retarded by the adopted definition (birthweight $\leq$ 10 centile). Furthermore, the birthweight of two-thirds of the pulmonary haemorrhage infants did not exceed the 25 centile of the standard distribution. A closely similar distribution of birthweights was found when only babies with massive haemorrhage were stud-

TABLE II

Frequency of pulmonary haemorrhage in newborn infants with different gestational age (gestational age-specific percent frequency in parentheses)

Gestational age (week)	≤30	31—33	34—36	37—38	≥39	Total
All deaths	105(100)	73(100)	60(100)	32(100)	45(100)	315(100)
Pulmonary haemorrhage	35(33.3)	17(23.2)	8(13.3)	9(28.1)	14(31.1)	83(26.3)
Massive haemorrhage	10(9.5)	5(6.8)	4(6.6)	2(6.2)	1(2.2)	22(6.9)
Focal haemorrhage	25(23.8)	12(16.4)	4(6.6)	7(21.8)	13(28.8)	61(19.3)

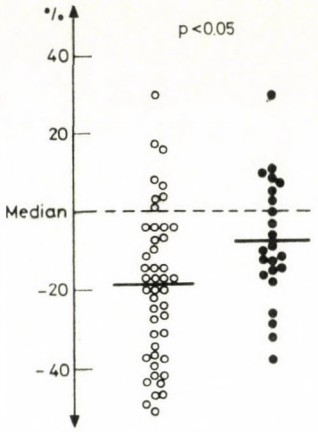

FIG. 2. Percentage deviation of birthweight of preterm (○) and term (●) infants with pulmonary haemorrhage from the median appropriate for their gestational age and sex

TABLE III

Birthweight centile distribution of newborn infants with pulmonary haemorrhage (percentage values in parentheses)

Centile zones	Preterm	Term	Total
≤10	15(32.6)	6(26.0)	21(30.4)
11–25	13(28.2)	7(30.4)	20(28.9)
26–50	10(21.7)	3(13.0)	13(18.8)
51–75	5(10.8)	3(13.0)	8(11.5)
76–90	3(6.5)	3(13.0)	6(8.6)
>90	—	1(4.3)	1(1.4)
	46(100.0)	23(100.0)	69(100.0)

TABLE IV

Gestational age, birthweight, ponderal index and survival time (mean±SEM) of preterm and term infants suffering from pulmonary haemorrhage, associated with hypoxia and/or infection

	Gestational age, week	Birthweight, g	Ponderal index	Survival time, h
Preterm				
Hypoxia(41)	29.2±0.45**	1190±58**	2.20±0.04	72.6±8.2***
Infection (19)	31.3±0.59	1542±100	2.27±0.06	213.9±2.16
Full term				
Hypoxia (13)	38.2±0.39*	2792±162	2.41±0.12	88.4±14.1**
Infection (10)	39.4±0.37	3180±141	2.55±0.06	189.5±32.3

Student's t test: *$p < 0.05$, **$p < 0.01$; ***$p < 0.001$

TABLE V

Clinical characteristics of the study patients

	Preterms (60)				Full terms (23)			
	Hypoxia No/total	(41) percent	Infection No/total	(19) percent	Hypoxia No/total	(13) percent	Infection No/total	(10) percent
Pathologic delivery	14/41	34.1	3/19	15.7	3/13	23.0	2/10	20.0
Resuscitation	20/41	48.7	5/19	26.3	5/13	38.4	2/10	20.0
Oxygen therapy (head-box)	9/41	21.9	8/19	42.1	5/13	38.4	6/10	60.0
Oxygen therapy (CPAP)	17/41	41.0	7/19	36.8	5/13	38.4	1/10	10.0
Oxygen therapy (IPPV)	33/41	80.4	12/19	63.1	8/13	61.5	2/10	20.0
RDS-I – RDS-II	41/41	100.0	16/19	84.2	11/13	84.6	0/10	0.0
Pneumonia	0/41	0.0	13/19	68.4	0/13	0.0	6/10	60.0
Septicaemia	0/41	0.0	4/19	21.0	0/13	0.0	4/10	40.0
Meningitis	0/41	0.0	2/19	10.5	0/13	0.0	0/10	0.0
Severe congenital malformation	2/41	4.8	1/19	5.2	3/13	23.0	6/10	60.0
Clinical symptoms with pulmonary haemorrhage	8/41	19.5	2/19	10.5	2/13	15.3	0/10	0.0
Massive pulmonary haemorrhage	12/41	29.2	7/19	36.8	2/13	15.3	1/10	10.0
Focal pulmonary haemorrhage	29/41	70.7	12/19	63.1	11/13	84.6	9/10	90.0

ied in this respect (5/18, 27.7 % $\leq$ 10 centile and 7/18, 38.8 % $\leq$ 25 centile). Figure 2 clearly shows the shift in weight of pulmonary haemorrhage infants towards the lower ranges. The mean ($\pm$ SEM) percentual deviation from the median was $-18.5 \pm 2.8\%$ and $-7.4 \pm 3.2\%$ in the preterm and term infants ($p < 0.05$). 14/60 (23.3 %) and 5/23 (21.7 %) preterm and term babies had a ponderal index of < 2.00 and < 2.20, respectively, indicating that a similar part, nearly one-fifth of both preterm and term pulmonary haemorrhage infants were growth retarded on the basis of these criteria too.

Distribution by sex. Of the total population of infants with pulmonary haemorrhage 66.2 % were male (55/83) and 33.7 % (28/83) were female. The twofold numerical dominance of male infants remained unchanged when preterm (m/f = 39 /21) and term babies (m/f = 16/7) were considered separately, and also if only those with massive haemorrhage were studied in this respect (m/f = 15/7).

Some clinical details and associated conditions are shown in Tables IV and V. In reviewing the clinical courses and evaluating them in the light of the necropsy findings, two fundamental aetiopathogenetic factors emerged from the chain of multiple pathologic events leading finally to death, specifically a perinatal hypoxia of shorter or longer duration, and some types of infection. When the patients were grouped according to these two main pathogenic factors, it was found that babies who had suffered hypoxia were considerably lighter and less mature and had a significantly shorter survival time than those whose death could primarily be ascribed to infection, in the group of both preterm and term infants (Table IV). By correlation

TABLE VI

Association of subependymal, intraventricular and spinal-epidural haemorrhage with pulmonary haemorrhage in preterm and term infants who had had hypoxia and/or infection

	Preterm (60)				Full term (23)			
	Hypoxia (41)		Infection (19)		Hypoxia (13)		Infection (10)	
	MPH (12)	FPH (29)	PMH (7)	FPH (12)	MPH (2)	FPH (11)	MPH (1)	FPH (9)
Subependymal or intraventricular haemorrhage	8	17	1	5	—	2	—	—
Spinal-epidural haemorrhage	5	7	1	3	2	2	—	4

Abbreviations: MPH = massive pulmonary haemorrhage; FPH = focal pulmonary haemorrhage;

analysis, however, no statistically significant relationship was found between birthweight, gestational age, ponderal index and survival time. Table V shows that both massive and focal pulmonary haemorrhage were in association with either severe cardiorespiratory adaptation disorders which needed oxygen therapy, or with different kinds of infection.

It was, however, notable that massive pulmonary haemorrhage occurred more frequently in preterm than term infants, independently of whether hypoxia or infection was the main operating factor causing the death of the patient. In vivo symptoms indicating pulmonary haemorrhage like bleeding or haemorrhagic discharge from the upper respiratory tract were detected only in 10—20% of the cases. Table VI shows how frequently hypoxia-related intracranial haemorrhage was associated with either massive or focal pulmonary haemorrhage.

DISCUSSION

The pathogenesis of neonatal pulmonary haemorrhage continues to be a controversial topic in the literature. The wide variety of diseases thought to be related to pulmonary haemorrhage, like perinatal asphyxia [3, 12, 14, 17, 23], hyalin membrane disease [23], central nervous system lesions [3, 11, 12], aspiration [14, 23], pneumonia [8, 16], hypothermia [5, 7], septicaemia [4, 14], congenital heart disease [3, 4, 13, 17], coagulation disorders [1, 9, 21], tranfusion and infusion therapy [17], oxygen [6, 15, 18, 24] and ventilation therapy [19] reflect much uncertainties. A possible synthesis of the numerous divergent theories seems to emerge on the basis of suggestions made by Adamson et al [2] and Cole et al [10], according to which pulmonary haemorrhage is basically a haemorrhagic oedema of the lungs, caused primarily by hypoxia and acidosis, and secondary left heart failure.

The data of our study patients convincingly proved that nearly all formerly suggested aetiologic factors may have had a role in the clinical course of the infants, many of them acting in combination. All babies were oxygen-therapy dependent and needed respirator therapy, transfusions and parenteral fluid administration by drip infusion. In a considerable number of the patients infection was a complication of decisive importance, either due to major surgery because of malformations or to a iatrogenic infection in consequence of the intensive care.

Neonatal pulmonary haemorrhage is a postmortem diagnosis since pathognomonic clinical symptoms do not occur regularly before death. In the present series of observations haemorrhagic discharge or bleeding from the upper respiratory airways was recorded in 10—20% of the cases, though some authors reported an incidence of 50%. Considering the great number of neonatal diseases which occasionally are associated with pulmonary haemorrhage and also the wide variability of their outcome, the incidence of pulmonary haemorrhage can only be estimated postmortem. In the 3 year study period, an incidence of 6.9% of massive pulmonary haemorrhage was found which corresponded well to formerly published frequencies [3, 14, 16, 17, 25, 26]. Focal pulmonary haemorrhage could be observed in a further 19.3% of the infants. It is worth to note that both massive and focal haemorrhages had complicated very similar diseases, furthermore that hypoxia and infection played a determining role in the final outcome of all patients, independently of whether they had massive or focal haemorrhage at necropsy (Table V). No difference in survival time was found between babies with massive (122 ± 22 hours) or focal (112 ± 14 hours) haemorrhage, which finding proves again the primary importance of the basic pathology and the secondary role of pulmonary haemorrhage, be it massive or focal. In support of this conclusion is that no significant correlation existed between birthweight, gestational age, ponderal index and survival time of the babies. Within the groups of preterm and term babies, those whose death may have primarily been attributed to infection were heavier, more mature and survived for a longer time than those with pulmonary haemorrhage due first of all to hypoxia (Table IV). It is supposed that the more favourable outlook certainly related with the advanced maturity of these babies had been altered by infection, malformation or surgery.

When examining the relationship between pulmonary haemorrhage and the maturity of the newborn infant, preterm and in general low birthweight babies were found to be at higher risk. It should be emphasized at the same time that preterm infants are much more predisposed to every condition frequently associated with pulmonary haemorrhage. The increased frequency of focal haemorrhage in term babies was remarkable. The cause remains

obscure, but to suppose the role of maturity-related pathophysiologic responses does not seem to be unrealistic.

Intrauterine growth retardation has been known to predispose to pulmonary haemorrhage, most likely due to a reduced intrauterine oxygen supply via the disturbed placental circulation. In fact, one-third of the preterm and term infants with pulmonary haemorrhage were small for dates by the definition used, and nearly 60% of them had a birthweight of equal or less than the 25th centile (Table III). The mean deviation from median weight was significantly greater in the preterm than in the term newborns (Fig. 2). All these observations are, however, to be evaluated with caution. They could never prove a direct relationship between retarded fetal growth and pulmonary haemorrhage but raised the possibility of some relation existing between fetal body growth and composition and neonatal pathology, elucidation of which would need further research. We think that the same applies to the 2/1 male/female ratio in babies who died with pulmonary haemorrhage.

In conclusion, it is suggested that neonatal pulmonary haemorrhage is not a clinical entity but much rather a preterminal complication of severe disorders of cardiorespiratory adaptation, which are in cause-and-effect relation with hypoxia. Closely similar final pathomechanisms must be acting when pulmonary haemorrhage presents itself in the final stage of severe pulmonary or generalized infections. Massive pulmonary haemorrhage develops much more frequently in small birthweight, preterm infants than in more mature ones. The frequency of focal pulmonary haemorrhage — in contrast with that of massive haemorrhage — rises again in term babies, after a fall with advancing maturity. The numerical preponderance of small for gestational age and male babies amongst pulmonary haemorrhage patients was remarkable. The questions remain, however, unanswered whether these babies are more susceptible to pulmonary haemorrhage or retarded intrauterine growth, and male sex was predisposing to conditions of which a complication would be the pulmonary haemorrhage.

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The effect of unprocessed wheat bran on blood glucose and plasma immunoreactive insulin levels during oral glucose tolerance test in obese children

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Blood glucose and plasma immunoreactive insulin concentrations were measured during oral glucose tolerance test in 10 obese children. Oral glucose was given by itself or combined with 15 g unprocessed wheat bran. Bran significantly reduced the blood glucose and plasma immunoreactive insulin concentrations at 30 min of the tolerance test. It is concluded that supplementation of obese children's diet with unprocessed bran is advantageous.

The effect of dietary fibre on carbohydrate metabolism has widely been studied in adults. Most studies seem to agree that fibre or at least some types of it (guar, gum, tragacanth, pectin) lower the glucose level during glucose tolerance tests [4, 8] and after meals [3, 9, 10]. The results concerning the glucose-lowering effect of cellulose are, however, contradictory [2, 6, 8] and there is even less agreement on the influence of dietary fibre on plasma insulin levels. Some of the studies showed that lower blood glucose levels were accompanied by lower insulin levels [3, 4, 9] while others denied this [8, 10]. Such investigations have not yet been carried out in obese children. The purpose of the present study was to investigate the blood glucose and plasma insulin concentrations in obese children when unprocessed wheat bran was given at the beginning of a standard oral glucose tolerance test.

Patients and Methods

Oral glucose tolerance test (1.75 g/kg b.w.) was carried out in 10 obese children (4 girls and 6 boys) after 10—12 h fasting. Oral glucose was given by itself or combined with 15 g unprocessed wheat bran (21% cellulose, 26% hemicellulose, 3% pectin, 4% lignin). The most important data and anthropometric parameters of the children are shown in Table I. Capillary blood samples were obtained by fingerprick before and 30, 60, 90, 120 and 180 min after the consumption of wheat bran and/or glucose solution.

Blood glucose was measured with the orthotholuidine method, plasma immunoreactive insulin (IRI) with radioimmunoassay using the charcoal separation technique. Relative body weight and body fat were calculated as described earlier [7]. Normal range of blood glucose and plasma IRI were used as given by Guthrie et al [1]. The statistical significance between the means was evaluated with the paired *t*-test.

Results

Glucose tolerance was normal, mean blood glucose levels fell into the normal range (Fig. 1). Glucose-induced plasma IRI concentrations were above

TABLE I

Anthropometric values (M±SE) of the investigated children

Age, yr	Body weight, kg	Height, cm	Rel. body weight, percent	Body fat percent
12.04 ±0.61	63.39 ±3.46	151.2 ±3.31	148.4 ±5.04	38.59 ±1.92

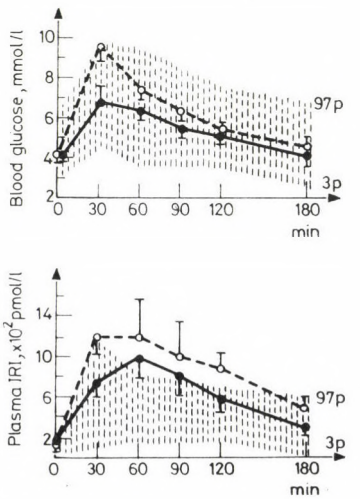

FIG. 1. Blood glucose and plasma immunoreactive insulin (IRI) concentrations in 10 obese children during oral glucose tolerance test with ——— and without – – – added bran. Vertical bars represent standard errors. An asterisk shows where the difference between the means is significant ($p < 0.02$). The range between the 3rd and 97th percentile of the distribution of normal values [1] is shown by the shaded area

the 97th percentile of the distribution of normal values. Unprocessed wheat bran significantly reduced blood glucose levels and plasma IRI concentration at 30 min, but all blood glucose and plasma IRI values tended to be lower when bran was consumed with glucose.

DISCUSSION

Wheat bran caused a moderate but significant decrease in blood glucose and plasma IRI concentration in the early phase of glucose tolerance test. Similar results were obtained in adults by Jenkins et al [4]. Bran possibly reduces or delays the absorption of glucose and this leads to a secondary decrease in IRI concentration. By reducing carbohydrate absorption wheat bran may help weight reduction. Hyperinsulinaemia is not a primary cause of obesity although it may play a role in the development of severe obesity [5]. Hypertriglyceridaemia, which is a frequent finding in childhood obesity [7], has also a bearing on hyperinsulinaemia.

Considering the above-mentioned facts the blood glucose and plasma IRI lowering effects of wheat bran seem to be advantageous in childhood obesity. In addition, volume for volume, fibre-rich foods provide less available energy than fibre-depleted foods. The increased bulk and low calorie density may be advantageous in reducing energy intake by displacing foods of high caloric density from the diet. However, before recommending the use of bran in the treatment of overweight children, the long-term effects of high fibre diet on carbohydrate and lipid metabolism have to be investigated.

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Book reviews

R. WITKOWSKI, O. PROKOP: *Genetik erblicher Syndrome und Mißbildungen für die Familienberatung.* 3., erweiterte Auflage in 2 Bänden. 1502 Seiten. Akademie-Verlag, Berlin 1983. Preis M 95,—

Die vorangehenden zwei Auflagen dieses Buches waren populäre Hilfsmittel für jene genetischen Ratgeber, die im deutschen Sprachgebiet arbeiten. Die jetzige Auflage widerspiegelt die rapide Vermehrung der Kenntnisse und den angewachsenen Anspruch auf genetische Beratungsstellen.

Der erste Band enthält die lexikale Beschreibung der einzelnen Krankheiten, während der zweite Band aus literarischen Angaben und auf 165 Seiten einem permutierten Symptomregister besteht.

Der Aufbau des lexikalen Teiles hat sich in der vorliegenden Ausgabe nicht geändert. Die Besprechung eines jeden Syndroms besteht aus einer Beschreibung des genetischen Ursprungs (oder dessen Mangels), der wichtigsten Symptome, Therapiemöglichkeiten, Häufigkeit und Vorkommen; abschließend wird die Familienberatung besprochen. Der Gebrauch des Buches wird dadurch erleichtert, daß ein jedes Synonym, das in der Literatur unter mehreren Benennungen erwähnt wird, in alfabetischer Reihenfolge bei den Schlagwörten steht. Ein weiteres Verdienst der Autoren ist, daß neben der langen Reihe der dysmorphischen Syndrome und Enzymopathien auch jene öfters vorkommende Krankheiten (z.B. Polyarthritis, Rheumatismus, Reye Syndrom usw.) angeführt werden, wegen deren die genetische Beratungsstellen oft in Anspruch genommen werden.

Ein relativer Nachteil der zweibändigen Auflage besteht darin, daß man zum Heraussuchen der literarischen Daten ein zweites Buch benützen muß, statt diese unter den Schlagwörten direkt auffinden zu können. Vielleicht könnten in einer nächsten Auflage die zwei Bände in der Reihenfolge der Stichwörter redigiert werden. Auch hätte man gerne ein erfrischtes Literaturverzeichnis gesehen, da unter den zitierten Arbeiten sehr wenige nach 1980 erschienen sind.

Das permutierte Symptomregister bietet eine bedeutende Hilfe bei der Diagnostik, da die Diagnose oft bei der Beratung gestellt oder wenigstens angenähert werden muß.

Die Autoren hielten es vor Augen, daß sie dem genetischen Berater ein leicht und gut verwendbares Handbuch in die Hände geben sollen. Das ist restlos gelungen, und das ausgezeichnete Syndromen-Lexikon ist für alle, die in der Krankheitsversorgung beschäftigt sind, ebenfalls nützlich, da die Resultate und Methoden der praktischen Humangenetik heute bereits in sämtlichen Gebieten der Medizin täglich angewandt werden.

MAGDA OSZTOVICS

Aktuelle Probleme der pädiatrischen Allergologie. Herausgegeben von U. WAHN. X+180 Seiten mit 44 Abbildungen und 29 Tabellen. Gustav Fischer Verlag, Stuttgart 1983. Preis DM 58,—

Der Band enthält das Material der im Oktober 1982 in Bochum veranstalteten Konferenz, an dem drei Schwerpunktthemen diskutiert wurden:

a) atopische Erkrankungen und Zusammenhang zwischen erblichen und exogenen Faktoren;

b) Kuhmilchallergie;

c) obstruktive Bronchitis im Säuglings- und Kleinkindesalter.

a) Das einleitende Referat von König und Mitarb. befaßt sich mit der Anti-IgE-Synthese und vor allem mit der Funktion der die IgE-Produktion regulierenden Suppressor T-Zellen. In diesem Zusammenhang wurden auch die Möglichkeiten zur therapeutischen Beeinflussung der IgE-Produktion angedeutet. Der Beitrag von Siraganian behandelt die aus den Mastzellen und basophilen Zellen frei werdenden Mediatoren; nach der gegenwärtigen Einteilung können diese in der Zelle präformiert anwesend sein oder aber unter Wirkung von Aktivität entstehen. Die neuen Kenntnisse der vergangenen Jahre ermöglichten dem Autor, ein hypothetisches Scheme hinsichtlich des Freiwerdens der Mediatoren zu konstruieren. In dem Beitrag von Marsh wird die Steuerung der IgE-Regulation erörtert. Nach dem heutigen Wissen steht die atopische Erkrankung unter genetischer Kontrolle, wobei jedoch auch äußere Reize hinzukommen. Die genetische Regulation dürfte die Folge eines zum HLA-System gehörenden und eines von diesem unabhängigen Systems sein. Hervorzuheben sei ferner der Beitrag von Bousquet und Mitarb. über die Bestimmung des Risikos einer späteren allergischen Krankheit bereits im Säuglingsalter. Eine positive familiäre Anamnese soll auf die 35—80% Wahrscheinlichkeit einer späteren Erkrankung hindeuten. Der IgE-Gehalt der Nabelschnur und die atopische Krankheit der Mutter sollen einzeln und zusammen die Wahrscheinlichkeit einer späteren Atopie beim Kind erhöhen. Ein Beitrag von Saarinen ist der Prophylaxe gewidmet; bei Kindern aus atopischen Familien kann das verlängerte Stillen und verzögerte Gemüsezufuhr, Verminderung des Haushaltsstaubes und tierischen Epithels zur Herabsetzung der Probleme dieser »Risikosäuglinge« beitragen.

b) Mehrere Vorträge beschäftigen sich mit der Kuhmilchallergie; so fassen Savilahti und Mitarb. die intestinalen morphologischen Veränderungen zusammen, während die Beobachtungen von Freier und Mitarb. jene Theorie unterstützen, daß die Kuhmilch-Intoleranz einer immunregulatorischen Störung zuzuschreiben ist; ob dabei die Störung der Helperzelle oder der Suppressorzelle dominiert, ist fraglich. Hill und Mitarb. fassen das Klinikum der Kuhmilch-Intoleranz zusammen. Es sei bemerkt, daß hier zwischen Text und Tabelle 8 ein Widerspruch besteht und die Angaben in bezug auf die spotane Heilung schwer zu bewerten sind.

c) In diesem Themenkreis wird im Referat von Geubelle und Mitarb. festgestellt, daß es bei etwa 10—20% der Säuglinge mindestens einmal zu einer obstruktiven Bronchitis kommt. Warum ein Teil der Infektionen zu einer dieses Krankheitsbild hervorrufende Ursache wird, und warum aus einem Teil dieser sich Asthma entwickelt, ist jedoch ein ungeklärtes Problem. Riedel und Mitarb. weisen darauf hin, daß die atopische Beschaffenheit in sich zu Bronchiolitis prädisponiert, Reinhardt und Mitarb. darauf, daß im Säuglingsalter die Beta-Rezeptoren der Bronchusschleimhaut noch unreif und folglich die Betamimetika relativ wirkungslos sind und schließlich schreibt von der Hardt, daß im Hintergrund der rezidivierenden Bronchitis häufig anatomische Abnormitäten des Bronchialsystems stehen, was auch die Beobachtungen von Székely bekräftigen.

Das Buch bietet ein umfassendes Bild über den gegenwärtigen wissenschaftlichen Stand der angeführten drei Themen.

E CSERHÁTI

W. CATEL: *Das gesunde und das kranke Kind* 12., neubearbeitete Auflage. Herausgegeben von E. GLADTKE, J. OEHME, J. SCHAUB. XXVIII + 868 Seiten mit 489 Abbildungen, 10 Farbtafeln und 104 Tabellen. Georg Thieme Verlag, Stuttgart 1983. Preis DM 89,—

Dieses von W. Catel — dessen Diagnostik zu seiner Zeit mit Recht zu den berühmtesten pädiatrischen Büchern gehörte — begründete Lehrbuch für Kinderkrankenschwestern erschien zuerst im Jahre 1939 während seiner Leipziger Tätigkeit und erlebte bis 1977 elf Ausgaben. Die vorliegende zwölfte, völlig neue Bearbeitung wurde notwendig durch die erhebliche Vermehrung des Wissensstoffes, den heute eine Kinderkrankenschwester beherrschen sollte. Der grundsätzliche Aufbau des Buches wurde beibehalten. Die bewährte Darstellung der Spezialgebiete innerhalb einer Kinderklinik wurde auch beibehalten, doch neu bearbeitet.

Das Lehrbuch dient in erster Linie zur Ausbildung, aber auch die im Beruf stehenden Kinderkrankenschwester und Kinderkrankenpfleger können es als Nachschlagwerk benützen, und in der Fortbildung kann es ebenfalls eine bedeutende Rolle spielen. Das Buch ist in 42 Kapitel gegliedert. Es fängt mit dem Berufsbild der Krankenschwester und einem geschichtlichen Rückblick an. Dann folgen die Grundkenntnisse: Physik, anorganische und organische Chemie, Anatomie und Physiologie, klinische Chemie, Radiologie einschließlich Computertomographie, Sonographie, Nuklearmedizin und Strahlenschutz, Hygiene und Mikrobiologie, und Medikamentenlehre. Weitere Kapitel sind die körperliche, geistige und seelische Entwicklung des gesunden Kindes, Ernährungs- und Stoffwechsellehre, Humangenetik, Sozialpädiatrie, Gynäkologie und Geburtshilfe.

Mit dem 16. Kapital fängt die eng genommene Kinderheilkunde an, mit der Beobachtung des kranken Kindes. Nach den Neugeborenen und Frühgeborenen, den speziellen Erkrankungen des Säuglings und der Säuglings- und Kinderernährung behandelt das Buch die Krankheiten der verschiedenen Organe und Organsysteme. Es fehlen aber auch nicht die Kinder- und Jugendpsychiatrie, Infektionskrankheiten, Intensivmedizin, Kinderchirurgie, Kinderorthopädie, Narkose und Erste Hilfe bei Zwischenfällen, Hals-, Nasen-, Ohrenkrankheiten, Augenkrankheiten, Gynäkologie des Kindes- und Jugendalters und Unfallverhütung. Zur raschen und guten Orientierung im Lehrbuch verhilft das Inhalts- und Sachverzeichnis.

Die einzelnen Kapitel sind klar, gut verständlich und leicht erlernbar. Dazu helfen viele Abbildungen, Tabellen und schöne Farbtafeln. Die am Ende der einzelnen Kapitel angegebene Literatur bietet eine große Hilfe für ausführlichere Kenntnisse.

Zusammenfassend: dieses Werk hat die Grundrisse, die Tradition der früheren Ausgaben beibehalten und hat es mit den neuen Kenntnissen ergänzt und somit sein vorgesetztes Ziel, die Aus- und Fortbildung der Kinderkrankenschwestern und Kinderkrankenpflegern weitgehend und restlos erreicht.

K SCHMIDT

W. KRAUSE: *Aktuelle Probleme der Geburtsmedizin*. 267 Seiten mit 130 Abbildungen und 56 Tabellen. Georg Thieme, Leipzig 1983. Preis M 65,—

Das Buch befaßt sich mit den geburtshilflichen Beziehungen der perinatalen Medizin und dürfte als Nachfolger des 1972 publizierten Buches von Tosetti-Krause: »Der intrauterine Patient« betrachtet werden.

Die vorliegende Arbeit ist in drei Hauptteile gegliedert, 1. moderne diagnostische Methoden in der Geburtsmedizin, 2. mit erhöhtem mütterlichen und fetalen Risiko einhergehende Krankheitsbilder, und 3. Fragen der Geburtsleitung und Geburtsbeendigung.

Im ersten Teil findet man ein zusammenfassendes Kapitel über die Themen: antepartale Kardiotokographie, Ultraschalldiagnostik, Plazentaperfusionsdiagnostik mit Radioisotopen. Von den diagnostischen Methoden wird auch die Plazentabiopsie nach der 35. Schwangerschaftswoche erwähnt, die bei fortgeschrittener Schwangerschaft jedoch nicht brauchbar ist; daß dem Verfahren eine genetisch-diagnostische Rolle in der frühen Schwangerschaft zukommt, wird nicht angedeutet. Hervorzuheben wäre das Kapitel über die fetale Überwachung, die telemetrischen Möglichkeiten, deren vorteilhafte Anwendung in der Eröffnungsperiode beim Stehen oder Gehen der Patientin unschätzbar sind, ferner die kontinuierliche transkutane fetale Sauerstoff- und pH-Messung während der Geburt, die Mikroblutgasanalyse als Grundverfahren zur Einschätzung der feto-maternen Stoffwechselsituation; die Mikroelektronik und ein entsprechend geplantes Computerprogramm dürften bei der Analyse der diagnotischen Angaben besonders wertvolle Hilfe leisten.

Im zweiten Teil werden einige schwangerschaftspathologische Krankheitsbilder geschildert, wie z.B. Schwangerschaftstoxikose, Frühgeburt, Diabetes mellitus. Die übrigen hier behandelten Zustände wie z.B. Schwangerschaftsikterus oder megaloblastische Anämie sind heute keine Hauptprobleme der perinatalen Medizin. Diese Kapitel sind entsprechend verfaßt, doch erhalten sie wenig Neues. Das Kapitel über Diabetes und Schwangerschaft muß jedoch hervorgehoben werden.

Der dritte Teil faßt praktische Fragen zusammen. Bei der Induktion wird gegenüber Oxytocin das Methyloxytocin bevorzugt. Prostaglandine sind bei Plazentainsuffizienz oder deren Verdacht mit Recht als kontraindiziert beurteilt. Bei Frühgeburten in Beckenlage wird der Kaiserschnitt nur nach der 31. Woche empfohlen, dieser Zeitpunkt wird als die Grenze der realen Lebensfähigkeit angesehen.

Das Buch ist gut überblickbar. Aus dem Schrifttum ist es festzustellen, daß das Manuskript bereits 1980 abgeschlossen wurde.

L Kovács

Beger, Annelies und Autorenkollektiv: *Rehabilitative Bewegungserziehung.* 176 Seiten mit 20 Abbildungen und 19 Tabellen. Verlag Volk und Gesundheit, Berlin 1983. Preis M 35,—

Die Monographie, die auch als Lehrbuch zu dienen scheint, ist das Ergebnis langjähriger Zusammenarbeit zwischen Mitarbeiter der Sektion Rehabilitationspädagogik und Kommunikationswissenschaft der Humboldt-Universität zu Berlin und der Fakultät für Defektologie Zagreb. Das Ziel ist die Bedeutung der Bewegungserziehung für die Rehabilitation geschädigter Kinder den Lesern zu vermitteln und theoretisch zu begründen. Von den Auffälligkeiten geschädigter Kinder ausgehend wird die Spezifik der rehabilitativen Bewegungserziehung abgeleitet. Die Darlegungen rücken die für alle Kategorien der Geschädigten gültigen Aspekte in den Vordergrund. Um die theoretischen Ausgangspunkte, die die Autoren für alle Schädigungsgruppen gültig halten, zu verdeutlichen, wählen sie drei Gruppen — gehörlose, stotternde und schwachsinnige Kinder — aus, an denen sie exemplarisch die Zusammenhänge aufzeigen. Nach ihrer Überzeugung müssen bei der Bestimmung von Zielen, Inhalten und Methoden immer die dominant gestörten Äußerungsbereiche mitberücksichtigt werden. Unter diesem Aspekt ist die Bewegung als Mittel anzusehen, mit dessen Hilfe auf Sozialverhalten, Wahrnehmungs-, Denk- und Sprachtätigkeit eingewirkt wird. Die spezifische Bedeutung ihrer rehabilitativen Bewegungserziehung liegt darin, daß sie auf Beseitigung, Minderung oder Verhütung der Lernbehinderung gerichtet ist, aber auch zur Aktivierung der Entwicklung der Gesamtpersönlichkeit dient. Erfolgreiche Arbeit auf dem Gebiet erfordert vom Päda-

gogen fundierte Kenntnisse von anatomisch-physiologischen Gesetzmäßigkeiten, sport- und rehabilitationspädagogischen Methoden usw., vor allem aber die Einsicht in Zusammenhänge und gegenseitiges Bedingen. Die Wirksamkeit des rehabilitationspädagogischen Prozesses hängt ab vom Engagement des Pädagogen für die rehabilitative Zielstellung, von seiner Fähigkeit, Bewegungsfreude wecken und entwickeln zu können, von seiner Beobachtungsgabe, qualitative Veränderungen in allen Äußerungbereichen der Lernbehinderung erkennen zu können und von seinen schöpferischen Möglichkeiten, immer neue Wege zu finden, um für das geschädigte Kind adäquate Bedingungen zu schaffen.

JUDITH FALK

Anorektale Fehlbildungen. Herausgegeben von S. HOFMANN-v. KAP-HERR. X+251 Seiten mit 89 Abbildungen und 108 Tabellen. Gustav Fischer Verlag, Stuttgart 1984. Preis DM 72,—

An der Kinderchirurgischen Klinik zu Mainz wurde im Juni 1983 ein wissenschaftliches Treffen mit Teilnehmern aus 18 Ländern über das Thema Anorektale Fehlbildungen veranstaltet. Der ungewöhnlich schnell, schon Januar 1984 publizierte vorliegende Band faßt die Vorträge dieser viertägigen Konferenz zusammen. In dem im Aufbau einer Monographie nahestehenden Buch werden die Erfahrungen und Überlegungen in diesem Themenkreis von 60 Fachspezialisten bzw Arbeitsgruppen angeführt, angefangen von der Historik dieser Fehlbildungen, über Diagnostik und Therapie, bis zu den psychischen Störungen.

Von den Vorträgen seien hervorgehoben die Besprechung der verschiedenen Behandlungsmöglichkeiten, die Betonung der pränatalen Ultraschalldiagnostik, die Nachuntersuchungen bei den sog. erfolgreichen Eingriffen, ferner die Schilderung der die Kontinenz fördernden Methoden.

Wie jede derartige Publikation, weist auch diese die Zeichen des Kongreßmaterials auf, d.h. daß ein einheitlicher Leitfaden und Stil nicht zum Ausdruck kommen kann. Dies wird jedoch durch die Aktualität der einzelnen Vorträge und auf den Erfahrungen der verschiedenen Zentren basierenden wertvollen theoretischen und praktischen Richtlinien und die instruktiven Abbildungen reich kompensiert.

Ein weiterführendes Schrifttum ist am Ende eines jeden Beitrages zu finden, und ein Stichwortverzeichnis am Ende des Buches verhilft zur raschen Orientierung.

T VEREBÉLY

Paediatric Oncology edited by W DUNCAN X+116 pages with 28 figures and 38 tables. Springer-Verlag Berlin—Heidelberg—New York—Tokyo 1983. Price DM 98,—

This volume of the Recent Results in Cancer Research series contains the proceedings of the fourth symposium of the Royal College of Radiologists held in London in 1982 on the actual problems of paediatric oncology. Most of the participants were widely known experts on the subject and the clear and concise style of their papers has to be emphasized. The guiding principle of up-to-date treatment and its results are discussed in the common forms of tumour such as the leukaemias, malignant lymphoma, brain tumours, neuroblastoma, Wilms tumour, bone tumours and soft tissue sarcomas, mainly rhabdomyosarcoma. There is an excellent review of the epidemiology of children's neoplasmas from the Manchester Tumour Registry, and of the pathology, natural history and localization of tumours. In every chapter it is not so much the detailed results that are in the centre, the main emphasis being on up-to-date classification, the guiding principles of therapy and its possible side effects. Separate chapters deal with the diagnostic significance of radiography, scintigraphy and sonography, and with

supportive treatment. Late side-effects and their prevention and treatment are also discussed, a question that gains increasing importance with the rapidly improving results of therapy.

The book does not aim at supplementing the text and data of previous monographs or at presenting new knowledge. At the same time it will be useful for practising physicians, paediatricians and oncologists who wish to have directives and a short overview of the now actual questions of paediatric oncology.

D SCHULER

Fetal physiology and medicine. Eds: RW BEARD, PW NATHANIELSZ. Second, revised edition. XI + 823 pages with illustrations. Marcel Dekker Inc. New York, and Butterworth, London, 1984. Price £ 70.00

This revised volume has been edited within a series of textbooks and monographs on reproductive medicine. The majority of the authors are Anglosaxons from both sides of the Atlantic, but smaller countries are also represented. The aim of the editors has not been to offer a full, didactic review of maternofetal medicine but to select the most exciting topics. They succeeded in presenting a nearly complete spectrum of new data of perinatal medicine.

The book is excellent. All its parts are concise, logical and up-to-date. The content of the volume can hardly be criticised, one can only wonder whether there has not been progress enough in intrauterine diagnosis of inborn errors and in research of intrauterine growth retardation to devote individual chapters to these topics. It is true that the former has been fully discussed in the second volume of the series, and much knowledge on growth retardation is scattered in various chapters of the book.

There are some useful overlappings (diabetes, for instance); this is not at all disturbing since the different descriptions of the same problem are not at variance. It is hard to choose the best section; from a practical point of view the chapters on ultrasound in antenatal diagnosis of structural anomalies, diabetes mellitus, the prevention of preterm delivery and especially that on maternal and fetal infection are of utmost importance and excellence. But even the seemingly most theoretical chapters have bearings to present or future neonatal practice.

This book should not be missing from the shelf of all doctors interested in the care of pregnant mothers and newborn babies.

P. CHOLNOKY

Giardia and Giardiasis. Edited by STANLEY L ERLANDSEN, ERNEST A MEYER. XXIV+ 407 pages. Plenum Press, New York 1984. Price $ 65.00

This exemplary monograph consists of 22 chapters divided into three parts. These deal with the morphologic features and biology of Giardia, the diagnosis, pathology and treatment of giardiasis, and the third section with epidemiology. The most impressive of the three sections is the first one with its many TEM and SEM pictures of the trophozoite and its individual parts, and the cysts of the parasite. A single look at the multitude of trophozoites attached to the microvillous border and covering the surface of the villi almost completely will suffice to make the reader to understand why severe giardiasis must cause malabsorption. After a detailed chapter on the metabolism of Giardia, the methods of its isolation and cultivation are described extensively.

The reader, if he is a paediatrician, will be less impressed by the second section. The data cited are correct and up-to-date but most of them relate to adults as if the incidence in children would not be overwhelmingly higher than in adults and also some symptoms different from theirs.

Thus, steatorrhoea and weight loss, very important sequels of giardiasis in the growing child, are not at all or only quite cursorily mentioned and a number of questions which nowadays intrigue the paediatrician, e.g. the incidence of the infection in different parts of the world, the underlying cause of giardial malabsorption and expecially that of fats, the relation of blood groups and giardiasis, etc., cannot be found in the book. Nor is it mentioned, what materials the Giardia need and where — through their ventral disk or their back — they ingest them, in other words whether they obtain their food from the host's tissues or from the intestinal contents. As to treatment, it is difficult to understand why in the USA the otherwise quite common metronidazole is not approved for use in giardiasis when this was recommended nearly ten years ago by Ament in the 1975 edition of Nelson's Pediatrics, and the best of all current drugs, timidazole, is still not available on the other side of the Atlantic.

The third and largest section deals with epidemiology. The waterborne epidemics in Leningrad and at different locations in the USA, the detection of cysts in drinking water, the methods of water filtration, transmission by foods, sexual contact and by various animals are discussed in extenso. Since the different Giardia species cannot be distinguished and we do not know whether the many animals found to be passing cysts have been contaminated by each other or by humans, or the humans by the same animals, we hope that the proposed extermination of such likable animals as the beaver will not take place. The more so as the total number of those infested in the waterborne outbreaks in the USA in the last 20 years does not amount to more than 20,000 people, while for instance in England there are now about 3 million hosts and among the 10 million inhabitants of Hungary the frequency in adults is 3 to 10% and in children, 10 to 20%, in any case without the intervention of beavers.

Summing up, the book is an excellent monograph which is perhaps more interesting for those working in public health and epidemiology than for clinicians, but the legion of data to be found in it and the lists of references that include all the up-to-date literature up to 1984 will make it useful also for clinicians and especially paediatricians.

PV VÉGHELYI

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Erythrocyte enzyme allotypes in the X-linked recessive disorders, Duchenne muscular dystrophy and haemophilia-A hemizygotes and heterozygotes

ARANKA LÁSZLÓ, F. KÓSA, ILONA ZIMÁNYI, Ágnes EGYED

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Institute of Forensic Medicine, Semmelweis University Medical School, Budapest,
Pál Heim Children's Hospital, Budapest, and
Blood-Transfusion Centre, Szombathely, Hungary

The erythrocyte enzyme-systems acid phosphatase, phosphoglucomutase, glutamate pyruvate transaminase, adenosine desaminase, adenylate kinase, glyoxase, glucose-6-phosphate dehydrogenase and esterase-D-isoenzyme phenotypes were studied for their percentile distribution and were compared with their incidence in the diseases with X-linked recessive heredity, Duchenne muscular dystrophy (DMD) and haemophilia-A, in hemizygous male children and heterozygous mothers.

Considering the frequency distribution of the above mentioned isoenzyme phenotypes of the enzyme-systems in DMD, the phenotypes proved to be homogeneous, only the X transmitted 6-phosphogluconate dehydrogenase (6-PGD) isoenzyme types were found to be genetic markers in DMD hemizygotes and heterozygotes. In these genotypes the 6-PGD A phenotype showed a decrease while the phenotypes 6-PGD AB and B were significantly increased.

The adenylate kinase (AK) 2-1 isoenzyme phenotype was increased to 25% against the population frequency of 6.34%, while the AK 1-1 phenotype occurred in 75% against its population frequency of 93.59%, showing a significantly decreasing tendency in haemophilia-A hemizygotes and heterozygotes.

The frequency distribution of phenotypes of erythrocyte enzyme-systems may have an informative value in population genetics and forensic medicine, as in genetically determined disorders it may serve as a marker concerning the different genotypes (homo- and heterozygotes) [6, 30, 16, 9]. Lamm et al [18] showed the close connection between glyoxase I and the chromosomal HLA-B region. In insulin dependent diabetes a connection was found between age and the frequent occurrence of a rare allele of the properdin factor [15] but the frequent occurrence of the same allele could not be proved in Graves disease [28].

In DMD and haemophilia-A hemizygous male patients and in gene-carrier mothers the frequency distribution of erythrocyte enzyme allotypes was therefore investigated with the aim of searching for genetic markers.

METHODS

The red cell enzyme allotypes acid phosphatase (AP), glutamate pyruvate transaminase (GPT), phosphoglucomutase (PGM), adenosine desaminase (ADA), adenylate kinase (AK) [20] and their Hungar-

ian frequency data were compared to those observed in hemizygote and heterozygote groups of X-linked disorders of recessive heredity. The frequency of glyoxalase phenotypes in the healthy control group was compared to that reported by Wenzel et al. [28].

In the DMD group, the AP phenotype was studied in 19, PGM in 18, GPT in 18, ADA in 14, AK in 14, GLO in 19, EsD (esterase-D) in 17, G-6 PD (glucose-6-phosphate dehydrogenase) in 26 DMD hemizygote male children, and AP in 8, PGM in 9, GPT in 12, ADA in 12, AK in 10, GLO in 10, EsD in 10 and G-6 PD in 14 DMD gene-carrier mothers.

In the haemophilia-A group the AP phenotype was studied in 36, PGM in 38, GPT in 34, ADA in 23, AK in 24, GLO in 32, EsD in 36, G-6-PD in 28 hemizygote male children, and in gene carrier mothers the AP phenotype was studied in 26 cases, PGM in 26, GPT in 27, ADA in 12, AK in 12, GLO in 19, EsD in 25 and G-6-PD in 18 patients.

The isoenzymes of the erythrocyte enzyme systems were separated by starch-gel electrophoresis, and PGM by gel-electrophoresis [25], AP according to Hopkinson et al. [14], GPT according to Chen and Gibblett [4a], ADA according to Spencer [24], AK according to Fildes and Harris [7], GLO according to Kömpf et al. [17], EsD according to Hopkinson [13] and G-6-PD according to Fildes and Parr [8].

The frequency of individual isoenzyme-phenotypes was compared to the population frequency and evaluated by the X^2 test.

Results

In the case of DMD-hemizygotes (Table I) and heterozygotes (Table II) the frequency distribution of erythrocyte enzyme-phenotypes proved to be homogeneous with the control group (Table V), only the X-linked transmitted G-6-PD phenotype A showed a decrease, while phenotype AB and B were significantly increased. In the haemophilia-A male children (Table III) and the gene carrier mothers (Table IV) the percentile frequency of the AK 2-1 heterozygote phenotype was as high as 25% against 6% in the control group, and the frequency of the AK 1-1 phenotype was only 75% against the 93% frequency in the population.

Discussion

Among the DMD genotypes (hemi- and heterozygotes), from the erythrocyte enzymes only the 6-PGD phenotype proved to be a genetic marker. Phenotype 6-PGD A was decreased while the phenotype AB and B was increased.

Fildes and Parr [8] described the inherited electrophoretic variations of human erythrocyte 6-PGD (NADP oxidoreductase: E.C. 1.1.1.44) and several papers [4, 5, 10] reported on further genetic data by comparing the enzymatic properties of some of the variants.

According to a study of 461 families with 864 children from the area of Marburg and Tübingen [21], the frequencies of X-linked inherited 6-PGD alleles were as follows: $6\text{-PGD}^a = 0.9775$; $6\text{-PGD}^b = 0.218$; $6\text{-PGD}^f = 0.0003$; $6\text{-PHD}^r = 0.0002^x$; $6\text{-PGD}^h = 0.0002$, where f stands for the Freiburg, r for the Richmond and h for the Hackney variant. In Southern Germany the distribution of 6-PGD phenotypes was reported as A: 95.83% AB: 4.02% and B: 0.15%,

TABLE I

DMD-hemizygotes

AP	n	per cent	x^2	PGM	n	per cent	x^2	GPT	n	per cent	x^2	ADA	n	per cent	x^2
B	6	32	0.02	1–1	10	56	0.001	2–2	3	17	0.25	1–1	12	86	0.02
AB	6	32	0.40	2–1	6	33	0.004	1–1	3	17	0.38	2–1	2	14	0.01
BC	1	5	0.001	2–2	2	11	0.39	2–1	12	66	1.47		14		
AC	2	10	0.21		18				18						
A	4	21	0.56												
	19														

AK	n	per cent	x^2	GLO	n	per cent	x^2	Es D	n	per cent	x^2	G-6-PD	n	per cent	x^2	p
2–1	2	14	0.44	2–1	13	68	0.87	1–1	14	82	0.02	A	20	76.92	8.07	<0.01
1–1	12	86	0.42	2–2	6	32	0.01	2–1	2	12	0.12	AB	4	15.38	1.88	>0.05
	14			1–1	0	0	2.25	2–2	1	6	0.44	B	2	7.69	29.7	<0.01
					19				17				26			

TABLE II

DMD-carriers

AP	n	per cent	x^2	PGM	n	per cent	x^2	GPT	n	per cent	x^2	ADA	n	per cent	x^2
B	1	12	0.70	1–1	6	67	0.03	2–2	0	0	0.85	1–1	10	83	0.08
AB	3	39	0.02	2–1	2	22	0.31	1–1	4	67	0.005	2–1	2	17	0.06
BC	0	0	0.02	2–2	1	11	0.01	2–1	8	33	0.20		12		
AC	1	12	0.01		9				12						
A	2	25	0.27												
AD	1	12													
	8														

AK	n	per cent	x^2	GLO	n	per cent	x^2	Es D	n	per cent	x^2	G-6-PD	n	per cent	x^2	p
2–1	2	20	0.11	2–1	4	40	0.40	1–1	6	60	1.57	A	12	86	0.35	>0.05
1–1	8	80	0.11	2–2	3	30	0.12	2–1	4	40	1.93	AB	1	7	0.21	>0.05
	10			1–1	3	30	0.77	2–2	0	0	1.11	B	1	7	8.92	<0.01
					10				10				14			

TABLE III

Erythrocyte enzyme markers in haemophilia-homozygotes

AP	n	per cent	x^2	PGM	n	per cent	x^2	GPT	n	per cent	x^2	ADA	n	per cent	x^2
B	9	25	0.62	1–1	24	63.16	0.17	2–2	9	26.47	0.000	1–1	22	95.65	0.64
AB	19	52.78	1.34	2–1	11	28.95	0.63	1–1	9	26.47	0.03	2–1	1	4.35	1.42
BC	2	5.56	0.03	2–2	3	7.89	0.17	2–1	16	47.06	0.002		23		
AC	5	13.89	3.06		38				34						
A	1	2.78	0.83												
	36														

AK	n	per cent	x^2	GLO	n	per cent	x^2	Es D	n	per cent	x^2	G-6-PD	n	per cent	x^2	p
2–1	6	25	10.5*	2–1	15	46.88	0.54	1–1	25	69.44	2.18	A	26	93	0.08	>0.05
1–1	18	75	10.36*	2–2	14	43.75	2.18	2–1	11	30.56	2.93	AB	2	7	0.07	>0.05
	24		* $p<0.05$	1–1	3	9.38	0.40	2–2	0	0.00	0.005		28			
					32				36							

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TABLE IV

Erythrocyte enzyme markers in haemophilia-heterozygotes

AP	n	per cent	x^2	PGM	n	per cent	x^2	GPT	n	per cent	x^2	ADA	n	per cent	x^2
A	5	27.78	2.39	1—1	13	50	0.37	2—2	8	29.63	0.1	1—1	12	100	0.07
B	6	33.3	0.04	2—1	9	34.62	0.001	1—1	9	33.33	0.34				
AB	7	38.89	0.0004	2—2	4	15.38	3.25	2—1	10	37.04	0.99				
	18				26				27						

AK	n	per cent	x^2	GLO	n	per cent	x^2	Es D	n	per cent	x^2	G-6-PD	n	per cent	x^2	p
2—1	3	25	4.12*	2—1	13	68.42	0.87	1—1	22	88	0.43	A	17	94.44	0.08	>0.05
1—1	9	75	4.05*	2—2	3	15.79	1.19	2—1	3	12	0.26	AB	1	5.56	0.09	>0.05
	12		* $p<0.05$	1—1	3	15.79	0.05	2—2	0	0	0.11		18			
					19				25							

TABLE V

Controls

AP *(n = 631)	per cent	PGM *(n = 330)	per cent	GPT *(n = 314)	per cent	ADA *(n = 1234)	per cent	AK *(n = 734)	per cent	GLO (n = 1194)x	per cent
B	32.80	1—1	58.18	2—2	24.84	1—1	88.00	2—1	6.34	2—1	55
AB	41.51	2—1	36.96	1—1	26.11	2—1	11.58	1—1	93.59	2—2	30
BC	7.75	2—2	4.86	2—1	49.05	2—2	0.42	2—2	0.06	1—1	15
AC	5.39									x by Wenzel	
A	12.52										
C	0.00										

Es D **(n = 1480)	per cent	G-6-PD **(n = 1080)		per cent
1—1	80.74	A	1008	93.35
2—1	17.97	AB	71	6.57
2—2	1.28	B	1	0.1

* See reference
** See reference 16a.

the gene frequencies of PGD^A were 0.9784 and of PGD^B, 0.0216 [1]. The red cell PGD polymorphism in the population of West-Berlin was reported by Smerling [23]; the detected gene frequencies were $PGD^A = 0.9775$ $PGD^B = 0.02295$, the phenotypes were PGD (A) 95.59%, PGD (AB) 4.23% and PGD (B) 0.18%.

The frequency of PGD^B gene varies among different populations; it is in England 0.021 [19], in whites in USA and Mexico 0.024—0.039 [4, 5, 10], among Australians 0.041 [2], in Chinese populations 0.066 [22] and in Hungarian populations 0.0395 [16a]. Inherited variants of the enzyme also occur. A new variant is for instance the Freiburg one with a frequency of 0.0004 [27]. Beside the forms named Hackney, Richmond and Friendship, three different new 6-PGD variants have been discovered in Greece with a frequency between 0.2 and 0.5% [26]. They characterize the permissive enzyme tolerance to changes of amino acid composition without an important loss of function. The Lod scores for the linkage relations of 6-PGD and ADA loci with each other and with 15 other loci have also been studied [29]; little evidence was found for the linkage of 6-PGD and Rhesus loci, the probability was one of 0.69.

In the haemophilia-A hemi- and heterozygotes, the frequency of phenotype AK 2-1 was significantly higher than in the general population. The enzyme AK is a genetically determined myokinase (EC: 2, 7, 4, 3), phosphotransferase, catalysing reversibly the transformation of 2 ADP to ATP + AMP. AK is present mainly in muscle [7] but also in other tissues and erythrocytes. The isoenzyme composition is the same in every tissue. The sex difference is not significant, the 2-1 phenotype occurred in English

males in 11.1%, and in females in 8.3%. Two autosomal alleles (AK^1 and AK^2) are responsible for its formation, the AK individuals are homozygotes (AK^1 AK^1), the AK 2-1 individuals are heterozygotes. The AK^2 gene frequency in the English population is 0.05, AK^2 AK^2 occurs in 1 : 400.

The sera of DMD patients contain high AK (ATP-AMP phosphotransferase, EC: 2.7.4.3) activity, in addition to their characteristically high creatine kinase (ATP: creatine N-phosphotransferase, EC: 2,7,3,2) activity. An aberrant adenylate kinase isoenzyme has also been detected in the serum of DMD patients [12]. The mobility of their enzyme on agarose gel appeared to be similar to that of the normal human hepatic AK. The presence of this aberrant liver-type AK could be demonstrated by characteristic inhibition patterns with P^1P^5-di(adenosine-5')-pentaphosphate, 5,5'-dithio bis (2-nitrobenzoate) and phosphoenolpyruvate, but structurally it is a muscle type enzyme or one derived from a muscle type enzyme, as shown immunologically by inhibition reactions with anti-muscle-type AK. Whether this is a fetal type isoenzyme of AK, will require further investigation.

We observed a high AK 2-1 phenotype frequency against the population frequency in the hemi- and heterozygotes for haemophilia-A. In the latter genotypes we could not detect aberrant AK isoenzyme. Semi-quantitative determination of AK on the basis of AK isoenzyme densitometry will only supply some further biochemical information.

PGM (phosphotransferase, EC: 2.7.5.1) catalyses the transfer of the phosphate group between glucose 1 and 6. Its physiological role in erythrocytes is not known [25]. Three autosomal loci have been described to determine the isoenzymes PGM_1, PGM_2 and PGM_3. These isoenzymes are codified by two alleles, PGM^1 and PGM^2, the phenotypes are marked by the allele compounds PGM_1 1-1, PGM_1 1-2 and PGM_1 2-2.

The GPT phenotypes show a significant population-ethnical deviation [4a]. GPT as an alanine-transferase (EC: 2.6.1.2.) catalyses the reversible transformation of L-alanine and alfa-ketoglutarate into L-glutamate and pyruvate. The phenotypes are GPT 1-1, 2-1, 2-2 and their gene-frequency in the Caucasian population is 0.496, in Afro-Americans 0.814, and in Oriental Americans 0.598. GPT has two molecular forms: one is a soluble cytoplasmatic enzyme, the other is mitochondrial [3]. There is a great amount of soluble GPT in the liver and the heart muscle [11]. The molecular weight is about 100,000 daltons. The serum GPT level is a useful indicator of cellular liver destruction. On starch-gel electrophoresis the isoenzymes move towards the anode, their markers are GPT_1, GPT_{2-1}, and GPT_2. GPT 1 has 1 stripe, GPT 2 has 2 stripes and GPT 2-1 consists of 3 stripes. The former phenotypes are regulated by the 2 autosomal allele genes GPT 1 and GPT 2. GPT 1 and 2 are homozygote phenotypes and GPT 2-1 is a heterozygote type.

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Subacute sclerosing panencephalitis in twins

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Development of subacute sclerosing panencephalitis after measles has been observed in twins although the disease seems to be quite exceptional in members of the same family, and no report has been found on its occurrence in twins.

Subacute sclerosing panencephalitis (SSPE) or Dawson's encephalitis seldom occurs in members of the same family. In spite of this we have observed SSPE in two boys, 8 and 9 years of age, respectively, who were cousins [4]. Since then we observed the disease in twin sisters who earlier had had measles.

Case Reports

Case 1 Girl C. A., born with a birthweight of 2450 g on 24th February 1972 as the third child of a family was admitted to our Institute at the age of 11 years with the suspicion of SSPE. Her two brothers were healthy and had not had measles. The patient at the age of 15 months had had measles with a severe clinical course. After the measles her somatic and psychomotor development had been excellent and unchanged until January 1983, when the first signs of SSPE were observed. Then abnormalities of behaviour, aggression and troubles in school were noted, so far she had been an excellent pupil. At admission psychomotor slow motion, bewilderment and jerks of limbs were observed. Psychological examination showed low spirits and sensitivity, misunderstanding of oral information, weakening of memory and a significant decrease of sight perception.

During her stay in hospital the girl's clinical condition became gradually from bad to worse. The motor signs (tremor, myoclonic jerks) increased considerably and so did the pyramidal signs. Ophthalmological examination did not reveal any abnormality, the EEG showed a typical Rademecker type record (Fig. 1).

The level of anti-measles antibodies, as estimated by the H.I. method, was 1 : 32 in serum and 1 : 8 in CSF. The CSF immunoglobulin level was increased to 33.2%, and the IgG to 28 mg/dl. The gold chloride curve was of paralytic type. Other laboratory tests of the CSF gave normal values.

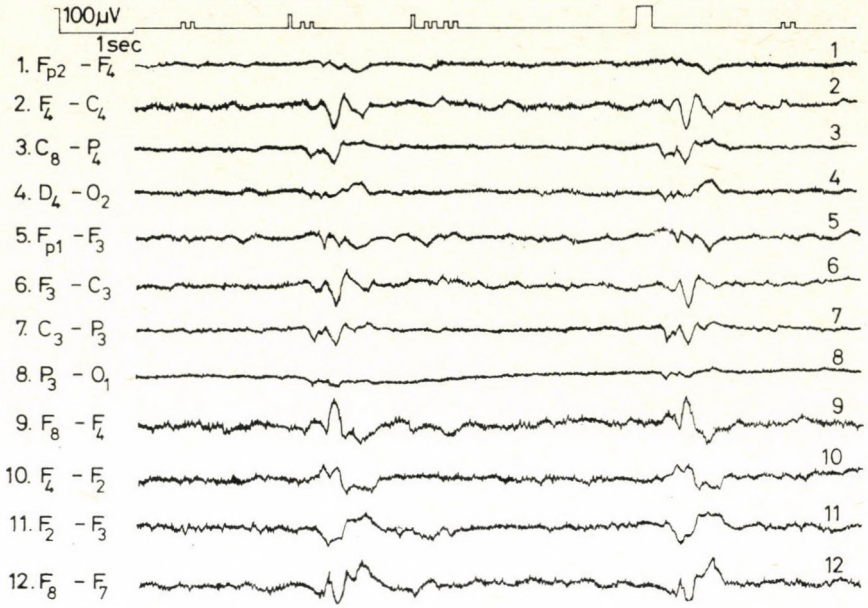

FIG. 1. EEG of Case 1. Typical Rademecker type record

Blast transformation test with PHA and ConA, specific activators of T cells, were normal (66 000 cpm and 45 000 cpm, respectively), in agreement with our currently done studies on other patients with SSPE (data not shown). The serum level of immunoglobulins, estimated by the Mancini technique, was in the normal range (IgG, 1960 mg/dl; IgA, 167 mg/dl). The amount of IgG antibodies in CSF was 128 mg/dl, much higher than normal (2.71 $\pm$ 47 mg/dl). IgM antibodies were not present.

In the brain, computer tomography showed focal demyelination and a narrow ventricle system. All other tests, blood and urine analyses gave normal values. The patient's blood group was A_1Rh^+.

On the basis of the laboratory tests and clinical findings, SSPE in Jabbour's stage II/III [1, 2, 3] was diagnosed.

Case 2 Girl C. Ag. was the twin sister of Case 1. The neonatal and infantile periods had been normal. She had contracted measles at the age of 15 months simultaneously with her sister. The clinical course of the disease was less severe than of Case 1.

In August 1981, psychomotor slow motion, troubles at school, difficulties in memorizing, reading and writing were observed, and she had disturbances of balance and of speech (paraphasia). Somewhat later epileptic seizures, myoclonic jerks and spastic paralysis of the limbs occurred. Ophthalmological examination revealed oculomotor palsy. The EEG showed a lack of basic functions typical of her age and the presence of delta type waves (1—3/sec). The level of anti-

measles antibodies, estimated by the H.I. method, was 1 : 64 in serum and 1 : 8 in CSF. The amount of immunoglobulins in the CSF was increased up to 18% and β-globulins up to 18%. Other laboratory tests were in the range of normal values. She belonged to blood group A Rh^+. On the basis of laboratory tests and the clinical course SSPE in stage II/III was diagnosed.

Both patients were subjected to methisoprinol and amantadine treatment; these drugs had no effect, they even failed to slow down the course of the process. The first child is still alive but in a very poor condition, the second died a year ago.

We have reported these cases of SSPE in cousins and especially those in twins not only because we could not find any publications concerning the problem, but we thought that these observations might prove useful in investigations into slow virus diseases and into the role of genetic factors in the aetiology and pathomechanism of SSPE.

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Isolation and physicochemical and functional properties of a calcium binding gluten fraction

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Each milligram gluten protein isolated from bread contains 0.03—0.06 μmol calcium. On theoretical grounds we have concluded that this calcium quantity is bound to the free carboxyl groups not participating in peptide bonds of dicarbonic aminoacids, especially glutaminic acid, making up a large proportion within the aminoacids of gluten. After treatment with EGTA, a well-known calcium complex forming compound, two gluten fractions can be distinguished: water-soluble gluten-ES, and gluten-EP soluble in acetic acid.

The aminoacid composition of gluten-ES is similar to that of unfractionated gluten. It is rich in aminodicarbonic acid (glu), aminodicarbonic acid amide (gln) and proline. Further properties of gluten-ES are: immunological similarity to gluten; a molecular mass of 36 000 dalton; an absorption maximum at 275.6 nm; a Ca^{2+}-binding capacity of 0.72 μmol Ca^{2+}/mg protein as measured by atomic absorption spectrophotometry and by Ca^{2+} ion selective electrode; inhibitory effect of a small quantity (25—30 μg) of the compound on the Ca^{2+}—Mg^{2+} dependent ATPase and Ca^{2+}-uptake of fragmented sarcoplasmatic reticulum. Preliminary experiments have demonstrated that gluten-ES has an influence on other calcium ion mediated systems like actomyosin superprecipitation.

We put forward the hypothesis that by its Ca^{2+}-binding capacity, gluten-ES is capable of influencing the level of free calcium and may thus play a part in the pathomechanism of coeliac disease.

In previous publications [15, 20] we have shown that gluten proteins isolated from bread contain 45—47% aminodicarbonic acids (glu, asp) and aminodicarbonic acid amides (gln, asn). 40—44% of the total aminodicarbonic acid content is free acid or simple salts, the rest occurs as amides. In other words, 20—21% of all aminoacids of the gluten proteins are aminodicarbonic acids (glu + asp).

It is a well-known fact that the free carboxyl groups not participating in peptide bonds of aminodicarbonic acids are capable of binding calcium: in the case of troponine-C [8], parvalbumin [5], thermolysine [2], calsequestrine [12] and staphylococcus nuclease [1] the free carboxyl groups of the aminodicarbonic acid units have been shown to be capable of binding calcium. Thus, it follows that also gluten protein might possess this property and play a part in the pathomechanism of coeliac disease e.g. by influencing the tonicity of intestinal muscles or the membrane structure of the microvilli.

On the basis of our previous experiments [14, 15, 20, 21] we have at-

tempted to find, isolate and characterize the physicochemical properties of gluten protein components capable of altering the concentration of compounds acting under physiological conditions (adenosine, Ca^{2+}, etc) and of playing thus a secondary or even primary part in the pathomechanism of coeliac disease.

For this purpose a gluten fraction capable of binding calcium was prepared from gluten by the use of EGTA (ethylene glycol-bis-)2-aminoethyl-ether)-N,N,N',N'-tetraacetic acid), a compound forming a complex with calcium. Homogeneity, molecular mass, absorption spectrum, amino-acid composition, calcium ion binding capacity and influence on Ca^{2+}-Mg^{2+} dependent ATPase and calcium uptake of fragmented sarcoplasmic reticulum (FSR) of this gluten fraction were investigated. In this paper we shall describe our results.

Materials and Methods

Isolation of gluten proteins from bread

This was carried out as described in a previous paper [21].

EGTA treatment of gluten proteins

To 10 ml of a gluten protein solution containing 4 mg protein/ml, 12 ml ion-free water, 8 ml 100 mmol/l aqueous solution of EGTA pH 7.0, and 0.2 ml 500 mmol/l TRIS-buffer was added and the pH was adjusted to 6.0—6.5. This corresponded to a ratio 20 μmol EGTA/1 mg protein, the optimum found in previous experiments described in Results. All manipulations were carried out in plastic vessels previously soaked in 1 mol/l HCl and washed in ion-free water. The protein-EGTA suspension was stirred and then kept at 37°C for 2 hours, this was followed by centrifugation in a Lh-413 (BVG) centrifuge, at 1800 *g*, 25°C, 60 minutes. The supernatant, called further *gluten-ES*, was dialysed against several samples of cold ion-free water of a 400-fold volume in order to eliminate EGTA. The efficacy of dialysis was checked by arsenazo III [19]. The precipitate obtained by centrifugation (gluten-EP) was dissolved in a solution containing 10 mmol/l acetic acid and 0.8 mmol/l sodium azide and dialysed against the same solution to remove eventual EGTA residues.

Aminoacid analysis

This was performed as described in a previous paper [14].

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) at pH 7.0 and polyacrylamide gel electrophoresis without sodium dodecylsulphate

was done as described in a previous paper [14].

Immune diffusion combined with polyacrylamide gel electrophoresis (immunodisk electrophoresis)

The solutions necessary for preparation of gels and for electrophoresis were made as described in a previous paper [17]. The acrylamide was polymerised in the shape of an empty cylinder. Each protein sample was run double, after polyacrylamide gel electrophoresis of gluten and gluten-ES each one gel was taken from the gel tube and directly stained with Coomassie brilliant blue (R-250) in a medium containing trichloroacetic acid. The parallel gel tube was provided with a plug without a hole and its lumen filled up with original or diluted immune serum produced against gluten. After 24—48 hours incubation the immune serum was removed from the lumen of the gel and stored at —18°C until next use. Then the gels were removed

from the glass tubes and the proteins that had not participated in the immune reaction were washed out by several samples of physiological saline over 72—96 hours. After washing the gels were stained again with Coomassie brilliant blue, densitometry was performed and the samples were photographed. This method elaborated by us [17] has successfully been used for demonstration of the related immune structure of other proteins [23].

Production of immune serum

5 mg protein/ml gluten solution isolated from bread was emulsified in a ratio 1 : 1 with complete Freund's adjuvant (DIFCO) and injected to rabbits. The animals were killed by exsanguination on the sixth day after the last injection. The sera were inactivated at 56°C and stored at —18°C in deep-freeze.

Determination of the absorption spectrum and the protein content

The absorption spectra of the proteins were established in a double-beam photometer Specord M-40. The protein content was measured by the method of Lowry et al. [11]. For calibration, bovine serum albumin (Serva) was used.

Determination of calcium content of gluten preparations

This was performed in an atomic absorption spectrophotometer AAS 1 Carl Zeiss, Jena.

Determination of calcium binding capacity of the gluten fractions by calcium ion selective electrode

A miniature Ca^{2+}-selective electrode Nuestro Research Inc. was used, for reference a Radelkisz calomel electrode was applied. An OP 208 Radelkisz digital pH-meter was used as amplifier, connected with an OH-814/1 Radelkisz potentiometric recorder for registration of mV values. In these experiments, 0.5 ml samples of gluten-ES, ion-free water or dialysing medium were mixed with 0.5 ml 20 mmol/l TRIS-HCl buffer (pH 7.2). The calcium ion selective and reference electrodes were dipped into this mixture. Under continuous stirring by magnetic mixer, 0.02—0.04 μmol Ca^{2+} was added through a micropipette every two minutes and the potential changes (mV) due to free calcium were registered.

Preparation of fragmented sarcoplasmic reticulum (FSR)

FSR was isolated from rabbit muscle by the method of Suko and Hasselbach [13].

Measuring ATPase activity of FSR

For the measurement an incubation solution containing 100 mmol/l KCl, 20 mmol/l TRIS-maleate, 5 mmol/l $MgCl_2$ 5 mmol/l potassium oxalate, 0.0—0.1 mmol per l $CaCl_2$ and 5 mmol/l ATP, pH 6.75, was used. The solution was designated as ATP-containing incubation solution. 1 ml of this solution contained 0.1 mg FSR protein, and — in inhibition experiments — 28 μg gluten-ES or 640 μg gluten protein in addition. The samples were incubated at 23°C for 10 minutes, then 0.1 ml 5% sodium dodecylsulphate was measured to the samples in order to inactivate and solubilize FSR ATPase. The inorganic phosphorus content (P_i) was determined by the method of Taussky and Shorr [22].

Measuring the calcium ion uptake by FSR absorption increment

The measurements were carried out at 350 nm under continuous stirring in an Opton PM 2 DL single-beam photometer. Each 2 ml sample of ATP-containing incubation solution contained 0.2 mg FSR protein and in inhibition experiments additional 28 μg gluten-ES protein. Incuba-

tion was started at the addition of FSR, the extinction was continuously registered by a Radelkisz potentiometric recorder. The principle of estimation of calcium uptake by FSR was as follows. In previous studies [16] we observed that if the sulphhydryl groups of FSR are titrated at 412 nm by the method of Ellman [7] under controlled Ca-uptake conditions, Ca^{2+}-uptake makes the FSR shrink and thereby its absorption increases. This increment in absorption is even more pronounced at lower wavelengths — e.g. at 350 nm — than at 412 nm. Therefore, the measurements were carried out at 350 nm because at this wavelength there is no more absorption in the ATP containing incubation medium used in these experiments. Our method for investigating the effect of various factors on FSR calcium ion uptake was based on this principle [18].

Results

First, gluten protein was prepared by the method described in the previous section. The calcium content measured by atomic absorption spectrophotometry was found to be 0.03—0.06 μmol/mg gluten protein. Then we attempted to remove the calcium from gluten by addition of EGTA. Then the effect of the EGTA/protein ratio on the fractions and their calcium content was investigated. Table I demonstrates the composition and quantity of solutions used in these experiments. After mixing the measured volumes, the suspensions containing EGTA and gluten were kept

Table I

Composition of reaction mixtures at fractionation of gluten proteins using various quantities of EGTA

Solutions		Ratio μmol EGTA: mg gluten						
		0	2.5	5.0	10	15	20	40
100 mmol/l EGTA,	ml	0	0.05	0.1	0.2	0.3	0.4	0.8
4.0 mg/ml gluten,	ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5
ion-free water,	ml	1.2	1.15	1.1	1.0	0.9	0.8	0.4
500 mmol/l TRIS,	ml	0.01	0.01	0.01	0.01	0.01	0.01	0.01

at 37°C for two hours, centrifuged on a Lh-413 centrifuge at 1800 *g*, 25°C for 60 minutes. The protein and calcium content of gluten-ES and gluten-EP was determined; the results are illustrated in Figure 1. As can be seen, with EGTA/protein ratios between 5 and 20 μmol/mg, 20% of the protein and 96% of the calcium appeared in the supernatant. The rest, 80% of the protein and 2—4% of the calcium, was in the precipitate.

A large quantity of gluten-ES was then prepared using a ratio of 20 μmol EGTA/mg protein and its properties were examined. After fractioning, gluten-ES was dialysed against ion-free water, the efficacy of dialysis was checked by the use of arsenazo III [19].

Homogeneity of EGTA-free supernatant (gluten-ES), gluten-EP and unfractionated gluten and their molecular mass distribution was investigat-

ed by SDS PAGE. Figure 2 shows the values. Untreated gluten (Gel 1) and gluten-EP (Gel 5) contained components of 73 000, 66 000 and 36 000 dalton while gluten-ES (Gels 2, 3 and 4) only a component of 36 000. PAGE without SDS (Figure 3) resulted in gluten with 5 components (I—V) near the catode (Gel 1). A similar picture was obtained for gluten-EP (Gel 4). Gluten-ES consisted mainly of components I, IV and V while III was present in a reduced quantity and II was hardly visible (Gels 2 and 3).

In immunodisk electrophoretic studies of gluten, (the immune serum produced in rabbits by injection of unfractionated gluten), precipitation of the components I, II and III occurred (Figure 4, Gel 2). Similar results were obtained with gluten-ES (Figure 4, Gels 4 and 6). This proves that gluten and gluten-ES are related immunologically.

Table II shows the results of amino-acid analysis of our preparations. In this respect there was a striking similarity between gluten and gluten-ES. The composition of gluten-EP differed markedly: its asparaginic acid + asparaginic acid amide (Asp + Asn) and methionine (Met) content was markedly increased, and so was the percentage of the less polar amino-

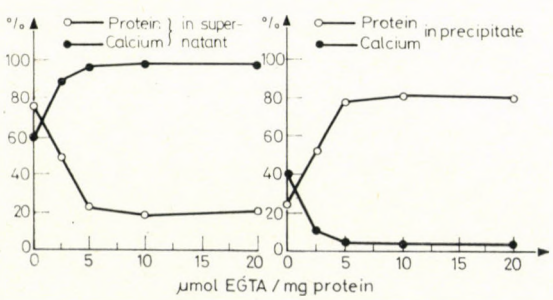

FIG. 1. Effect of EGTA on the partition of the calcium and protein content of gluten. The experiments were carried out under conditions described in Table I

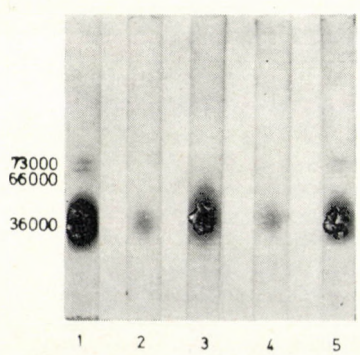

FIG. 2. Pictures of gluten (Gel 1), EGTA supernatants of various protein content (Gels 2, 3 and 4) and EGTA precipitate (Gel 5) after sodium dodecylsulphate polyacrylamide gel electrophoresis and staining

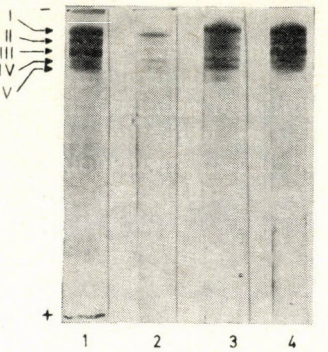

FIG. 3. Pictures of gluten (Gel 1), EGTA supernatants (Gels 2, 3) and EGTA precipitate (Gel 4) after polyacrylamide gel electrophoresis, without sodium dodecylsulphate treatment and after staining

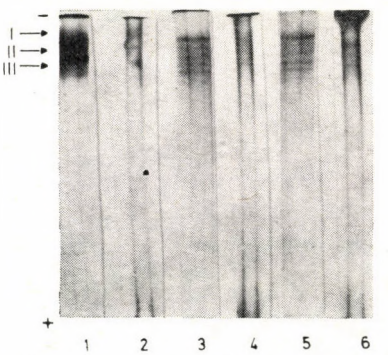

FIG. 4. Pictures of gluten (Gels 1 and 2), EGTA supernatants (Gels 3 and 5, and 4 and 6) after immunodisk polyacrylamide gel electrophoresis, incubation with immune serum (Gels 2,4 and 6), and staining

TABLE II

The aminoacid composition of gluten, the supernatant of gluten-EGTA (gluten-ES) and the precipitate of gluten-EGTA (gluten-EP)

Aminoacid	Gluten	Gluten-ES mol%	Gluten-EP
Lys	0.14	0.18	1.66
His	1.40	1.35	2.12
Arg	1.11	1.09	1.33
Asp + Asn	2.02	1.86	8.04
Thr	1.56	2.13	2.60
Ser	4.42	6.76	6.84
Glu + Gln	47.12	43.17	27.84
Pro	16.55	17.47	17.80
Gly	2.04	4.00	3.05
Ala	2.03	2.30	2.79
Val	2.76	2.40	3.12
Met	0.52	0.63	3.00
Ile	4.30	3.97	4.65
Leu	7.24	6.48	7.50
Tyr	1.96	2.46	3.00
Phe	3.64	3.77	4.65

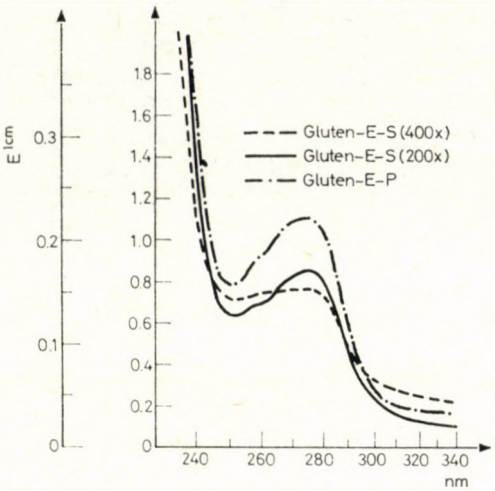

FIG. 5. Absorption spectra of EGTA precipitate of gluten (gluten-EP), EGTA supernatant (gluten-ES) dialysed against 200-fold resp. 400-fold volume of water. The outer scale on the ordinate refers to gluten-ES, the inner scale to gluten-EP

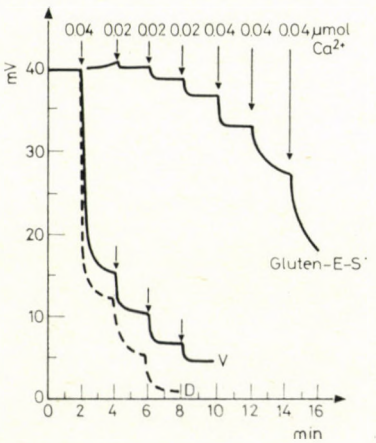

FIG. 6. Changes in potential (mV) measured by a calcium ion selective electrode in gluten-ES dialysed against a 400-fold volume of ion-free water (v), in ion-free water and in the dialysis fluid taken at completion of dialysis of gluten-ES (D) after addition of various quantities of calcium

acids (Pro, Gly, Ala, Met, Val, Ile, Leu, Phe), while the glutaminic acid amide (Glu + Gln) content was decreased. As demonstrated in Figure 1, this fraction had a markedly lower calcium content than gluten or gluten-ES.

Figure 5 shows the absorption spectra of our preparations measured by a Specord M 40 double-beam photometer. Both gluten and gluten-ES exhibited an absorption maximum at 275.6 nm. During dialysis of gluten-ES the spectrum had changed: after dialysis with a 200-fold volume of water the peak was pointed while it was rather flat after dialysis with

a 400-fold volume. Studies with calcium-ion selective electrode showed that gluten-ES preserved its Ca^{2+}-binding capacity even after dialysis with several 400-fold water samples. Figure 6 illustrates the changes in mV after the addition of exogenous calcium ions to gluten-ES, ion-free water or dialysis fluid after dialysis. As can be seen, 0.04 μmol Ca^{2+} already induced an increase by 20—25 mV in water and the dialysing medium. In contrast, a similar change in gluten-ES containing solutions was caused by quantities of calcium as large as 0.18—0.20 μmol. This clearly proved the calcium-binding property of gluten-ES.

The volume of dialysing medium affects the absorption spectrum of gluten-ES. Therefore, we investigated the effect of dialysis conditions on the absorption spectrum. After EGTA treatment of gluten, 25 ml gluten-ES preparation was dialysed against 2000 ml ion-free water for 24 hours. Then 2 ml of gluten-ES solution was removed and designated as dialysed once, the rest was exposed for a second dialysis against another volume of 2000 ml ion-free water for 24 hours. By this procedure gluten-ES dialysed one, two, three and four times was obtained. For each sample we determined the calcium content by atomic absorption spectrophotometry, the Ca^{2+}-binding capacity by an ion-selective electrode and the absorption spectrum (Figure 7). To characterize the flattening of the spectral peaks, the quotient of the extinction values measured at 276 nm (the maximum) and at 252 nm (the minimum) was calculated. The results were compared with those obtained for calcium-free ovalbumin void of calcium-binding capacity. As can be seen from Table III, the value of this quotient gradually decreased with the increasing degree of dialysis; this points to a flattening of the spectrum curve. It may be supposed that the calcium content of the gluten-ES preparation increases with dialysis and this in turn may be ascribed to an altered conformation of the molecule. It appears contradictory that the preparation should bind more calcium

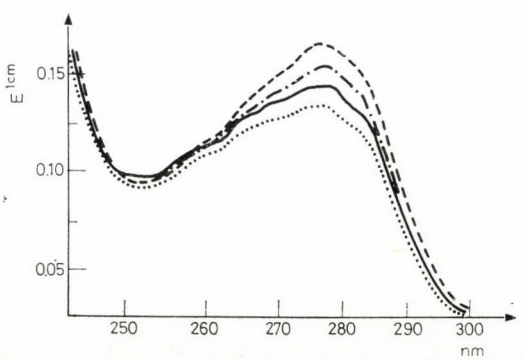

FIG. 7. The effect of dialysis with 1x (- - - -), 2x (·—·—·), 3x (———) and 4x (....) 80-fold volume of ion-free water on the absorption spectrum of gluten-ES

TABLE III

Effect of number and volume of dialysis portions on E 276/E 252 nm quotient, calcium content and Ca^{2+}-binding capacity of gluten-ES

Gluten-ES dialysed *n* times	E 276 nm / E 252 nm	Protein-bound Ca μmol Ca / mg protein	Calcium-binding capacity, μmol Ca^{2+} / mg protein	Protein-bound Ca plus calcium-binding capacity, μmol Ca^{2+} / mg protein
1×	1.71	0.0888	0.673	0.761
2×	1.67	0.160	0.680	0.840
3×	1.51	0.258	0.550	0.808
4×	1.47	0.341	0.396	0.737
Ovalbumin	1.85	0.0005	0.0005	

when dialysed against ion-free water but according to our calculations and measurements the 2000 ml ion-free water still contained 0.1—0.2 μmol calcium (20 ml ion-free water was evaporated to dryness in a quartz tube, the residue was dissolved in 1 ml ion-free water, and the calcium content was determined with AAS). With progressing dialysis the calcium content increased and the calcium-binding capacity decreased. In the fifth column of Table III the values exhibit a maximum of 0.840; this may have been due to EGTA remnants in the gluten-ES preparation where the EGTA concentration of the latter exceeded the value of 3 μmol/l.

Further experiments were carried out to see whether gluten-ES was capable of influencing the Ca^{2+}-level of the medium. Sarcoplasmic reticulum of the muscle regulates the free calcium concentration within the muscle fibre by a calcium pump sensitive to the free calcium ion concentration and operating by a Ca^{2+}-Mg^{2+}-dependent ATPase. We investigated the effect of gluten and gluten-ES on the ATPase activity and calcium ion uptake of FSR prepared as described in the chapter on Methods. The results are

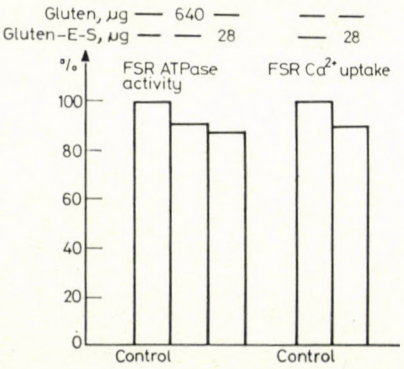

FIG. 8. The inhibitory effect of gluten and gluten-ES on the ATPase activity and Ca^{2+} uptake of fragmented sarcoplasmic reticulum isolated from rabbit muscle

demonstrated in Figure 8. 640 μg gluten inhibited the ATPase activity of FSR by 9—10%, while a much smaller quantity (28 μg) of gluten-ES did so by 13—15%. A similar inhibition is reflected in the diagrams illustrating Ca^{2+} uptake. From these findings it is clear that gluten-ES has a marked influence on calcium ion concentration.

Discussion

Since the first description by Dicke [6] of the causal relationship between wheat and rye gluten and coeliac disease, several attempts have been made to elucidate the aetiology and pathogenesis of the disorder [4]. Proof has been obtained that some enzyme deficiency associated with the presence of HLA-B8 tissue antigen may be responsible for the manifestation of coeliac disease [3]. In our opinion, however, the primary cause is not necessarily a lack of some protease but the ultimate defect may lie in one or more enzymes responsible for the synthesis of certain component(s) of the membrane of intestinal epithelial cells.

The presence of certain HLA antigens may predispose to abnormal immunological reactions to otherwise innocuous proteins like gluten [9]. The incidence of coeliac disease has decreased since the return of breast-feeding and the spread of gluten-free baby-foods [10]. Feeding with cow's milk predisposes to enterocolitis and this, together with the immaturity of the intestinal mucosa and the immunological defence system may precipitate coeliac disease at the moment of introduction of cereals into the child's diet. In a previous paper [14] we offered a hypothesis on the role of structure, composition and protease resistant particles of gluten in the pathogenesis of the disease. The large number of various gluten components, their similarity in aminoacid composition and sequence and in molecular mass, the difficulty of obtaining the components in pure homogeneous form and the lack of a suitable test model for toxicity research greatly hamper progress in this field.

Gluten-ES, prepared by us by EGTA, proved to be a homogeneous compound by SDS-PAGE, having a molecular mass of 36 000. However, by PAGE without SDS and immunodisk PAGE it appeared to be inhomogeneous. Undoubtedly, there is an immunological similarity between gluten and gluten-ES, as confirmed also by their similar aminodicarbonic acid plus aminodicarbonic acid amide and less polar aminoacid content.

Gluten-ES is water-soluble, remains in solution in the presence of electrolytes, its absorption maximum is at 275.6 nm. The spectrum curve, however, flattens if the volume of the dialysis fluid is increased. By use of a calcium ion selective electrode we have shown that gluten-ES dialysed against a 400-fold volume of water was capable of binding calcium, during dialysis the calcium content of the preparation paradoxically increases, it takes up calcium from the "ion-free" fluid still containing small

quantities of calcium (0.1—0.2 μmol), with sustained dialysis the calcium content of the preparation increases while its calcium binding capacity falls. Flattening of the spectrum curve may be related to these phenomena, the degree of flattening can be characterized by the extinction quotients measured at 276 and 252 nm. If the dialysis volume is increased, changes in conformation of gluten-ES may occur as indicated by the icreased calcium content and decreased calcium binding capacity.

In previous studies [20] we found that our gluten resp. gliadin preparations contained 20—21% aminodicarbonic acids (95—96% of these is glutaminic acid), one of the carboxyl groups does not participate in the chain-forming peptide bonds. In gluten-ES, having a molecular mass of 36 000 and being capable of binding calcium ion, there must be glutaminic acid of 7200—7600 molecular mass, corresponding to 49—52 mol free carboxyl suitable for the binding of calcium ion. If we anticipate that two free carboxyl groups bind one calcium ion, 1 mol gluten-ES can bind 24—26 mol Ca^{2+}. Further calculations lead to a figure of 0.667—0.720 μmol Ca^{2+} bound by 1 mg gluten-ES. This is in fair agreement with the figure obtained in our experiments and shown in Table III in the last column, where bound calcium plus calcium binding capacity is indicated: this is 0.737 μmol. The possibility, however, cannot be excluded that a proportion of glutaminic acid present in gluten-ES is gamma-carboxyglutaminic acid.

The calcium binding property of our preparation has been proved also by the fact that it is capable of inhibiting the ATPase activity and calcium uptake of FSR. This shows that gluten-ES may influence the free calcium level and thereby muscle function. This mechanism may play a certain role in the pathogenesis of intestinal muscle hypotonicity characteristic of coeliac disease. We are now investigating the effect of gluten-ES on various calcium ion mediated processes and membrane structures and could already show that the preparation markedly inhibits the superprecipitation of actomyosin.

Acknowledgements

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Experience based on 800 000 newborn screening tests of the Budapest Phenylketonuria Centre

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800 000 newborns were screened for hyperphenylalaninaemia by the Guthrie-test in the Budapest PKU Centre in the 10 and a half years since 1 May, 1973. The blood samples were taken from mature newborns on the fifth and from premature babies on the fourteenth day of life. All infants exhibiting a level equal to or exceeding 12 mg/dl were telegraphically invited to the Centre and those having a level of 15 mg/dl or higher were put on an appropriate diet. The patients were classified according to the result of the phenylalanine tolerance 73 were found to have classical phenylketonuria and 15 had atypical phenylketonuria. The total incidence of phenylketonuria was thus 1 : 9091. The mean age at introduction of diet was 30 ± 15 days during the first period, while 21 ± 11 days during the second period. Infants having an initial value of 4—12 mg/dl were kept under continuous control; among them 69 were found to have benign hyperphenylalaninaemia (HPA). The PKU/HPA ratio amounted to 1.28. Both screening and care were carried out by the Centre, and the practice of care is described in detail. A preliminary evaluation of the therapeutical results with a view of the patients' social class is offered. Phenylalanine levels during the diet were greatly influenced by the familial background and the sociocultural environment.

The first report on the prevalence of phenylketonuria (PKU) in Hungary was published in 1961: among 1500 mentally handicapped children 0.66% were found to have PKU. The disorder showed the highest prevalence, 2.2%, in residence institutions. Screening for PKU during the neonatal period was initiated on a national scale in 1968 using the Guthrie-test [7]. The University Children's Department in Szeged was charged to organize the screening. This centre gradually extended its activities over the Eastern part of the country. During the first five years 100 000 newborns were screened, the incidence was somewhat higher than 1 : 10 000. In 1973, a second PKU screening centre was established in Budapest, and by the beginning of 1975 continuous mass screening and care had become obligatory for the whole country. The National Institute of Child Health published a booklet describing the diagnostic and therapeutic principles of PKU, its essential features, the principles of diagnosis and treatment and a detailed formulary adapted to Hungarian conditions, supplying information for parents, health visitors and doctors.

Organization of the screening programme

In Hungary, having 10.6 million inhabitants, nearly all births take place in obstetric institutions and all mothers delivering at home are admitted to hospital as soon as possible. The mean number of births per annum was about 150 000 during the reported period, the rate of low-birth-weight newborns (under 2500 g at birth) was high, 10% throughout the period.

The introduction of the screening programme was preceded by information spread by mass media and circulars to the public. The obligatory character of screening was accepted without any difficulty. This may have been due to the fact that preventive vaccinations including BCG have been a common practice in the country.

The blood sample from mature babies is obtained on the fifth day of life, the usual day of discharge from hospital; for infants with a low birth weight blood sampling is postponed to the fourteenth day. Screening and care are carried out by the two centres supplied with up-to-date laboratory facilities. The centres maintain a close contact with the neonatal health institutions, practitioners, kindergartens, schools and especially with the parents.

The leader of the department caring for the newborn is responsible for the completeness of screening. From the centres an annual report on the number of blood samples obtained from each institution is sent to the responsible persons who can then check the figures for completeness. The centres mutually inform each other about all problems and report the number of screened newborns, test repetitions and the definite number of diagnosed cases to the National Institute of Child Health. Every year this institute organizes with the Ministry of Health a conference for the chief paediatricians of the counties where the results, difficulties and further tasks are reported.

The two centres have proved sufficient for this work; each of them receives 70 000 blood samples annually, a number thought to be the optimum. It seems advantageous that the centres work in the framework of paediatric institutions [10, 23]. The centres are now also charged with neonatal screening for galactosaemia, also performed by the microbiological Guthrie-test [8]; this screening has been obligatory since 1977.

In the Budapest centre the screening and care of PKU patients is carried out by a team comprising a full-time paediatrician expert in clinical genetics, a chemical engineer, a biologist, three technical assistants and a part-time psychologist. All members of the team make personal acquaintance with all affected families, possess appropriate practical knowledge on treatment and diet of the disorder and all share success and failure alike. In addition, the team participates in diagnostic tasks of other inborn errors of metabolism, research into clinical genetic problems, graduate and post-graduate teaching of medical students and doctors, health visitors and technical assistants.

Material

During the 10 years and 9 months from 1 May, 1973, to 31 January, 1984, a total of 800 000 blood samples was received on Schleicher-Schüll No. 2994 filter paper. During this time there was first a steep increase in birth rate followed by a pronounced but gradual decrease. During the first period, from 1 May, 1973, to 31 December, 1978, the number of samples increased every year, the highest figure, 92 000 was attained in 1976. The rate of completeness during the first period increased from 80 to 91%. During the second period, from 1 January, 1979, to 31 January, 1984, the demographic wave had abated, and this was reflected in a decrease of the annual number of blood samples, but the rate of completeness increased to 98.5% by 1983. This high rate was due to three facts: (i) The screening had become obligatory; (ii) obligatory training of all nurses working in neonatal institutions; (iii) in 1982, when hypothyroidism screening was also included into the programme, a large-scale organizatory and information campaign took place. Since then all institutions fulfil their obligation to post the samples twice every week. Thereby the age at introduction of treatment could markedly be lowered. The parents of newborns exhibiting a positive or suspect level receive a direct telegram, thus only 1—3 days elapse between abnormal reading and the institution of treatment.

Methods

Guthrie-test

The Guthrie-test is used for mass screening [7]. For semiquantitative evaluation, haemoglobin and the plasma proteins are denatured by heat for three minutes. Standard blood samples containing 4, 6, 10, 15 and 20 mg/dl of phenylalanine are used. They are prepared by addition of known quantities of phenylalanine to blood with phenylalanine level measured by spectrofluorometry. The filter papers containing the separate blood samples are dried on a horizontal grid [19]. The standard blood samples are stored in a refrigerated desiccator [14]. The optimum spore/inhibitor ratio is adjusted for every culture. The culture medium is poured into plastic plates, each plate is used for culturing 120 disks 7 mm in diameter. The standards and the blood samples originating from persons suspected to have high levels are placed to the centre of the plate, and the 4 mg/dl standards are placed into the corners.

An area exhibiting growth inhibition of *B. subtilis* develops in about 3% of the blood samples. In a minor fraction of these cases this is due to antibiotics taken by the mother or the newborn. In the majority of cases the filter papers must have been contaminated with some inhibitor during storage or transfer. Repeated use of autoclave heat reduces the percentage of inhibition to 0.2%. If growth inhibition is observed after repetition, the health visitor of the child is asked to send a second blood sample taken by her in the newborn's home.

Blood samples originating from children on diet were used for control. In these samples phenylalanine is routinely determined by spectrofluorometry. By sampling as late as the fifth day of life, by the use of appropriate standards to increase sensitivity of the semiquantitative Guthrie-test, and by the use of quality control all samples containing more than 4 mg/dl of phenylalanine are noted with certainty.

An increased level was encountered in two cases of galactosaemia. Two false positive results occurred; in one case the sample was contaminated with casein hydrolysate, in the other case two samples originating from an affected child were sent in under two different names. We were aware of two false negative findings. Both cases were recognized at the age of six years at family screening. One of them had atypical PKU manifesting itself with severe behavioural disturbances and emotional lability, his

IQ was 90. The other false negative case at screening had severe classical PKU with a very low IQ.

Supplementary tests

Serum phenylalanine is measured by the spectrofluorometric method of McCaman and Robins [16]. For this purpose the Sigma kit No. 60-F, based on the same principle, has been used since 1980. A comparison between these findings and those obtained by the Guthrie-test has shown that the difference does not exceed 10% with blood samples containing about 4 mg/dl. In 29 blood samples containing 15 mg/dl out of the 45, the microbiological test measured values higher by 2—4 mg/dl. Three quarters of the blood samples with a phenylalanine level exceeding 20 mg/dl by the Guthrie-test were found to contain more than 20 mg/dl by spectrofluorimetry. This was in agreement with the findings of Belton et al. [1].

Serum tyrosine was determined quantitatively by the Sigma 70-F kit based on the spectrofluorimetric method of Waalkes and Udenfriend [27]. In earlier years the method of Efron et al. was used for serum and urine aminoacid chromatography [6], and more recently thin-layer chromatography has been applied [13]. For demonstration of phenolic acids in urine, the ferrichloride reaction and thin-layer chromatography were used; preparation of the material was carried out by the Koch-Light method [13], and the samples were run according to Schmidt [24]. By this method phenylpyruvic acid and/or o-hydroxy-phenylacetic acid were found in all urine samples of affected children before the age of five weeks.

Results

Differential diagnosis of hyperphenylalaninaemia

Figure 1 shows the strategy for further tests depending on the results of the first Guthrie-test. Negative results are not sent to the local health workers. A second blood sample is asked for if the result of the first test was 4—12 mg/dl; if a similar result is obtained, monthly checking is demanded. If two subsequent blood samples furnished normal phenylalanine levels within the first year of age, the condition was designated transitory hyperphenylalaninaemia (HPA). If the level falling between 4 and 12 mg/dl persisted beyond the first year of life, sustained hyperphenylalaninaemia was diagnosed and regular checking was maintained. Whenever a level of 12 mg/dl or more is found the parents are asked in a telegram to come and present the child. If in such a child a level of 15 mg/dl or higher is found with an increasing intermittent tendency, a phenylalanine-poor diet is immediately prescribed. If the blood sample taken from the presented child is lower than 15 mg/dl, the possibility of atypical (variant) phenylketonuria is considered and regular checking of the child on normal protein intake (2—2.5 g/kg/day) is recommended. In some cases treated like this the phenylalanine level increased again and then a phenylalanine-poor diet had to be prescribed. In other cases the phenylalanine falls rapidly and stabilizes at a level of 4—10 mg/dl, pointing to sustained mild HPA.

As early as 1976, a loading test with natural protein [3] was introduced for differential diagnostic and prognostic purposes. Since this test proved to be of no practical value it was abandoned

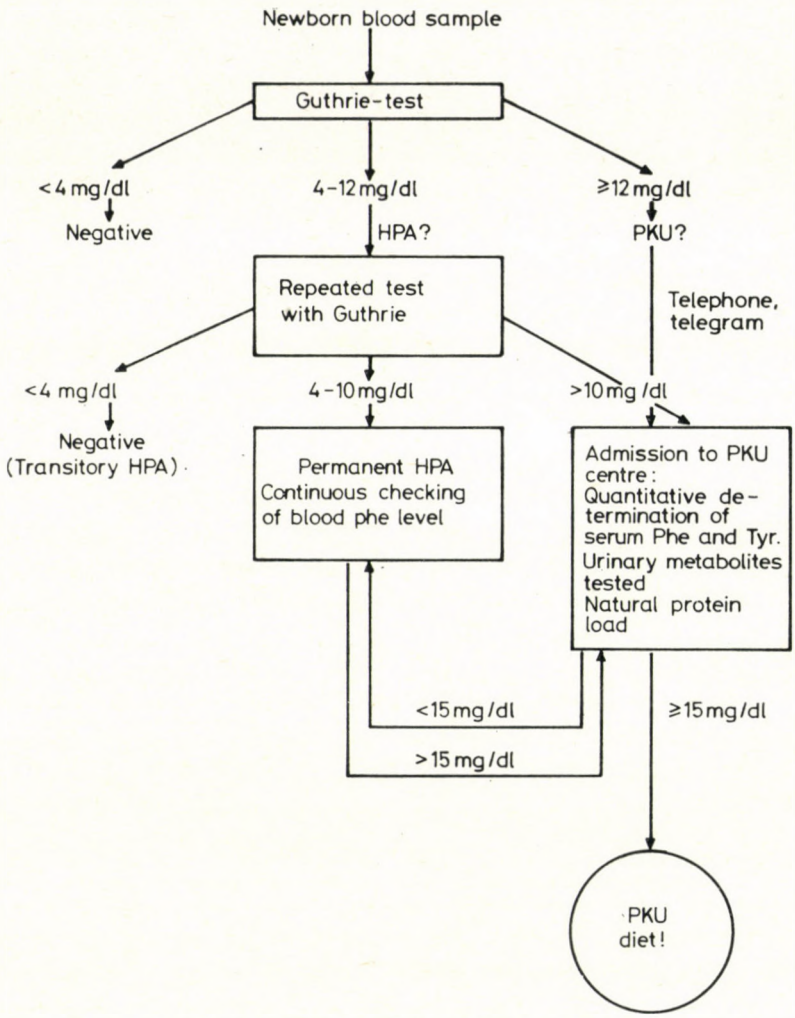

FIG. 1

in 1979. Now the following parameters are used for estimating phenylalanine tolerance: (i) Speed and decrease in serum phenylalanine after initiation of a diet with restricted phenylalanine content. (ii) The rate by which natural protein could be reintroduced. (iii) The quantity of natural protein, principally cow's milk, tolerated during the second year of life for maintenance of the desirable phenylalanine level of 4—8 mg/dl. (iv) Severity and duration of derangements of phenylalanine metabolism elicited by intercurrent diseases.

Classification of our patients on diet has been based on phenylalanine tolerance. Patients aged between 1 and 2 years with a phenylalanine

tolerance of daily 30–60 mg/kg (daily total intake less than 500 mg) were classified as classical phenylketonuria (severe or mild), those tolerating daily 75–100 mg/kg (daily total intake more than 500 mg) were regarded as atypical (variant) PKU as can be seen in Table I.

Classical PKU

72 patients kept on diet during the neonatal period have been registered, 41 boys and 31 girls; 4 of them were low-birth-weight infants. Most were breast-fed prior to the first blood-sampling. The results of the first Guthrie-test are shown in Table II.

Table III illustrates the levels obtained during diet, as grouped according to the periods within the 10 years. The result obtained at admission exceeded 20 mg/100 ml in all infants, by spectrofluorimetry the mean phenylalanine level was 44.5 ± 18.3 mg/dl and the mean tyrosine level, 2.1 ± 0.9 mg/dl. This is in agreement with the findings obtained by O'Flynn et al [17] in 216 classical PKU patients in U.S.A. The mean phenylalanine/tyrosine ratio was 23.6 ± 12.8 in our material.

At first admission to our department, 61% of the patients were exclusively breast-fed, mixed feeding was given to 11%, only a high-protein

Table I

Distribution of various types of hyperphenylalaninaemia among 800 000 screened newborns

Type	*n*
Classical PKU	72
Transitory PKU	1+
Atypical (variant) PKU	7
Late-onset atypical PKU	7
Intermittent PKU	6
Permanent HPA	69
Transient HPA	111

+: died

Table II

Result of Guthrie test in classical PKU patients at screening

Phenylalanine, mg/dl	*n*
4–14	3
15–20	44
Higher than 20	25

TABLE III

Data of classical PKU patients concerning diet during two periods, means and standard deviations

	Period 1 1 May 1973— 31 Dec 1978	Period 2 1 Jan 1979—31 Jan 1984
Age at admission, days	27±12	20±10
Age at introduction of diet, days	30±15	21±11
Length of hospital stay for diet adjustment, days	28±10	27±14
Time needed for introduction of 100 ml cow's milk into the diet, days	21±16	12± 6
Quantity of tolerated milk at discharge, ml	148±49	
Phe tolerance at discharge from hospital, mg/kg/day	65±28	
Formula at discharge	Berlophen (Berlin Chemie) only	50%: Berlophen 30%: Nofelan (Polfa) 10%: Albumaid XP (Maizena) 10%: P-AM (Maizena)

formula was administered to 15% and a low-protein formula to 13%. There was no difference in the phenylalanine level among the groups at admission.

Transitory PKU with an extremely rapid improvement of phenylalanine tolerance was encountered in one case. By the sixth month of life the phenylalanine level was stable below 4 mg/dl in spite of a normal diet. This child, offspring of caravan-dweller Gypsies, died of pneumonia when one and a half years old.

Atypical PKU

During the neonatal period, 7 cases of atypical (variant) PKU were found, 6 girls and 1 boy. All were mature babies. In two, the phenylalanine level was very high by the Guthrie-test, 15 and 30 mg/dl, respectively, both were put on diet on the 17th day of life, then they had a phenylalanine level of 20.2 and 28 mg/dl, respectively. The other five patients had a level between 6—8 mg/dl; four of these were put on diet between six weeks and three months of age because of an increasing level by the time of the first control, then they had levels between 35—44 mg/dl. In the seventh child the increase was slower, 15 mg/dl was exceeded as late as by the third repetitive control, she was put on diet when two and a half months old.

At discharge, after adjustment of the diet, their mean phenylalanine tolerance was 75 mg/kg/day, with a range of 65—125. At the age of two years all were tolerating daily 200—250 ml cow's milk with a serum phenylalanine level of 4—8 mg/dl. There has been no relapse in their phenylalanine tolerance ever since, their daily intake is above 500 mg. The oldest child is now 8 years and

the youngest 3 years old; all have shown a normal intellectual and behavioural development.

Table III shows the phenylalanine tolerance of classical PKU patients at discharge after prescription of the diet. As can be seen, there was no difference in this respect between classical and atypical PKU at this age.

In the course of continuous checking of the group affected by permanent HPA, we encountered biological variants hardly known in the international literature; in these cases, indication of the diet was a great dilemma. These patients could be classified into two subgroups. In late-onset atypical PKU (seven cases) a rapid increase of the phenylalanine level occurred between six and ten months of age, and all biochemical parameters fulfilled the criteria of atypical (variant) PKU. In intermittent PKU (6 cases) extreme fluctuations of the phenylalanine level were observed, with unpredictable periodicity and intensity. The characteristics of these patients will be reported in a separate paper.

Permanent benign hyperphenylalaninaemia

This group consisted of 69 cases, 35 boys and 34 girls. All had an initial screening value of 4—12 mg/dl. At immediate repetition the level was 4 mg/dl or higher. They were checked monthly during the first year of life, every three months during the second year, and annually twice after the end of the second year. In half of the patients there was a single peak of about 15 mg/dl at the age of 3—4 or 8—11 months, on all other occasions they had a level not exceeding 10 mg/dl. The peak could be related with the introduction of protein-rich food.

All children were seen by us around their first birthday, and clinical and laboratory examinations were then conducted. In the meantime we kept a close contact with the child's health visitor. Mean age of the patients now is 5.9 years, the majority attends school or kindergarten. We receive regular information about their achievements, they have made a problem-free intellectual and personality development not deviating from the mean.

Incidence of hyperphenylalaninaemia

The area covered by our screening comprises the capital with more than two million inhabitants, and 8 out of the 19 counties of Hungary with about 3 million inhabitants. Table IV shows the incidence of the various forms of hyperphenylalaninaemia as calculated from the 800 000 screening results, the false negative cases included. The early and late-onset forms of atypical phenylketonuria are in the same group.

The two centres, in Budapest and Szeged, have screened 1 760 042 newborns up to 31 December 1983. Among these, 212 cases of PKU have been found [22], corresponding to an overall incidence of 1 : 8302. According to comparative studies [9, 26]

TABLE IV

Incidence of hyperphenylalaninaemia types, based on 800 000 screening test results

Type	*n*		Incidence
	Budapest	Country	
1. Classical PKU*	18	55	1 : 10 959
2. Early- and late-onset atypical PKU**	3	12	1 : 53 333
All PKU (1 plus 2) on diet	21	67	1 : 9 091
3. Intermittent PKU	—	6	1 : 133 333
4. Permanent HPA	13	56	1 : 11 594

* negative neonatal result: one case
** negative neonatal result: one case

this incidence is somewhat higher than in some adjacent countries like Austria, Czechoslovakia or the German Democratic Republic and comparable to the 1 : 8956 observed in Poland. In the area of the Budapest centre the ratio PKU/HPA is *1.28*, much higher than in the above mentioned countries, especially in Poland where this value amounts to *9.94*. Exact statistical comparison is, however, greatly impeded by differences in terminology.

As can be seen in Table IV, all types of HPA have a higher incidence outside Budapest than in the capital although they were almost equally represented in the material (53 and 47%, respectively). It is noteworthy that the overwhelming majority of the parents, and even more of the grandparents, were born in small settlements irrespective of the birthplace of the child. Quite strikingly, a single case has only been found among Gypsies, a minority still isolated within the Hungarian population; their number is estimated at 500 000.

Current practice of care

In Hungary medical help is gratuitous for every citizen. In the case of phenylketonuria this can be translated into the following terms: PKU dietary formulas are gratuitous; starch containing foods are gratuitous; expenses of travel for examinations of the child and both parents are repaid to the family; child benefit is paid also for a single child (otherwise it is only paid for two or more children); the mother of a PKU child may have paid leave until the child is six years old (with healthy children this is due for three years).

The blood samples of the children registered in our centre are taken at home and sent to us together with a diet control sheet. This is filled out by the parents assisted by the health visitor, registering the child's weight,

appetite, intellectual and motor development, eventual intercurrent diseases, etc. Questions concerning the diet may be asked by the parents. The result of the test is sent back to the parents, the letter usually includes advices. Children under three years of age are seen by us at least every half year, in case of difficulties more frequently. From three years on all children undergo psychological examination at least once a year. All children above three years are sent to kindergarten, initially for a half day, later for a whole day. In such cases the parents supply the foods. This type of activity has proved excellent for the personality development of the child and his subsequent entry to school is facilitated.

For the parents of PKU children, meetings are organized by our centre. For schoolchildren affected by the disorder there are separate reunions. Meetings between the parents are promoted, an experienced skilled parent may be most helpful in giving advice especially in the details of diet. A report is supplied by the teacher of each child annually, preferably at the time of the psychological examination.

Mean age of our patients now is six years. They were classified into one of five social classes according to their father's educational level, as shown in Table V. The children's condition was then scored as good, medium or bad according to the following points: educational level of both parents, dwelling conditions, monthly income, cultural claims, the child's other diseases, quality of child-parent bond, educational methods of the parents, cooperation, keeping the diet, reports released by the health visitor or leader of the kindergarten or teacher, progress in school, and IQ. As seen from Table V, there was a strong correlation between the score and the social class, especially in the 36 children who were regularly subjected to an intelligence test. Unsatisfactory keeping of the diet only occurred in the social classes IV and V with three exceptions.

The effect of the strictly kept diet was unsatisfactory in two cases. In one of these, lack of movements, muscular hypotension, hyperflexibility of the joints were striking from

TABLE V

Preliminary evaluation of therapeutic effect

Social class (Paternal educational level)	n	Good	Medium	Bad	IQ, mean ± SD
I. University or equivalent degree	11	9	2	0	108±17
II. Secondary school (graduation at grammar school or secondary technical school)	15	12	2	1	n=10
III. Skilled worker (Eight years elementary school plus 3 years technical school)	31	12	17	2	100±12 n=12
IV. Semiskilled worker (Eight years elementary school plus course)	18	1	12	5	88±12
V. Unskilled worker (Primary school with or without final examination)	11	1	4	6	n=14

the first month of life. The damage may have been due to perinatal lesion but also to maternal hyperphenylalaninaemia, since the mother's level was found to be as high as 10 mg/dl in repeated tests. In the other child, keeping the diet was made difficult by frequent vomiting, intercurrent febrile infections and recurrent hypercalcaemia of obscure origin during the first ten months of life. No hypertonicity of the muscles or progressive neurological symptoms characteristic of malignant PKU were encountered in this patient.

Discussion

The well-known clinical and biochemical variability due to genetical heterogeneity has been reflected in our material. In recent years much progress in classification has been achieved by the advent of in vitro and in vivo determinations of phenylalanine hydroxylase activity. However, the rest activity of the enzyme still divides groups overlapping between classical and variant forms. In addition, there is no method predicting the further individual development of the untreated child [2, 4, 5, 11, 12, 15, 18].

Over the whole world phenylalanine levels are the best indicator for therapy. It appears that, in contrast to the USA where the limit value for therapeutic intervention is 20 mg/dl [21], many European countries, including the U.K., have become more conservative, proposing therapy for neonates with a level of 15 mg/dl; this is already near to the 10 mg/dl initially proposed by Bickel [4]. In consequence, the number of newborn found by screening to have atypical variant PKU is increasing; this is, however, not indicated in the national statistics of the individual countries [4, 9, 26].

In our practice, 15 mg/dl is the limit value of blood phenylalanine for therapeutic intervention. The course of phenylalanine tolerance is still a primary tool in classifying PKU into severe, mild and atypical PKU, and this, too, has an impact on the quality and duration of the diet. In spite of lowering the age at introduction of therapy (Table I) our results are negatively influenced by the fact that nearly half of the parents had low sociocultural and psychosocial ratings. In a large number of families the lack of parental cooperation and responsibility led to failure of our efforts to maintain the phenylalanine level at 4—8 mg/dl, especially after the age of one and a half years.

Our experience suggests that the efficacy of care must be improved by the principles as follows:

— PKU children, their parents and grandparents need individual care in view of their variable familial background.

— A description of the familial environment should be asked for immediately after confirmation of the diagnosis.

— Organization of a parent patronation system seems promising; expert parents of successfully treated PKU children should be encouraged to visit or accept beginners in their

homes, the sight of a successfully treated child is a most effective emotional tool.

— Parents who cannot cope with the problems should be encouraged to give their child to State care, special homes should be set up for this purpose.

— Early education of the child should be extended to special knowledge about his or her own disorder, the limitations imposed by it and their reason. If this does not happen, the child will brake the diet by stealing or other surreptitious self-feeding, especially after the early years. The disorder should not be kept in secret from the relatives and the neighbours.

— Knowledge of the diet should be extended to all family members, especially to the father and grandparents.

— For children in whom aversion against casein hydrolysate has developed, products consisting of synthetic amino acid mixtures should be secured.

— The parents' attention should increasingly be drawn to the fact that the diet has to be kept also during periods of intercurrent febrile diseases; thereby the severity and duration of a hyperphenylalaninaemic period can be shortened.

Gradual omission of the diet of patients with classical PKU is recommended after the age of 10 years [5, 20, 25, 28], in children with weak school performance or with parents willing to go on with the diet even later. The period of relaxation is planned for two years. During this period the children are checked twice yearly, when EEG and psychological examinations are carried out and the teacher is asked for a report. Special attention is paid to the child's behaviour. After this period of transition, a decision is made by us together with the parents as to a change for a natural diet poor in protein; when its risks and the eventual necessity of a return to the diet are discussed. Female children are told about the necessity of diet before conception of a future child of her own. For atypical PKU, relaxation is instituted from the age of six years but until the age of ten years the blood level must never exceed 15 mg/dl.

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Influence of beta-receptor stimulation on catecholamine and phospholipid concentrations in lungs of fetal rabbits

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It was studied whether the beta-receptor stimulating fenoterol had any influence on endogenous catecholamines in the lung tissue of fetuses. On the 23rd, 27th and 31st days of pregnancy, concentrations of noradrenaline and dopamine in the lung homogenate of mature and immature rabbit fetuses were determined by fluorometry, and the basic surfactant components total phospholipids and lecithin by colorimetry, in a group receiving beta-mimetic treatment and in a control group receiving physiological salt solution. The concentration of noradrenaline decreased with the progression of pregnancy but the concentration of dopamine did not change significantly. Application of fenoterol caused an increase in total phospholipids and lecithin in the lungs of 23 and 27 day old rabbit fetuses and decreased the concentration of catecholamines, especially of noradrenaline. The drug had no such effect in mature (31 day) fetuses.

Insufficiency of surfactant, anoxia and coagulation disturbances are essential factors determining the incidence of idiopathic respiratory distress in premature babies [2, 3, 9, 10, 20, 27]. Anoxia and subsequent tissue acidification inhibit lecithin synthesis [16, 28] on the one hand and on the other evoke a release of catecholamines, especially noradrenaline [20, 21] which increases resistance of the pulmonary vessels [1, 5]. Anoxia and the high circulatory resistance enhance the permeability of blood vessels for proteins and plasma, and result in protein-rich exudate in the lung.

Synthesis of surfactant is realized by stimulation of adrenergic beta-receptors [13, 24], hence all beta-mimetics intensify the process. Clinical and experimental examinations have confirmed the beneficial influence of beta-receptor stimulating drugs on maturation of the lung tissue [4, 6, 7, 8, 14, 22, 23, 30].

The aim of the present experiments was to clarify the influence of fenoterol on the behaviour of phospholipids and catecholamines in the lungs of fetal rabbits.

Material and Methods

Examinations were carried out on 176 Belgian rabbit fetuses obtained from 22 females. Dead fetuses were excluded from the examinations. Duration of pregnancy was calculated from the date of female covering. Examinations were carried out on the 23rd, 27th and 31st days of pregnancy. The rabbits were divided into two groups (Table I). The experimental group included 11 pregnant rabbits which

TABLE I

Grouping of rabbits

Day of gestation	Examined subjects		Controls	
	Number		Number	
	pregnant rabbits	fetuses	pregnant rabbits	fetuses
23	4	34	4	35
27	4	30	4	31
31	3	22	3	24

received 0.1 mg/kg body weight of fenoterol in intravenous drip infusion. The control group consisted of 11 pregnant rabbits which received physiological solution intravenously. Then 24 hours after administration of the last dose of fenoterol all the fetuses were taken out in general anaesthesia In this way, 86 fetuses were obtained in the experimental group and 90 fetuses in the control group.

After removing the lungs, they were dried on Whatman paper, weighed, and subsequently homogenized in ice bath. Total phospholipids and lecithin in extracts of the lung tissue were determined by means of the colorimetric method of Yoshida et al. [31]. Extraction of phospholipids and lecithin from the lungs was carried out by the method of Folch et al. [15]. The obtained results were analysed using Student's t test.

Catecholamines (noradrenaline and dopamine) in lung tissue homogenates were determined by the fluorimetric method of Spano and Neff [29] and Chang [11], respectively.

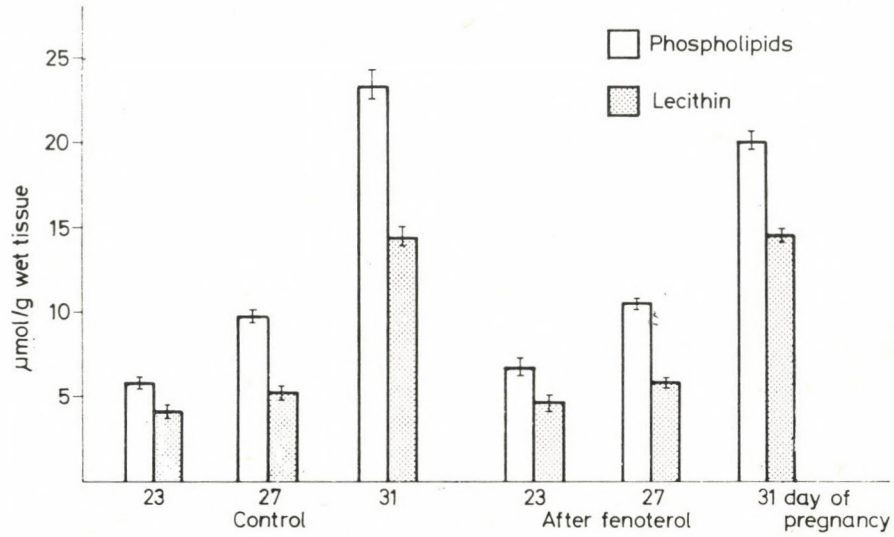

FIG. 1. Concentration of total phospholipids and lecithin in lung homogenates before (control group) and after fenoterol administration

Results

Total phospholipids and lecithin

Concentration of total phospholipids and lecithin in fetal lung homogenates was found to increase with the age of pregnancy (Fig 1).

In 23 day fetuses, mean concentration of total phospholipids in the lung amounted to 5.78 ± 0.319 μmol/g and lecithin to 3.93 ± 0.306 μmol/g. On the 27th day of pregnancy, the mean value of total phospholipids was 9.51 ± 0.452 μmol/g and of lecithin 5.34 ± 0.411 μmol/g. In mature fetuses i.e. on the 31st day of pregnancy, total phospholipids in the lung homogenate amounted to 20.10 ± 0.876 μmol/g and lecithin to 14.50 ± 0.495 μmol/g tissue.

After administration of fenoterol, in 23 day fetuses the level of total phospholipids amounted to 6.84 ± 0.553 and the level of lecithin to 4.80 ± 0.396 μmol/g. In 27 day fetuses the mean of total phospholipids was 10.66 ± 0.155 and of lecithin 5.97 ± 0.170 μmol/g. The increase was statistically significant in both groups. In the mature fetuses, however, fenoterol caused no change, total phospholipids amounted to 20.29 ± 0.598 and lecithin to 14.68 ± 0.354 μmol/g.

Catecholamines (noradrenaline and dopamine)

The behaviour of catecholamines was different from that of phospholipids and lecithin. The concentration of noradrenaline in the lung homogenates decreased with the age of pregnancy while the dopamine concentration was unchanged (Fig 2).

On the 23rd day of pregnancy, noradrenaline amounted to 1.27 ± 0.197 and dopamine to 1.09 ± 0.23

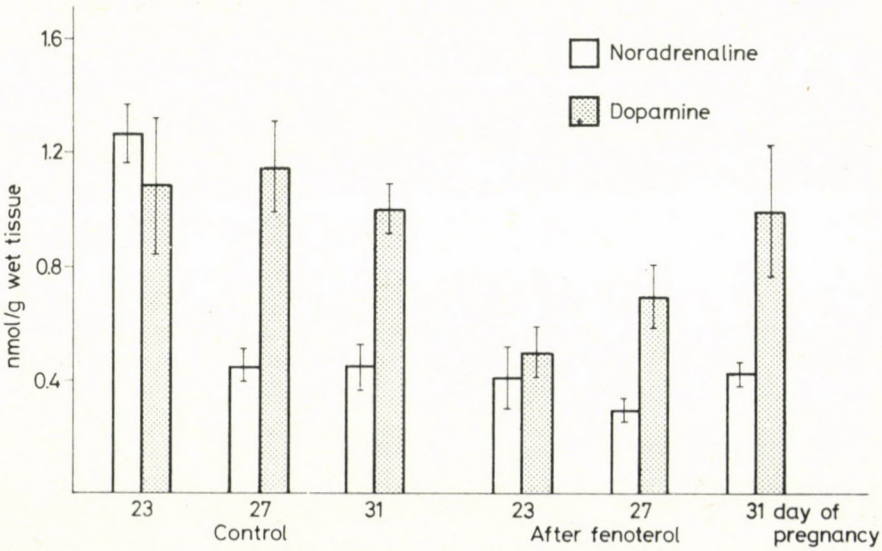

Fig. 2. Concentration of noradrenaline and dopamine in lung homogenates before (control group) and after fenoterol administration

nmol/g. In 27 day fetuses the concentrations were 0.452 ± 0.06 nmol/g and 1.150 ± 0.156, respectively, while in mature fetuses 0.450 ± 0.06 and 1.0 ± 0.156 nmol/g respectively. After administration of fenoterol, concentration of dopamine and noradrenaline on the 23rd and 27th days of pregnancy was lower than in the control group, while in the lungs of mature fetuses i.e. on the 31st day of pregnancy no significant changes occurred in the concentration of catecholamines (Fig 2).

Discussion

The examinations showed that the concentration of total phospholipids and lecithin increases whereas the concentration of noradrenaline decreases in the lungs of the fetal rabbit in the course of pregnancy. The dopamine content, on the other hand, showed no change. These results have confirmed the findings of other reports on the role of the adrenergic system in maturation of pulmonary tissue [13, 17]. It should be emphasized that our lecithin values did not differ considerably from the data of other workers who used alternative techniques of lecithin estimation [18, 19]. Similar observations were done by Hallman and Raivio [18] and Hayden et al [19] in lungs of rabbit fetuses on the 27th and 30th days of gestation, respectively. Administration of betamimetics was found to intensify the observed changes, i.e. to stimulate the maturation of lung tissue.

Decrease of noradrenaline concentration when the dopamine concentration is relatively high, improves the blood supply to the lungs. Like in the placenta, dopamine decreases the blood vessel resistance and thus increases the pulmonary blood flow [26]. High concentrations of dopamine when the noradrenaline content is low, are observed in placenta and lungs i.e. the organs where the blood flow plays an essential part. Improvement of the blood supply in the lungs ensures the adequate metabolism of the lung tissue which is reflected by an increased concentration of total phospholipids. The decrease of the noradrenaline concentration may be explained by the effect of fenoterol on the endogenous inhibitor of dopamine, beta-hydroxylase [25].

The obtained results confirmed the hypothesis that application of betamimetics in the treatment of prematurity simultaneously serves the prevention of neonatal respiratory disturbances.

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Bronchial hyperreactivity after infantile obstructive bronchitis

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A follow-up study was performed on 406 patients treated for infantile obstructive bronchitis during the period between 1964 and 1973. Their mean age was 12.6 years at the time of the study. The male : female ratio was 1.7. Forty-three patients (11%) became asthmatic within 10 years after onset of the wheezy episode of infancy. In one-third of the 363 non-asthmatic children, bronchial hyperreactivity was shown by acetylcholine and histamine provocation. There was a significant correlation between the number of recurrent obstructive episodes and the length of the period of recurrent wheezing on the one hand and bronchial hyperreactivity on the other hand.

Obstructive bronchitis is a common disease during infancy and early childhood. It is characterized by fever, dyspnoea, expiratory wheezing, sometimes crepitation, dry, later productive cough, pulmonary hyperinflation, depressed position of the diaphragm, on X-rays hyperaeration of lungs, and sometimes atelectasis. Obstructive bronchitis is usually accompanied by upper airways catarrh and not infrequently by bronchopneumonia.

The condition is also termed spastic, asthmatic or wheezy bronchitis; Clark and Godfrey wrote of a "wheezy baby syndrome" [2]. Some Anglosaxon authors apply the term bronchiolitis to all forms of wheezing, but most experts distinguish the disease caused by respiratory syncytial virus from obstructive bronchitis [2, 5, 14, 22]. Williams and McNicol regard obstructive bronchitis as one extreme of the broad spectrum of asthmatic diseases [20]. Still, the phrase "all that wheezes is not asthma" [19] means that the symptom does not unequivocally mean asthma. Indeed, wheezing during childhood may be a manifestation of cystic fibrosis, tracheal or bronchial malformation, foreign body, heart defect, gastrointestinal reflux, etc. About 10% of all children experience at least one obstructive episode before their second birthday [1].

Ten to 27% of children affected by obstructive bronchitis during infancy develop asthma in subsequent years [1, 11, 12, 13]. Host factors may here play an important role [5, 14]. There may be hereditary factors at work since allergy is more frequent in the families of patients with infantile obstructive bronchitis; its prevalence, however, is not as high as in families of asthmatic children [2, 19, 20].

A characteristic feature of bronchial asthma is a hyperreactivity of the bronchial system [3]. Bronchial hyperreactivity has been found in both short and long-term follow-up studies performed in patients with infantile obstructive bronchitis; the provocative factor may be exercise [6, 7, 9], histamine [13] or acetylcholine [12]. Similarly, bronchial hyperreactivity has been observed after infantile bronchiolitis [4, 17]. Heredity of bronchial hyperreactivity occurring after "wheeze disease" has been assumed [4, 5, 16] and confirmed by family and twin studies [7, 8].

The present follow-up study on children having experienced obstructive bronchitis during infancy was initiated in 1979; its aim was to see whether bronchial hyperreactivity could be demonstrated 10 years or more after the primary wheezing episode and to examine its relationship to certain factors.

Material

During the period between 1964 and 1973, 27 724 infants and children were admitted to our department; in 744 instances (about 3%) the diagnosis was obstructive bronchitis, 660 patients were involved. 406 of these 660 patients (62%) answered positively to our request to participate in the follow-up. Their mean age was 12.6 years (range, 9—18 years) at the time of the study. The male : female ratio of the 406 participants was 1.70 while among the original 660 infants this was 1.73. No fatality due to obstructive bronchitis occurred. Two children had died of heart defect and one of eclampsia; in one child cystic fibrosis, bronchial anomaly, chronic foreign body, in another a heart defect was found. 98 healthy children, free from any chronic complaint and matching in age served as controls.

Methods

The children were asked in a letter to visit our outpatient department. A detailed case history was then taken, data of earlier admissions were reconsidered, physical examination and lung function tests were carried out. Provocation tests were performed in children free from symptoms and complaints for at least two weeks. Bronchial reactivity was examined after acetylcholine and histamine provocation. Acetylcholine provocation was carried out in all 406 participants, while histamine provocation only in 313 out of the 363 non-asthmatic patients.

First, resting FEV_1 (forced exspiratory volume in the first second) and PEFR (peak expiratory flow rate) were determined; the best value of three determinations was chosen. A 1% solution of acetylcholine, 0.02, 0.05 and 0.10% solutions of histamine were applied step-wise by a Tur-Usi 50 ultrasound nebulizer, each solution for maximum 3 minutes. Each time the loading test was interrupted after 4—5 initial inspirations in order to see if an early reaction occurred. The above mentioned parameters were measured 3, 5 and 10 minutes after the cessation of provocation. A value exceeding the initial value by more than 15% was regarded as a sign of bronchial hyperreactivity [15, 18]; in the control group no increment larger than 12% was encountered.

The data of the 406 children were fed to a Videoton R-10 (VT-1010) computer and analysed statistically by the t and χ^2 tests.

Results

(i) 43 children out of the 406 participants (11%) became asthmatic some time after the initial obstructive bronchitis episode and the time of the study. The diagnosis of asthma was based on our own data or on data obtained in other hospitals. In earlier years the diagnosis rested on clinical data, later, with increasing frequency, on skin tests, aspecific or specific airway provocation, etc. The male : female ratio of asthmatic children was 1.86, in the non-asthmatic subjects only 1.68. Seventeen children had asthmatic complaints at the time of study, 26 of the asthmatics had been free from symptoms for at least one year and had not received any kind of treatment.

(*ii*) Bronchial hyperreactivity was observed in 122 children out of 363 non-asthmatic participants (33.6%). The distribution of positive results according to the kind of provocation was as follows.

only acetylcholine (363 children)	87	positive results
only histamine (313 children)	20	positive results
both (313 children)	15	positive results
all:	122	patients

The rate of hyperreactivity was identical for the two genders: 33.7% in boys, 33.3% in girls.

There were three pairs of twins, one of them uniovular. The monozygotic twins were concordantly negative, in one dizygotic pair both members exhibited a positive result, while in the other, one was positive and one was negative.

(*iii*) The number of episodes of obstructive bronchitis showed the following distribution.

one	91	children (25%)
two or three	118	children (33%)
more than three	154	children (42%)

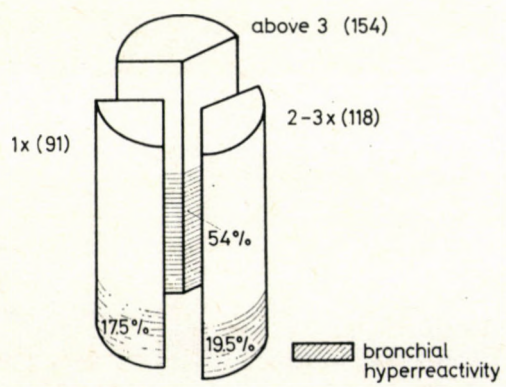

Fig. 1. Relationship between rate of bronchial hyperreactivity and number of episodes of obstructive bronchitis. In the group with more than 3 episodes, the rate is significantly higher ($p < 0.001$), $n = 363$

Figure 1 demonstrates the relationship between the number of obstructive episodes and bronchial hyperreactivity. In the group with more than three episodes bronchial hyperreactivity was significantly more frequent, $\chi^2 = 49.336$, $p < 0.001$. It is noteworthy that even in the group with a single obstructive episode, one fifth of the patients exhibited bronchial hyperreactivity.

(*iv*) The recurrent episodes disappeared by the age of ten years, only 7 children had obstructive symptoms accompanying respiratory infections beyond the seventh year of life. In 54% the obstructive symptoms disappeared by the end of the second year of life, in 85% by the end of the fourth year of life (see Figure 2). There was a certain relationship between the length of the period charged with obstructive episodes and the rate of bronchial hyperreactivity:

		Length of period	
		less than 4 years	more than 4 years
Bronchial hyperreactivity	present	97 (31%)	25 (47%)
	absent	213 (69%)	28 (53%)
	all	310	53

$\chi^2 = 5.114$, $p < 0.05$

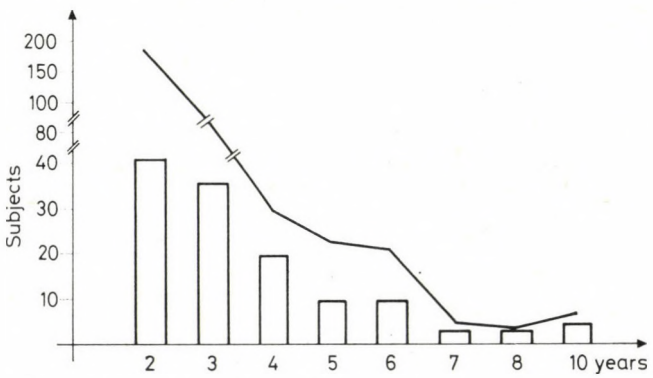

FIG. 2. Length of period with recurrent obstructive bronchitis and rate of bronchial hyperreactivity. In the group exhibiting recurrences beyond the fourth year of life there is a significantly higher rate of bronchial hyperreactivity ($p < 0.05$). The continuous line represents the patients in whom the recurrence ceased in the given year; this is 197 for two years and 84 for three years of age. The height of columns represents the rate of bronchial hyperreactivity

As can be seen, there was a significantly higher rate of bronchial hyperreactivity among children with recurrent episodes of obstructive bronchitis beyond the fourth year of life.

DISCUSSION

In a considerable proportion of infants affected by obstructive bronchitis, asthma develops during child-

hood. Conversely, in a proportion of asthmatic children the complaints begin before the age of two years [10]. The rate of asthma developing in subsequent years in infants with obstructive bronchitis has been found 11% in our study; this is in agreement with published data [1, 13], although the length of follow-up was not uniform.

Bronchial hyperreactivity is a characteristic feature of childhood asthma; it can be demonstrated by aspecific stimuli like provocation with cold air or body exercise. A similar hyperreactivity has been found in follow-up studies on infants with obstructive bronchitis, both shortly or a long time after the primary disease. Scislicki et al [13] found hyperreactivity on histamine provocation in 16% of 42 patients, König and Godfrey demonstrated hyperreactivity to exercise in 18 children [6, 7]. In patients with recurrent obstructive bronchitis, 87% hyperreactivity was found by Lenney and Milner while the patients were symptom-free [9]. The high rate (33%) of hyperreactivity found by us among 363 non-asthmatic children was significantly higher than that found among healthy children by acetylcholine and/or histamine provocation.

Inheritance of bronchial hyperreactivity has been suggested by König and Godfrey, on the basis of exercise provocation studies on family members and twins of patients with obstructive bronchitis [8]. The number of twins in our material was too small for conclusions in this respect.

Bronchial hyperreactivity was found to occur at a higher rate in children with more than three recurrences of wheezy bronchitis, respectively in those whose disposition persisted beyond the fourth year of life. Such a relationship has not yet been investigated according to the literature available to us. Further follow-up studies are necessary to determine whether this high rate of hyperreactivity, exceeding 50%, predicted an increased risk for chronic respiratory disease or bronchial asthma.

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Effect of oral and intravenous calcium load on glucose-induced insulin secretion in obese children

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The effect of intravenous (IV) (10 ml of 10% calcium gluconate) and oral (3 g calcium) calcium on plasma immunoreactive insulin (IRI) and blood glucose levels was investigated during intravenous (0.5 g/kg bwt. glucose) and oral (1.75 g/kg bwt. glucose) glucose tolerance test in 21 control (body fat 14.0 ± 0.5%) and 34 obese (body fat 36.1 ± 0.7%) children. Calcium given before IV glucose tolerance test and IV or oral calcium by itself did not alter blood glucose and plasma IRI concentrations in either group. Oral calcium load significantly increased the glucose-induced IRI response and decreased the blood glucose levels in obese children with impaired glucose tolerance (n = 7) compared to the levels without calcium. Since IV calcium did not alter the plasma IRI concentration, it has been assumed that oral calcium exerts its effect by influencing the secretion of an insulin secretogogue gastrointestinal factor (gastric inhibitory polypeptide ?). This effect, however, was observed only in obese children with impaired glucose tolerance.

The important role of calcium in cell activities, especially hormone secretion, has been widely recognized and numerous hormones were shown to require calcium for their secretion [6, 15, 20]. In vitro [5, 11, 13] and in vivo [9, 10, 23] studies have demonstrated that calcium is an essential requirement for insulin secretion induced by various stimuli. The present study was planned to investigate the effect of oral and intravenous calcium load on glucose-induced insulin secretion in obese children.

Materials and Methods

The investigations were carried out after an overnight fast in 21 non-obese and 34 obese children with body weights more than 20% in excess of the ideal body weight. Anthropometric measurements and the calculation of relative body weight and body fat were done as described earlier [17]. The relevant data of the children are given in Table I.

Oral load of 1.75 g/kg body wt. glucose was given with and without an oral load of 3 g calcium (calcium carbonate and calcium lactogluconate) in 18 obese and 6 lean children. In the second part of the study 0.5 g/kg body wt. glucose was administered intravenously with and without the intravenous injection of 10 ml 10% calcium gluconate in 6 obese and 5 control children. In addition, 5 overweight and 5 control children received a similar oral or intravenous calcium load without glucose. Capillary blood samples were taken by fingerprick for the measurement of blood glucose, plasma immunoreactive insulin (IRI) and plasma calcium concentration. Blood glucose was determined with the glucose oxidase method, plasma calcium with flame photometry. Plasma IRI levels

TABLE I

Physical characteristics of the investigated children (mean ±SE)

	Age, year	Height, cm	Weight, kg	Relative weight, per cent	Body fat, per cent
Control n = 21	11.9 ±0.5	150.5 ±2.6	42.4 ±2.2	99.5 ±1.6	14.0 ±0.5
Obese n = 34	11.8 ±0.5	153.7 ±3.0	68.7 ±3.6	150.1 ±3.7	36.1 ±0.7

were measured by radioimmunoassay, using commercially available kits (Isotope Institute of the Hungarian Academy of Sciences, Budapest). Normal (NGT) and impaired (IGT) glucose tolerance were defined according to the criteria of Guthrie et al. [12]. Statistical analysis was performed by paired and unpaired Student's *t* test.

RESULTS

Fasting blood glucose and plasma calcium levels were similar, but fasting plasma IRI concentrations were elevated in obese children as compared to controls (Fig 1).

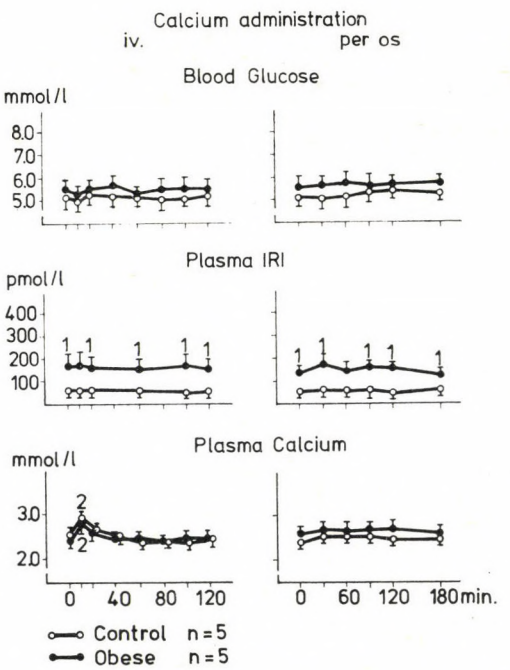

FIG. 1. Effect of intravenous (IV) and oral calcium load on blood glucose, plasma IRI and calcium levels (mean ±SE). 1 $p < 0.05$ Control vs Obese; 2 $p < 0.05$ Preload vs Postload values

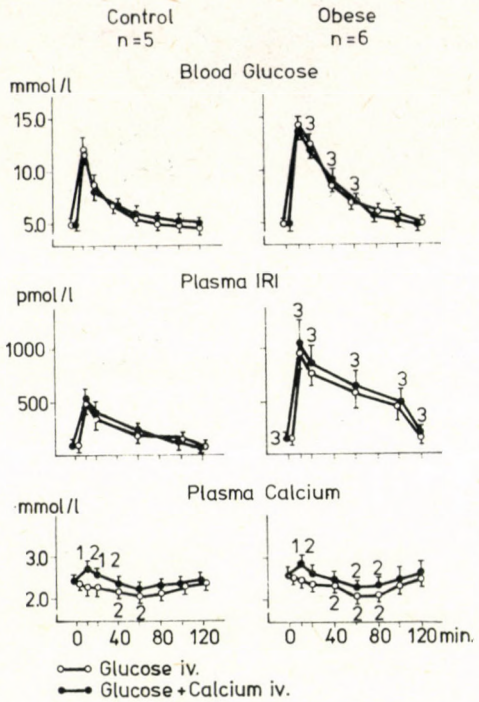

FIG. 2. Effect of intravenous glucose and intravenous glucose + calcium on blood glucose, plasma IRI and calcium levels (mean ±SE). 1 $p < 0.05$ Glucose vs Glucose + — calcium; 2 $p < 0.05$ Preload vs Postload value; 3 $p < 0.05$ Control vs Obese

Intravenous (IV) or oral calcium load did not alter blood glucose or IRI levels in either group. Plasma calcium rose significantly 10 min after IV administration of calcium while it was not affected by oral calcium load in overweight and lean children (Fig 1).

Blood glucose levels of obese children were significantly higher 20, 40 and 60 min following IV glucose injection and IRI concentrations were higher throughout the test than in controls (Fig 2). These two parameters, however, were not altered by the simultaneous IV injection of calcium in both obese and control children (Fig 2). IV glucose load decreased plasma calcium at 40, 60 and 40, and 60 and 80 min in the control and obese groups, respectively. When glucose and calcium were injected together, a transient rise of plasma calcium concentration was observed at 10 and 20 min in the controls and at 10 min in the obese children (Fig 2).

The glucose-induced increase of plasma IRI concentration was significantly higher at 30 and 60 min in the obese children when oral calcium was given, compared to the levels without calcium, whereas the comparison of blood glucose values revealed a significant decrease at 30, 60, 90 and 120 min in the group with

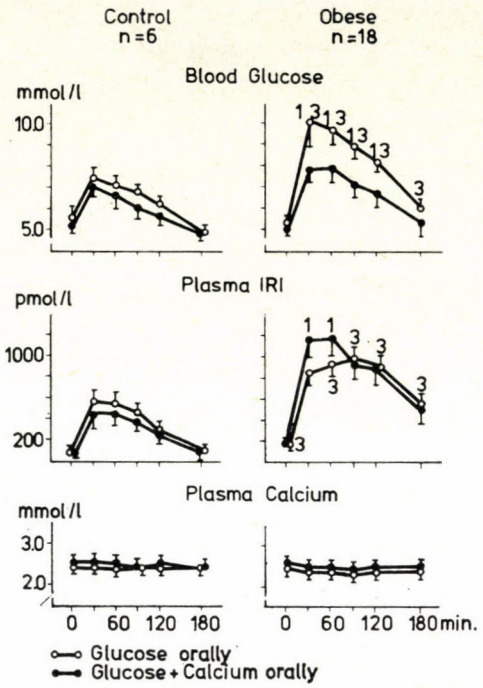

FIG. 3. Effect of oral glucose and oral glucose + calcium on blood glucose, plasma IRI and calcium levels (mean ±SE). 1 $p < 0.05$ Glucose vs Glucose + calcium; 3 $p < 0.05$ Control vs Obese

oral calcium load (Fig 3). None of these values showed significant changes after oral calcium load in 6 controls. No changes were detected in plasma calcium concentrations following oral calcium and glucose intake in either group (Fig 3).

The above demonstrated influences of oral calcium on plasma IRI and blood glucose levels during oral glucose tolerance test were, however, variable in the overweight children. The stimulation of glucose-induced IRI secretion and decreased blood glucose levels after oral calcium load were marked and occurred only in obese children with IGT (Fig 4), whereas in the group with NGT it had no effect whatsoever.

The early phase of glucose-induced IRI response was defective in the IGT group, demonstrating lower plasma IRI concentrations at 30 min (543.0 ± 76.8 pmol/l) than in the NGT group (1072.0 ± 154.5 pmol/l) ($p < 0.01$). Plasma IRI after oral glucose + calcium load was similar in the NGT and IGT obese subgroups (Fig 4).

Age (NGT: 12.3 ± 0.46 yr, IGT: 10.3 ± 1.8 yr), body fat (NGT: 36.9 ± 0.74%, IGT: 37.2 ± 0.78%) and duration of obesity (NGT: 6.4 ± 0.7 yr, IGT: 4.7 ± 0.78 yr) were not different in the two obese subgroups.

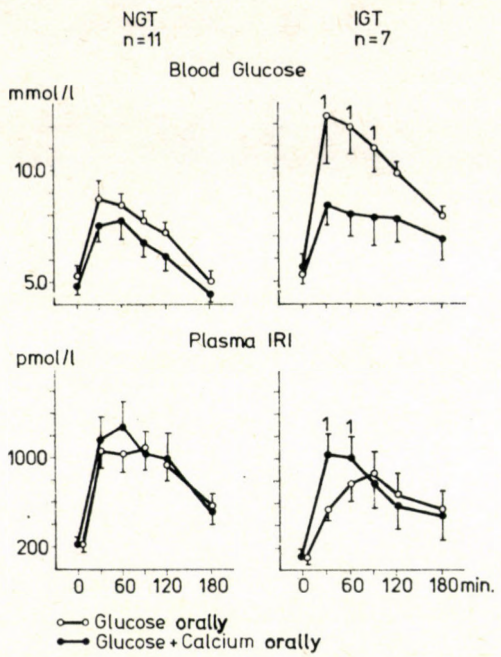

FIG. 4. Effect of oral glucose and oral glucose + calcium on blood glucose and plasma IRI levels (mean ±SE) in obese children with normal (NGT) and impaired (IGT) glucose tolerance. 1 $p < 0.05$ Glucose vs Glucose + calcium

DISCUSSION

In the present study IV or oral calcium by itself, or IV calcium in combination with glucose load had no effect on blood glucose and plasma IRI levels in obese and control children. Increased glucose-induced insulin secretion and a secondary fall in blood glucose concentration was observed in obese children with IGT, but not in children with NGT and controls after oral calcium load.

The role of extracellular calcium in the secretion of insulin is well documented. High and low extracellular calcium levels have been shown to enhance and suppress, respectively, glucose-induced IRI secretion in vitro [5, 11, 13] and in vivo [14, 23, see 22 for review]. In patients with islet cell tumour, IV calcium infusion caused a release of insulin and proinsulin, but no such effect was obtained in normal subjects [10]. Calcium apparently acts on β cell membrane or enters the cell before insulin release [16]. Such a direct effect of calcium on pancreatic β cells, however, was unlikely in the present investigation since an increase in plasma calcium concentration following IV calcium administration did not influence glucose-induced insulin response. An insulin secretogogue gastrointestinal factor stimulated by oral calcium might explain why IV calcium had no effect on insulin secretion whilst

oral calcium had such an effect. To date, gastric inhibitory polypeptide (GIP) best fulfils the criteria for such a factor [3]. GIP is released at the ingestion of glucose and fat [1, 2] and potentiates glucose-induced IRI release [1, 7, 19]. Exaggerated GIP response of obese subjects and animals [4, 8, 21] to glucose or standard test meal has been reported. The higher the GIP response to glucose, the more pronounced insulin response is observed [4]. The IV administration of purified GIP increased IRI secretion in the early phase of glucose tolerance test [7], resembling the effect of oral calcium in obese children with IGT observed in the present study.

On the basis of the present results and earlier data it can be hypothesized that oral calcium increases glucose-induced IRI secretion by stimulating GIP secretion in the obese subgroup with IGT and a low insulin response, whereas in the hyperinsulinaemic obese children with NGT the presumably high GIP level cannot be stimulated further by oral calcium. It is likely that the enteroinsular axis can be stimulated by oral calcium in obese children with IGT as it was observed in diabetics by Fujita et al [9]. The pathological significance of the present observation, however, is not known and needs further investigation.

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Chromatographic screening of 70,328 neonates for inborn errors of amino acid metabolism

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Between the years 1974 and 1984, amino acid chromatography was performed from dried blood spots and partly from urine of 70 328 neonates. Six cases of phenylketonuria, one histidinaemia, one hyperglycinaemia and three cystinurias were found. Since all these could have been detected by other methods, the regional screening was discontinued in agreement with international recommendations.

The enthusiasm elicited by the first results of neonatal mass screening for phenylketonuria (PKU) in the early sixties animated to the early detection of as many inborn errors of amino acid metabolism as possible [6]. This tendency was promoted by the elaboration of simple screening methods including a variety of chromatographic procedures from dried blood or urine spots [1, 7, 13, 14, 15, 16].

In accordance with the international trend, we also introduced a regional neonatal amino acid screening; its results are presented here.

Material and Methods

From January 1, 1974, to April 30, 1984, capillary blood samples dried on filter paper were collected from the liveborn neonates in the 5 obstetrical units of county Győr-Sopron (North-West Hungary). In the first two years of the programme urine samples were also obtained, from 1976 only blood spots were examined. The materials were taken on the 5th or 6th day of life, and the filter papers were sent to our laboratory.

Here two-dimensional thin-layer amino acid chromatography was performed according to White [16]. When the result was equivocal, the procedure was repeated from another disk of the original filter paper. If the finding was still uncertain or pathological, the infant was called to our department where fresh blood and urine samples were taken, the child was thoroughly examined, and hospitalized, if necessary. In these cases quantitative amino acid concentrations were determined with a Beckman Multichrom 4225 column analyser.

From January 1, 1975, parallel blood samples were analysed in the frame of the nationwide screening for PKU by means of Guthrie's bacterial inhibition test.

Results

A total of 70,328 newborn infants were screened. This covered 95.5% of all the liveborn neonates of the region in the given period.

In 1158 cases, i.e. in 1.65%, the chromatography had to be repeated, mainly because of insufficient separation of the individual amino acid spots. Only 79 infants, i.e. 0.11% of the whole material, had to be recalled for further investigation.

In 11 infants, some inborn error of amino acid metabolism could be verified with the following distribution:

PKU	6
Histidinaemia	1
Ketotic hyperglycinaemia (propionic acidaemia)	1
Cystinuria	3

Discussion

In the first years of our screening programme this organisation proved to be undoubtedly useful. It contributed to the high efficiency of the nationwide PKU + galactosaemia screening, and obviously helped to prepare the introduction of regional hypothyroidism screening in Hungary [10].

Having gathered more experience, it became evident that mass screening for all amino acid disorders was not rentable. In contrast to several surveys applying different methods, no aminoacidopathies other than PKU were discovered in the chromatographic screening of 40,454 neonates by Giovannini et al [9], whose organization and methods were almost identical with our ones.

In the roughly 70,000 babies of our study, 5 non-PKU cases were found. Three of them were cystinurias detected in the first two years of our programme, when urine specimens were also analysed. These would have been overlooked later when only blood spots were examined. The only patient with histidinaemia needed no therapy, the early diagnosis caused rather unnecessary anxiety in his family. The girl infant with hyperglycinaemia was a seriously ill neonate with vomiting and metabolic acidosis. On the basis of her grave symptoms this child would certainly have undergone more detailed examinations, and her disease could have been diagnosed even without chromatographic screening. All the 6 cases of classical PKU were also discovered by the centralized screening service; the diagnosis made a few days earlier in our local laboratory did not mean a significant advantage in treatment and prognosis.

Our results are in agreement with the well-established international recommendations according to which the only enzymopathies screened for on a population-wide basis should be congenital hypothyroidism, hyperphenylalaninaemia, galactosaemia, and maple syrup urine disease [3, 4, 5, 8, 10]. Since the screening of the first three conditions has been solved on a nation-wide basis in Hungary, and maple syrup urine disease seems to be as rare as about 1 : 150,000 to 1 : 200,000 [2, 4, 12], we have stopped our regional chromatographic screening programme on April 30, 1984.

Acknowledgement

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Coincidence of paternal 13pYq translocation and maternal increased 13p NOR activity in a child with arthrogryposis and other malformations

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Cytogenetic examination of a boy with congenital multiple arthrogryposis, VSD and dysmorphic facies revealed a probable t(Y; 13)(q?; p1) translocation and three NOR-positive dots on one of the chromosomes 13. The latter variant could be followed in the family of the mother, the 13/Y translocation was found in the relatives of the father. Since all the family members affected by one or the other cytogenetic anomaly were healthy, the abnormal phenotype of the propositus was interpreted as coincidence by chance.

Translocations of a Y chromosome to an autosome are rare, and involve most frequently a genetically inactive part of the Y chromosome translocated to chromosomes 15 or 22. Here we report a familial 13pYq translocation combined with a peculiar NOR variant of chromosome 13.

REPORT OF A CASE

K. G., a male infant was born at term weighing 3550 g after an uneventful pregnancy. At birth club foot, stiff joints, and a systolic murmur were recorded, and congenital multiple arthrogryposis and VSD were diagnosed.

At 8 months of age he was referred to our department because of failure to thrive and increased susceptibility to infections.

At admission he weighed 4220 g, his length was 58 cm, with a head circumference of 40 cm. He gave the impression of severe physical and mental retardation. His main symptoms were, a broad nasal bridge, epicanthic folds, strabism with exophthalmus on the right side and optic coloboma, high arched palate, large ears (Fig. 1).

Both arms and the right lower extremity were stiff with extension contractures of the elbows and the right knee, and with flexion of the hands and the right foot (Fig. 2). The testicles were not palpable. Cardiology revealed a ventricular septal defect. No characteristic bone anomalies were seen at radiologic examination. Blood and urine chemistry proved to be normal.

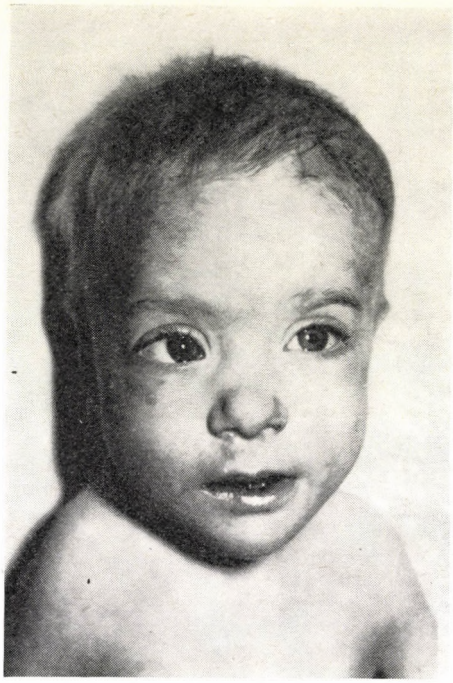

FIG. 1. The face of the propositus

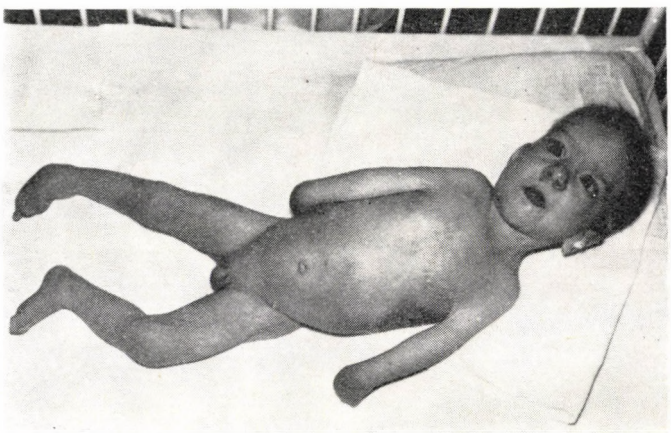

FIG. 2. Arthrogryposis and dystrophy of the patient

Cytogenetic analysis

In routine G-banded karyotypes of the proband the chromosome number was consistently 46, but one of the chromosomes 13 had longer short arms in each of the examined mitoses (Fig. 3). With Q- and C-banding the plus material on 13p proved to be positive with the intensity of Yq

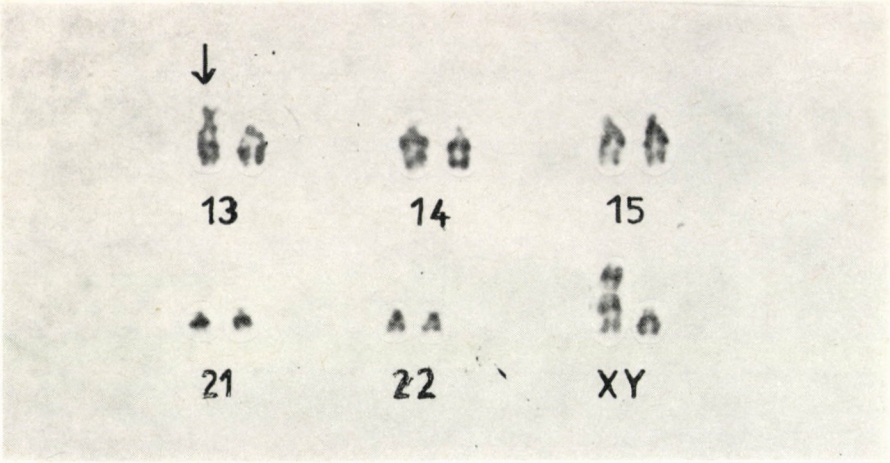

FIG. 3. Partial karyotype of the patient showing and extra material on 13p

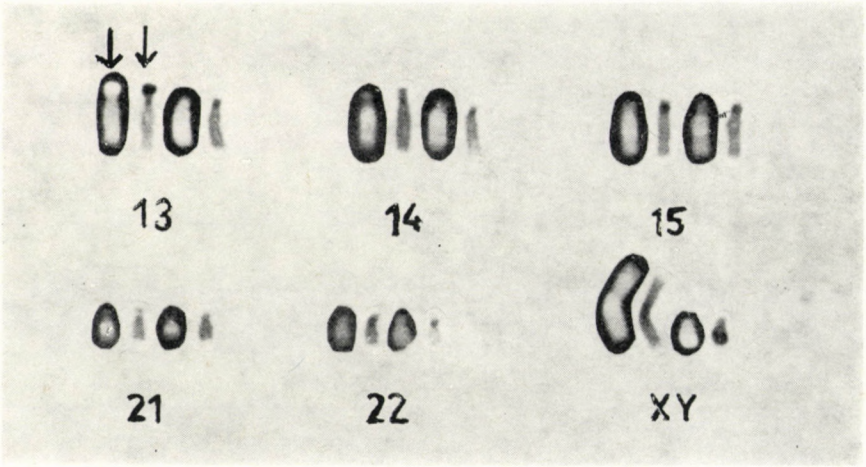

FIG. 4. Sequential Q and C-banding of the same chromosome

(Fig. 4). Since in 23% of interphase lymphocytes two fluorescent Y-bodies were found (Fig. 5), the anomaly was interpreted as t(Y;13) (q?p1) translocation. In NOR preparations a further peculiarity was noted: one of the chromosomes 13 had three darkly stained dots in each of the mitoses.

Family investigations

The pedigree is demonstrated in Fig. 6. As shown by the symbols, the lymphocyte cultures of 15 family members in three generations were analysed by means of G, C and Q bandings and NOR techniques. Ex-

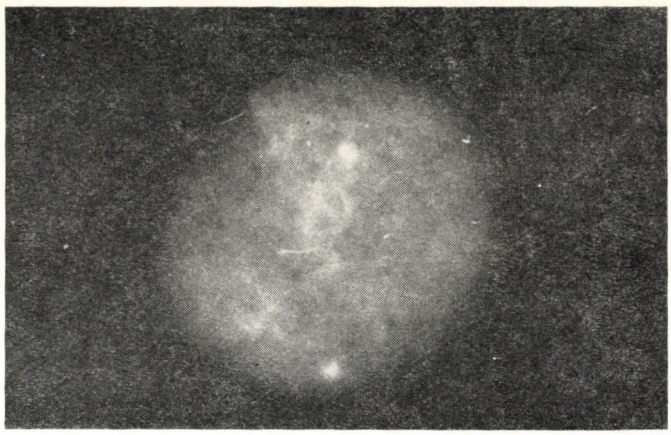

FIG. 5. Two fluorescent Y-bodies in the interphase lymphocytes

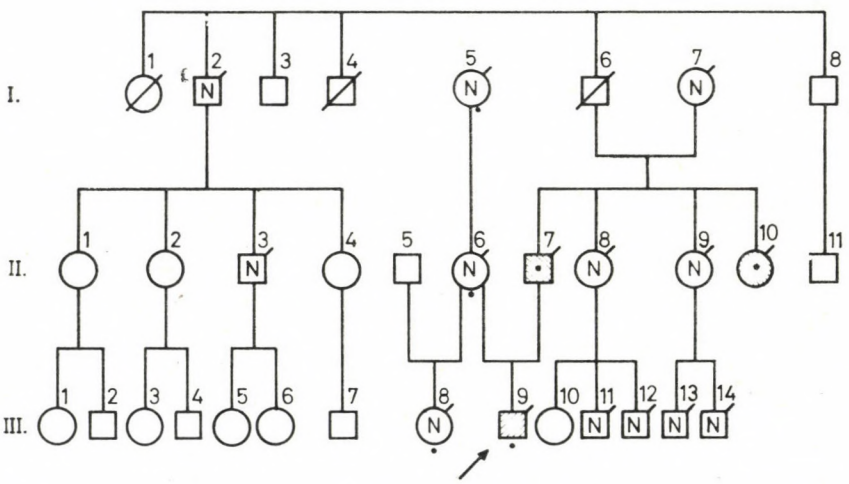

FIG. 6. Pedigree of the family. Symbols: personally examined, cytogenetically tested; ♂ □ subjects with 13pYq translocation; ⊙ family members with three NOR positive dots on 13p; N normal karyotype

cept for the proband all the subjects examined were phenotypically normal. The father (II/7) had the same t(Y;13) (q?p1) translocation as his son.

The father's youngest sister (II/10), a healthy 18-year-old girl, had the abnormal 13p+ with one fluorescent Y-body in her interphase lymphocytes. The other paternal relatives had normal karyotypes.

The mother (II/6), her 5-year-old daughter from her first marriage (III/8) and the maternal grandmother (I/5) had a normal karyotype, but in each of the mitoses an increased number of satellite association was noticed (Fig. 7). In the NOR preparations of

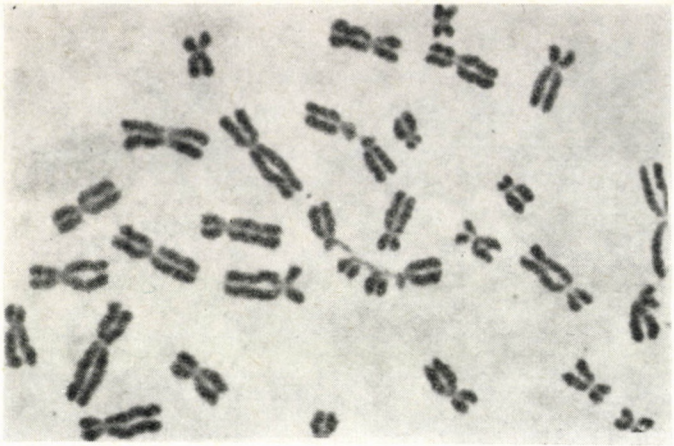

FIG. 7. Increased tendency to satellite-associations in the mother (II/6)

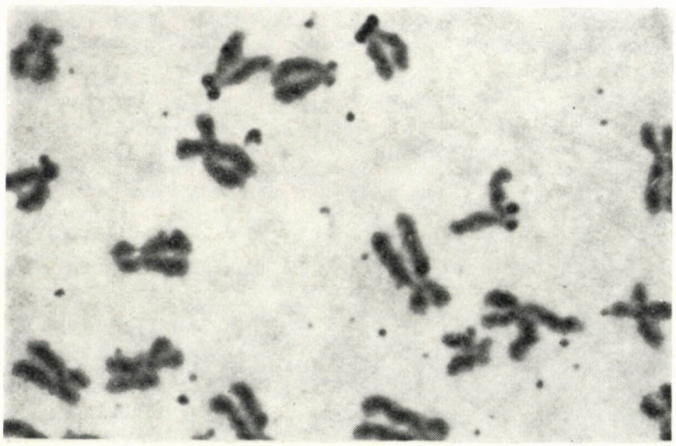

FIG. 8. Three NOR positive dots on one of chromosomes 13

these subjects the conspicuous three dots on one of the chromosomes 13 was observed (Fig. 8).

DISCUSSION

Investigation of the family could have been more reliable with the application of distamycin A-DAPI banding for distinction between Yq and possible extra large satellites [3], with determination of H-Y antigen for estimation of genetic activity of the presumed extra Y material, and with in situ r-RNA-DNA hibridization in order to clarify the nature of the peculiar three NOR dots on chromosome 13. Since, however, in the majority of the presumptive Y/autosome translocations called in question retrospectively [1, 4] the

autosomes involved were Nos 15 and 22, it was unequivocally No. 13 in our family. Since the carrier father and aunt were somatically and psychologically normal, we believe that they had a duplication of a genetically inactive part of the Y chromosome translocated to chromosome 13. This would mean that the symptoms of the proband had nothing to do with the 13/Y translocation.

We have no explanation for the familial NOR variant on the maternal side but, considering the observations of Schwarzacher et al [2], one may reckon with an unusually increased NOR-activity in this region. The phenomenon may be interpreted as a probably rare population variant, which has certainly no phenotypic consequences. However, the coincidence of two presumably harmless inherited anomalies of chromosome 13 in a severely malformed child seems at least curious.

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Ring chromosome 4 : Wolf syndrome and unspecific developmental anomalies

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A new case of ring chromosome 4 in a 18-month-old girl is described. The patient presented extreme growth failure, psychomotor retardation, and some features of 4p deletion or Wolf syndrome. No significant loss of genetic material could be seen by G-banding technique (breakpoints p16q35). The ring was found to be unstable both in lymphocyte and fibroblast culture and a substantial proportion of aneuploid cells containing derivatives of the ring could be observed.

An increased cell death-rate could be detected by cell viability determination with trypan blue in the first subculture of skin fibroblasts. It is suggested that this finding is a consequence of behavioural instability of the ring at mitosis existing probably in vivo as well.

The clinical and cytogenetic findings in this patient were compared with those in the other 16 cases with ring 4 published so far. It is suggested that the phenotype in patients with this chromosomal anomaly is a mixture of phenotypic abnormalities generated by both the chromosomal deletion prior to ring formation (features of Wolf syndrome) and the behavioural instability of the ring at mitosis (unspecific developmental anomalies).

A ring chromosome is generally believed to be the result of breakage in each of the terminal segments of a chromosome, the broken ends joining together to give a continuous ring. This mechanism presumes the loss of some genetic material from the end segments of a chromosome, leading to monosomy for the short and/or long arms.

Ring formation of chromosome 4 has been observed up to now in at least 16 cases [1, 2, 4, 5, 7—11, 13, 16—19, 21, 22]. The phenotypic abnormalities in some of these cases formed a clinical picture which resembled the 4p- or Wolf syndrome. Other patients had only a few features of Wolf syndrome, moreover, patients were reported who had practically ne features of the syndrome. Lack of uniformity of the phenotype changes in different cases may be caused by variations in breakpoints and the amount of deleted genetic material lost during ring formation. Since it is difficult precisely to locate the breakpoints in a ring chromosome, attempts to correlate the clinical picture to deletion of specific segments have been made only in 7 out of the 16 cases. Analysis of the clinical and cytogenetic data of these cases suggests that a phenotype resembling Wolf syndrome is present when the distal band of the short arm of

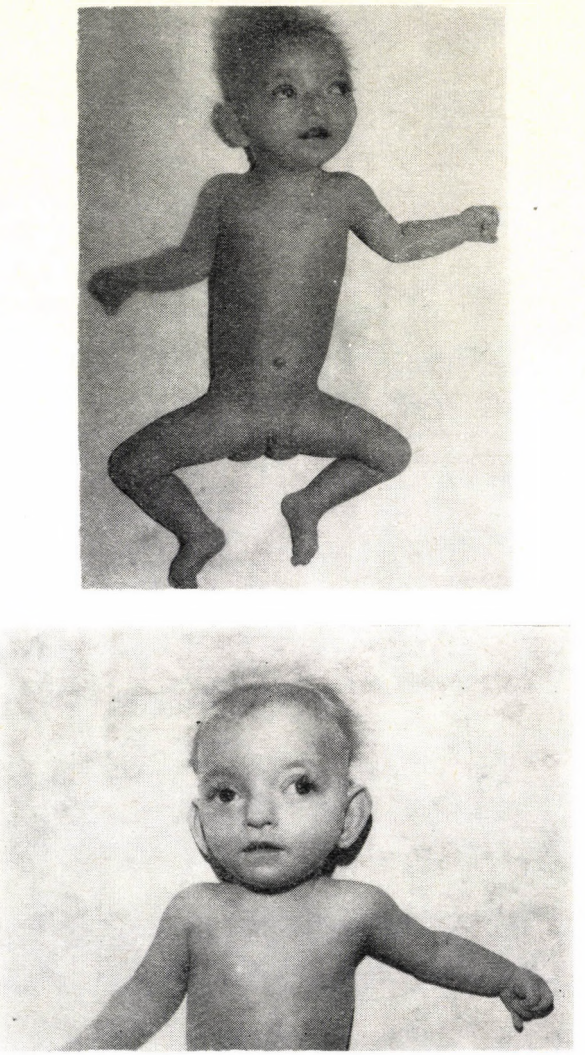

FIG. 1. The proposita at 15 months of age

chromosome 4 (band p16) is lost [8, 10].

This report describes a new case of ring chromosome 4 and summarizes the 17 cases (including the present one) with this anomaly published so far. A new approach is also described which was planned to throw some light upon the non-specific developmental anomalies usually present in cases with ring chromosome.

CASE REPORT

The proposita was the first-born child of unrelated healthy parents. The mother was 20 and the father

25 years of age at the time of the birth. The patient was born after an uneventful pregnancy of 35 weeks. Her birthweight was 1240 g. During infancy she was seen several times in a local hospital (poor feeding, vomiting, loose stools, respiratory tract infections, febrile convulsion). She was sent to us for diagnostic investigation at the age of 12 months because of severe unexplained failure to gain weight. Physical examination revealed an infant with proportionate somatic retardation (weight 2780 g, height 53 cm, head circumference 32 cm). There were only few dysmorphic stigmata (Fig. 1): down-turned mouth, beaked nose, relatively simple large ears, and protruding big eyes with divergent strabismus. Psychomotor development was severely retarded (developmental age was calculated to be 1 month). There were no clinical abnormalities in the cardiovascular, respiratory, urinary and alimentary systems. Laboratory data were all within normal limits (growth hormone was not determined). Radiographs of skull, chest, abdomen, and long bones were normal. The bone age was estimated to be 3 months. In spite of great efforts, her weight reached only 3400 g by the age of 17 months (at this age height was 56 cm, head circumference 33 cm). She died of pneumonia at the age of 18 months. Autopsy showed no abnormalities of the internal organs except a small atrial septal defect.

Cytogenetics

Cytogenetic studies from peripheral blood culture (conventional 72-h culture) are shown in Table I. Of the cells, 87.6% were diploid and contained one ring, its size being more or less constant. G-band analysis showed that the ring consisted of an apparently complete chromosome 4, without clear evidence of deletion prior to formation of the ring (Fig. 2). Breakpoints were considered to be in or distal to the terminal bands of the short arm (p) and the long arm (q). Thus, the patient's karyotype was 46,XX,r(4)(p16q35). Of the cells, 3.6% showed monosomy 4, without ring. In 8.8 per-

TABLE I

Distribution of cells with various chromosome counts and derivatives of ring chromosome 4 in lymphocyte culture

Karyotype	No. of cells	per cent
45, XX, -4	6	3.6
46, XX, r(4)	148	87.6
46, XX, dic r(4)	8	4.7
47, XX, two r(4)	3	1.7
two interlocked rings	2	1.2
pulverized ring	2	1.2
total	169	100

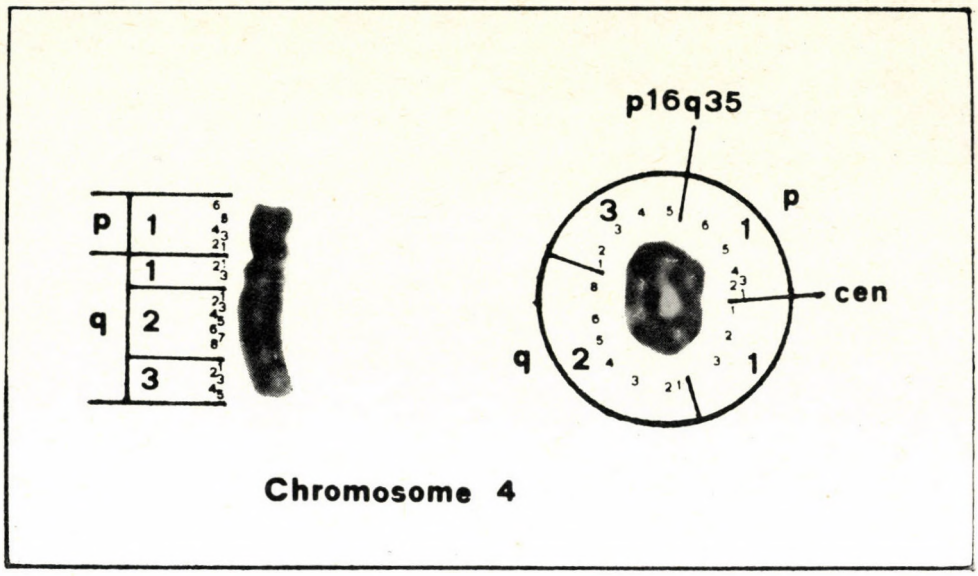

FIG. 2. Ring chromosome 4 and its homologue. *cen* indicates centromere. Ring is joined at p16q35

cent of the cells analysed various configurations of the ring chromosome could be found including dicentric rings of various sizes, interlocked dicentric rings, two rings, and pulverization of the ring. Micronuclei and nuclear projections were present at a frequency of 3.6% in lymphocyte culture (as compared to 0.18% in a control culture).

The karyotypes of the parents were normal.

A fibroblast culture was initiated from a skin specimen taken one hour after death and maintained in RPMI 1640 medium (GIBCO) with 20% fetal calf serum. Cultured fibroblasts were harvested for chromosome analysis after the first subculture. Six of the 29 cells analysed were aneuploid because of various ring derivatives.

Cell viability (in vitro experiment)

It was noted that the fibroblast culture grew poorly compared to normal ones. In an attempt to clarify the cause of poor growth, cell viability was determined. Cells of first subcultures from the patient and a matched normal control were stained in situ with 0.5% trypan blue and the rate of unstained (living) cells was determined by counting at least 500 cells in each subculture.

Cell viability of the patient was 72%, while that of the control was 93%. (Standard viability in the author's laboratory is 92 $\pm$ 5% in control cultures.) This result suggested an increased cell-death in the first subculture of our patient compared to controls.

Discussion

The main clinical symptoms of our patient were a very severe growth failure with proportionate somatic retardation and a profound psychomotor retardation. Apart from these non-specific features she had only few dysmorphic stigmata which belonged to the features of the 4p- or Wolf syndrome. Out of the 7 cases with ring chromosome 4 in whom the breakpoints are known (Table II), the patients described by Niss and Passarge [17], McDermott et al [16], del-Mazo et al [8], and Finley et al [10] showed few signs of the 4p deletion syndrome, while the patient of Chavin-Colvin et al [5] had practically none of the main features. All these cases including the present one had no obvious loss of terminal band on the short arm (p16) of chromosome 4. The patients of Perez-Castillo and Abrisqueta [19] and Friasse et al [11] in whom the entire p16 band (breakpoints in band p15) was missing, showed many of the features of Wolf syndrome. Only one case [5] had a detectable deletion on the long arm of chromosome 4. Since this patient showed no specific dysmorphic features, the role of the missing q34-35 terminal regions is difficult to determine.

These observations suggested that the terminal region of the short arm of chromosome 4 (p16) is significant in the final manifestation of Wolf syndrome. The fact that some features of the syndrome could be seen even in cases with no obvious loss of band p16 may be explained that even with high-resolution banding technique it is not possible to exclude the loss of some chromosomal material.

Many authors agree that at least some abnormalities of the phenotype in cases with ring chromosome are due to the specific behaviour of the ring in mitotic anaphase. Without sister chromatid exchanges (SCE) ring chromosomes can behave normally as long as the two chromatids can separate freely. However, ring chromosomes are usually subject to various difficulties at mitosis as a result of the normal occurrence of SCE. The behavioural instability of rings is reflected in the number of various ring configurations and of aneuploid cells, promoted by the peculiar ring mechanics. These cells are less likely to survive [3] and a certain proportion of cells with ring chromosome will presumably be lost at subsequent cell divisions.

In our patient, a high proportion of the cells with abnormal ring configuration was found in both lymphocyte and fibroblast cultures. In addition, we observed an increased cell death-rate in the first subculture of fibroblasts as compared to controls. This observation suggests that cells containing these abnormal configurations do not survive in vivo, and their elimination at subsequent divisions is likely to end with a reduction in the total number of viable cells. This assumption was proposed by Kjessler et al [15], Cote et al [6], and Jansen et al [14] as well studying cases with ring chromosome 1, 2, and 2, respec-

TABLE II

Comparison of physical features and chromosomal breakpoints

	Carter et al.	Dallaire	Hecht	Faed et al.	Bobrow et al.	Surana et al.	Bofinger et al.
Sex	♂	♀	♂	♂	♂	♀	♂
Age		16y			4y	5y2m	
Early death	+			+			+
Low birth weight	+	++	+	+++	++	+	++
Growth failure		+	+		+++	++	
Mental retardation		−	+		+	−	
Retarded bone age					+	+	
Beaked/broad nose							+
Down-turned mouth							+
Large simple ears							+
Short philtrum							+
Hypertelorism					+		
Abnormal thumbs and/or radii	+	+		+			+
Cleft lip/palate	+						+
Heart malformation	+		+				+
Sacrococcal dimple					+		
Hypospadias	+						+
Ring unstable	?	?	?	?	+	+	?
Breakpoints							

tively. Cote et al [6] found that these abnormal products were found after two or more cell cycles but not after one cycle in lymphocyte culture, suggesting that these anomalies do not survive in vivo. We think that our results with the cell viability testing in fibroblast culture have given further support to this assumption and are in favour of the opinion that aneuploid cells found in cases with ring chromosome are derived *de novo* in each instance and not perpetuated in a clonal manner proposed by the majority of authors observing "mosaicism" in cases with ring chromosome.

Concerning the extreme somatic retardation and severe growth failure, we are of the opinion that these symptoms are direct consequences of the persistent generation of cells with various aberrant configurations induced by ring mechanics. This process, followed by the elimination of aneuploid cells in vivo, will result in the long run in a reduction of the total number of viable cells expected for a given interval of proliferation and is

in 17 patients known to have ring chromosome 4

Parker et al.	Niss, Passarge	Perez-C., Abrisqueta	Mc-Dermott et al.	Fraisse et al.	Chavin-C. et al.	del Mazo et al.	Young, Zalneraitis	Finley et al.	Present case
♀	♂	♂	♂	♂	♀	♀	♀	♂	♀
9y	11y		4y	8y	5y		6y	2m	1y2m
		+				+			
+	+	++	++	+	++	++		+	+++
++	+	+	++	+++	++			+	+++
+	+		+	+	?		+	+	+
+			+	+	+				+
				+	+			+	+
		+		+					+
+		+		+		+			+
+				+	+				
		+				+			
+	+		+	+		+			
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				+					+
+			+	+					
	+			+				+	
?	+	+	+	+	+	+	?	+	+
	p16 q35	p15 q35	p16 q35	p15 q35	p16 q33	p16 q35		p16 q35	p16 q35

thought to account for the marked reduction of body mass in these patients. Extreme somatic retardation was found in cases with ring chromosome 4 both with and without features of Wolf syndrome (see Table II) indicating that this degree of growth failure is less related to the deletion of genetic material than to the specific behaviour of the ring during cell division.

Microcephaly is usually mentioned in the reports of cases with ring chromosome 4 as a dysmorphic stigma. In relation to their body size, however, the patients cannot be considered microcephalic. The head circumference found to be small on percentile graph is simply a manifestation of the proportional somatic retardation. Hence, "microcephaly" cannot be regarded as a distinct feature in cases with ring chromosome.

Analysing other features of the 17 cases with ring chromosome 4 (Table II), abnormal thumbs and/or radii have been observed in more than half of the patients. Although Haspes-

lagh et al [12] have recently reported on limb malformations in a case with 4p deletion, the other known cases with this chromosomal defect did not show abnormal thumbs and/or radii, and these malformations do not belong to the features of 4q deletion, either [20]. Thus, it is not unrealistic to assume that this limb defect, too, is caused primarily by ring mechanics, possibly reducing the number of viable cells at a critical point of morphogenesis rather than by terminal deletion of chromosome 4. That these features are less specific to chromosome 4 than to the ring per se, can be inferred from the fact that they occur also in cases with ring formation of other chromosomes.

Conclusion

Our observations and analysis of the clinical and cytogenetic findings in the 17 known cases with ring chromosome 4 suggest that the clinical picture consists of a mixture of anomalies originated from both the chromosomal deletion prior to ring formation (it is frequently undetectable even with fine techniques) and the behavioural instability of the ring chromosome. The former brings about symptoms of 4p deletion or Wolf syndrome depending on the lost amount of genetic material, while the latter generates unspecific anomalies like growth failure (occasionally very extreme) including "microcephaly", developmental delay, and supposedly some other developmental anomalies (perhaps limb defects).

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Book reviews

ODED BAR-OR: *Pediatric Sports Medicine for the Practitioner, from Physiologic Principles to Clinical Applications.* XXII + 376 pages with 124 figures. Springer Verlag, Berlin—Heidelberg—New York—Tokyo 1983. Price DM 98.—

This book of the series Comprehensive Manuals in Pediatrics has been written for the practising paediatrician and may be useful for all paediatric subspecialities.

The first chapter deals with the physiology of exercise. The principles are summarized in a concise and conceivable form, all new data are offered. The following chapter is dedicated to clinical aspects, and the benefits and consequences of physical load are described here. In the third chapter exercise of children affected by respiratory diseases, especially by asthma, is discussed together with all aspects of exercise-induced bronchoconstriction. Practically every open question and important practical principle is dealt with. In the chapter of heart diseases, concise facts and practical guidelines can be found. The endocrinological section illustrates the many problems connected with exercise of diabetics and several data hardly known by doctors treating children with diabetes are presented. In the chapter on nutritional diseases, anorexia nervosa, malnutrition and obesity are also discussed. Special interest is paid to epilepsy and haemophilia in the chapters on neurology and haematology, respectively, and again excellent practical guide-lines are offered. A separate chapter is devoted to adaptation of children to extreme climatic conditions. The quantification of physical load is illustrated with very useful tables of normal values. The loading tests are described in detail and their essential features are underlined. This chapter makes the book indispensable for experts performing physical loading in children.

This concise book offers a theoretical basis for every issue and excellent practical knowledge on exercise. Paediatricians will find that it fills a gap: up to now, almost all textbooks have neglected this aspect of child health.

J KELEMEN

U-F HABENICHT, F NEUMANN: *Hormonal Regulation of Testicular Descent.* VI + 55 pages with 39 figures. Springer Verlag, Berlin, Heidelberg, New York, Tokyo 1983. Price DM 48.—

This book represents Volume 81 of the series Advances in Anatomy, Embryology and Cell Biology.

The first part deals with development of the male genital organs. The LH induced androgen effect plays a decisive role in the persistence and differentiation of the Wolffian tube. In mammals this process is androgen-dependent while regression of the Müllerian tube is independent from the male hormone. Jost produced convincing evidence that the so-called x-factor or anti-Müllerian hormone (AMH) produced by the fetal testicle was responsible

for regression of the Müllerian tube. Activation of AMH sets in before and the appearance of Leydig cells, and it has been shown that the site of AMP production is the Sertoli cell.

The next chapters are devoted to testicular development and descent. Experiments with antiandrogens have shown that testicular descent is an androgen-dependent process: this has been further confirmed by the observation that treatment of testicular maldescent with human chorionic gonadotropine (HCG) or luteinising hormone releasing hormone (LH-RH) may be successful. From the clinical point of view it is noteworthy that 70% of all maldescended testicles are actually retractile, spontaneously descending to the scrotum during puberty, and only 30% are truly maldescended. According to Scorer, ectopy occurs in 1% of all cases of testicular maldescent, obstruction in 23%, inguinal position in 19%, high scrotal position in 49%; the testicles are abdominal or congenitally absent in 9%. Spontaneous descent can be expected until 9 months of age with the notable exception of retractile testicles. The inhibitory effect on testicular descent of the antiandrogen compound cyproterone acetate (CPA) and large doses of ethinyl oestradiol (EE) is discussed in detail. Wistar rats were treated with EE, CPA, or EE plus CPA during the 17—20th days of pregnancy: the offspring were killed immediately or 70 days after birth and subjected to thorough gross and microscopic examination. It turned out that inhibition of testicular descent was associated with suppression of the gubernacula. The most striking inhibitory effect was exerted by EE plus CPA, a strong but less marked effect by EE alone, but CPA alone also induced a weak but demonstrable action. Differentiation of the ligament system was undisturbed in all treated groups. Testicular descent was tardy in all groups and normal definite descent occurred only in the group treated with CPA alone.

L Barta

Endokrinologie des Kindes- und Jugendalters. Herausgegeben von V Hesse. 460 Seiten mit 162 Abbildungen und 78 Tabellen. Georg Thieme Verlag, Leipzig 1982. M 79.—

Der Herausgeber dieses Buches hat sich bemüht, das progressive Gebiet der pädiatrischen Endokrinologie in einer wissenschaftlich fundierten, doch praxisnahen Form darzulegen. In den unter Mitarbeit von zehn Fachwissenschaftlern verfaßten Kapiteln werden die endokrinen Organe und Organsysteme eingehend besprochen. In den entsprechenden Kapiteln werden stets die Embryologie, Anatomie, Physiologie und Biochemie erörtert und eine symptomorientierte Klassifikation angegeben. Die ausgezeichneten Kapitel über die Wachstumsstörungen und Hypoglykämien sind besonders hervorzuheben. Ein gesondertes Kapitel befaßt sich mit den genetischen Aspekten der endokrinen Erkrankungen, ein anderes mit der Hormontherapie nicht endokriner Krankheiten. Ein ganz ausführliches Kapitel ist der endokrinologischen Funktionsdiagnostik gewidmet, wo die verschiedenen Untersuchungsverfahren und deren Indikation, Kontraindikationen und Nebenwirkungen geschildert werden. Hormonpräparate sind in einem eigenen Kapitel zusammengefaßt und schließlich in einer tabellarischen Zusammenstellung die Normwerte angegeben. Man vermißt jedoch einige Themen, wie z. B. die parakrine Sekretion oder Gewebshormone bzw. die hormonartig wirkenden Enzyme und Mediatoren, oder aber die ektopische Hormonbildung.

Die schematischen Abbildungen sind weitgehend informativ, und die Bilder sind von guter Qualität. Nach jedem Kapitel ist ein reiches weiterführendes Literaturverzeichnis zu finden, leider dürfte aber zwischen der Fertigstellung des Manuskriptes und dem Erscheinen des Buches einige Zeit vergangen sein: es ist eine Ausnahme, eine Referenz aus 1980 zu finden.

E Cserháti
Anna Körner

Lehrbuch der Logopädie. Redigiert von K-P Becker und M Sovák. 333 Seiten mit 30 Abbildungen und 20 Tabellen. Volk und Gesundheit, Berlin 1983. 3. erweiterte Auflage. Preis M 36.—

Derjenige, der in diesem Buche einen praktischen Wegweiser, Beschreibungen logopädischer Therapien und Methoden sucht, wird sich täuschen. Solche Kenntnisse soll er vielmehr in dem kürzlich erschienen Buch von K P Becker und Mitarbeiter: Rehabilitative Spracherziehung, Volk und Gesundheit, Berlin 1983 suchen. Das jetzt zu besprechende Lehrbuch hat sich zum Ziel gesetzt, die Stellung der Logopädie unter den Wissenschaften genau zu definieren und die Sprachstörungen systematisch einzuordnen.

Dieses ausgezeichnete Buch erlebt jetzt seine 3. Auflage. Seit der ersten haben die Autoren ihre Auffassung über den Begriff der Sprachheilkunde weiterentwickelt; laut ihrem Standpunkt ist sie eine medizinisch-pädagogische Disziplin, deren pädagogischer Teil durch die Logopädie dargestellt wird, die zugleich ein Teilgebiet der Rehabilitationspädagogik bildet. Obwohl die Bedeutung der medizinischen Verbindungen von den Autoren mehrmals betont wird, bestreiten sie die Ansichten, daß das sich mit den Lautbildungs- und Sprachstörungen beschäftigende ärztliche Gebiet, die Foniatrie, die Gesamtheit der Sprachtherapie umfaßt. Nach ihrer Konzeption — womit doch der Rezensent nicht einverstanden ist — wären alle Arten der Sprachstörungen nur eine ihrer Untergruppen.

Das Buch gibt eine ausgezeichnete Übersicht über die Sprachentwicklung während der Ontogenese, die geschichtlichen und phonetischen Grundkenntnissen, die vielen Beziehungen der geschriebenen Sprache (eines der besten Kapitel im Buche!) und die speziellen Kommunikationsmöglichkeiten und -Mittel der Hörgeschädigten, und die Zusammenhänge der Zeichensprache und der Blindschrift.

Die Sprachstörungen werden in zwei Gruppen geteilt: die Abnormalitäten der Sprachbildung (verzögerte Sprachbildung, Alalie, Dyslalie, Agrammatismus) und die spät erscheinenden erworbenen Fehler. Die Letzteren werden unterteilt in nach impressiven Schädigungen auftretenden Störungen, zentrale Störungen (Agnosie, Dyslexie, Apraxie, Aphasie, infolge von neuropsychiatrischen Erkrankungen auftretende Störungen), expressive Fehler (Dysarthrie, Rhinolalie, Lautbildungsstörungen), reaktive und Sprachneurosen (Mutismus, Stottern usw.).

Das Buch enthält eine außerordentliche Fülle von Kenntnissen; manche der vielen Konzeptionen und Formulierungen sind jedoch schwer verfolgbar, obwohl die Orientierung durch die dezimale Einteilung etwas erleichtert wird. Die Arbeit wurde in erster Reihe dem Heilpädagogen geschrieben, es darf jedoch auf das Interesse von Ärzten, hauptsächlich Pädiater, Neurologen und Otorhinolaryngologen, und Psychiater rechnen.

J. Hirschberg

Diagnostik intrakranieller Blutungen beim Neugeborenen. Herausgegeben von U Haller, L Wille. XII + 152 Seiten mit 102 Abbildungen. Springer Verlag, Berlin, Heidelberg, New York, Tokyo 1983. Preis DM 79.—

Die Monographie enthält das Diskussionsmaterial eines in St. Gallen veranstalteten interdisziplinären Symposiums über die modernen diagnostischen Möglichkeiten.

Das erste Kapitel bespricht die intrakraniellen Blutungen aus der Sicht des Neonatologen. Seit der Einführung der zeitgemäßen kontrollierten Beatmung ist die subependymale-intraventrikuläre Blutung das Zentralproblem geworden, deren Häufigkeit mit der Gestationszeit in umgekehrtem Verhältnis steht und bei Frühgeborenen unter 1500 g Geburtsgewicht eine extrem hohe Letalität bewirkt.

In mehreren Beiträgen wird die normale und pathologische intrakranielle sonogra-

phische Anatomie erörtert und werden alle Möglichkeiten der Sonographie analysiert. Im Frühstadium ist die Blutung echodens, später von der Richtung des pathologischen Prozesses abhängig als hypodenses Bild in der Umgebung des Ventrikels zu sehen. Die frühzeitige Ermittlung und Kontrolle haben die Bestimmung einiger neuen klinischen Kategorien der Risikofälle ermöglicht.

Mehrere Vorträge befassen sich mit der zweidimensionalen Enzephalographie im Vergleich zur Computertomographie. Vorteile und Grenzen werden sorgfältig erwogen. Die Computertomographie bietet einen genaueren Überblick über das ganze Gebiet und ist vom Alter unabhängig, doch an der Intensivstation, bei kontinuierliche Monitorbeachtung oder maschinelle Beatmung beanspruchenden schweren Fällen bedeutet das Transport eine weitere Gefährdung, zu der die Strahlenbelastung noch hinzukommt. Die Sonographie kann hingegen auch unter den obigen Verhältnissen schnell, ohne Belastung und Sedation auch bei Frühgeborenen vorgenommen und wiederholt werden; das Säure-Basengleichgewicht und pO_2-Wert erleiden dabei keine Änderung, wie das bei anderen Manipulationen zu vermerken ist. Auch kann die Sonographie von einer einzigen Person durchgeführt werden, hängt aber von der Geschicklichkeit und Erfahrung dieser Person ab und muß so als eine ziemlich subjektive Untersuchung betrachtet werden. An der anderen Seite bietet das Verfahren keine entsprechende Informationen über Hygromen, Hydrocephalus externus, und auch nicht bei okzipitalen interhämispherischen Blutungen und infratentorialen Veränderungen.

Die Monographie kann auf das Interesse von Pädiater, Neonatologen, Geburtshelfer und Radiologen rechnen und dürfte sich als ein hervorragender diagnostischer Ratgeber erweisen.

G. Harmat

Trace metals and inherited metabolic disease. Editors: GM Addison, RH Harkness, RJ Pollit. MTP Press Ltd, Lancaster UK 1983. Price £ 19.95

This volume contains Supplements 1 and 2 of Volume 6 (1982) of the Journal of Inherited Metabolic Disease. They consist of selected papers read at the 20th symposium of the Society for the Study of Inborn Errors of Metabolism held in Manchester in September, 1982. The main issue of the meeting was trace elements and inborn errors of metabolism.

The first part of the volume offers a selection of the comprehensive reviews. A very interesting piece is Williams's review on the history of detection of relationships between trace elements and certain inherited disorders of metabolism. He stresses the importance of interactions between metals in the body and genes. Fell sums up the recent analytical methods for measuring serum levels of trace elements (mass spectometry, X-ray emission, neutron activation). The biochemical markers used for quantitative determination of trace elements (metalloenzymes) are also mentioned here. Laurie summarizes the issue of trace element transport and storage, primarily of iron, copper and zinc. He states that in most metabolic disturbances of trace elements, their transport and storage are involved. Unfortunately, knowledge of these aspects is rather scarce, the most reliable and voluminous data are available on iron metabolism. The next chapter deals with the physiology of metallothiones, compounds capable of binding zinc, copper and cadmium.

For the paediatrician a table, composed by Agett, comprising the daily needs of the individual trace elements according to the child's age appears most useful. The importance of repletion during total intravenous feeding is underlined.

A number of papers deals with the impact of zinc, with its role in gene expression and in the pathogenesis of acrodermatitis enteropathica, a consequence of a defect of intestinal absorption of zinc.

The chapters on copper primarily anal-

yse the disorders described by Wilson and Menkes. For both diseases there are several animal models available. Cell cultures from the animals are helpful in elucidating the molecular basis of the disorders. In Menkes's disease even prenatal diagnosis has been made possible, the ^{64}Cu uptake of amnial cells is increased but the degree of this phenomenon is in negative correlation with gestational age, and for this reason false negative results may occur after the eighteenth week of gestation.

From the chapter on haemochromatosis one can learn that its gene lies on the short arm of chromosome 6, next to the HLA locus. Among these patients there is a strikingly frequent occurrence of HLA A3, B14 and B7.

Molybdenum is the topic of the subsequent chapters. The combined defect of sulphite oxidase and xanthine dehydrogenase can be attributed to the absence of the so-called molybdenum cofactor; the most prominent clinical features of this entity are mental retardation, dislocation of the lens, abnormal muscle tone and impaired food uptake.

The second part of the volume contains a selection of short communications of the SSIEM symposium of 1982. An interesting method for differentiation between Wilson's and Menkes's disease has been described by Favier et al: In the latter the Cu/Zn quotient in fibroblasts is higher than 1. The same authors found that malformations of the nervous system occur at higher frequency in newborns whose mother had decreased Zn levels during pregnancy. Hyanek et al observed that the breast-milk of mothers affected by hyperphenylalaninaemia contains four times less phenylalanine than the plasma, these mothers can therefore breastfeed their infants. A case report of Trijbels et al describes a patient in whom lactacidosis was caused by the absence of cytochrome oxidase.

Many aspects of trace element metabolism are discussed in this book and much up-to-date information concerning clinical work and basic research is offered. All chapters are provided with detailed references.

A Arató

Berner Datenbuch der Pädiatrie. Herausgegeben von der Medizinischen Universitäts-Kinderklinik, Inselspital Bern. XVI + 766 Seiten mit 60 Abbildungen und zahlreichen Tabellen. Gustav Fischer Verlag, Stuttgart—New York 1984. Preis DM 34,—

Mit der Herausgabe dieses Taschenbuches werden die an der Berner Kinderklinik angewandten diagnostischen und therapeutischen Richtlinien und Referenzwerte der breiten Öffentlichkeit zugänglich gemacht. Mit einer ansehnlich großen Zahl von Angaben geben die insgesamt 55 Autoren dem klinisch und praktisch tätigen Arzt die Möglichkeit einer schnellen Orientierung für das Vorgehen bei den häufigsten Krankheiten.

Die 23 Kapitel sind didaktisch gut aufgebaut und übersichtlich. Besonders lobenswert sind die Abschnitte über Notfälle und Technik, wo man in Sekunden erfahren kann, was und wie in bestimmten Notfallsituationen gemacht werden soll. Ebenfalls nützlich ist die Beschreibung verschiedener Laboruntersuchungen und Belastungsproben, wo die manchmal umstrittene Beurteilung der Befunde klar definiert wird. Der Inhalt ist sehr reichhaltig: der Leser findet informative Tabellen sowohl über Abmagerungsdiät und perinatale Gerinnungsstörungen wie auch über Kleinwuchs und Hirnödem. Nur die morphologischen Aspekte kommen etwas zu kurz: Mißbildungen und Syndromatologie werden überhaupt nicht erwähnt.

Wie das Vorwort betont, trägt das Buch den Stempel der Kinderklinik Bern, die empfohlenen Richtlinien entsprechen aber den neusten internationalen wissenschaftlichen Kenntnissen und Erwartungen. Nur einige Kleinigkeiten sind etwas zu schweizerisch geworden: Znüni und Zvieri sind nicht jedem Deutschlesenden bekannte Begriffe, und die »Auflösung« mancher

lokalen Handelsnamen (wie z. B. Beba® auf Seite 317) wird nirgendswo angegeben.

Alles in allem, das Berner Datenbuch bietet eine moderne, rasche Nachschlagemöglichkeit und damit eine wertvolle Ergänzung der pädiatrischen Lehr- und Handbücher.

K MÉHES

H MANZKE: *Entwicklungsprognose von Kindern mit perinatalen Risikofaktoren.* XII + 265 Seiten mit 10 Abbildungen und 183 Tabellen. Gustav Fischer Verlag, Stuttgart—New York 1984. DM 98,—

Für den sich mit Frühgeborenen und pathologischen Neugeborenen Befassenden Neonatologen stellt die perinatale Mortalität eine wichtige Kennziffer dar, viel ausschlaggebender ist jedoch die Gestaltung des späteren Schicksals der mit perinatalen Risikofaktoren belasteten Neugeborenen. Hierzu sind eine langzeitige Beobachtungszeit und bis zum sechsten Lebensjahr regelmäßig durchgeführte Untersuchungen erforderlich. Die Bedeutung einer solchen Studie ist zweifaltig: daß sich der Neonatologe mit langsichtig geplanten multidisziplinären Methoden über die Entwicklung der in Neugeborenenalter behandelten Patienten, über eventuelle Schädigungen oder aber die Wirksamkeit der verschiedenen therapeutischen Maßnahmen informieren und ferner darüber überzeugen könne, welche der Methoden die Zeitprobe nicht bestanden hat. Diese Fragen werden von Professor Manzke aus der prospektiven Untersuchungsstudie klar beantwortet.

Der Autor und seine Mitarbeiter haben 1783 Neugeborene untersucht. Die aus mit den wichtigsten perinatalen schädigenden Prozessen einhergehenden Geburten stammenden Patienten wurden bis zum 6. Lebensjahr regelmäßig untersucht. Die Nachuntersuchungen erstreckten sich auf die somatographischen Angaben, auf die neurologische, psychische und motorische Entwicklung, bei denen bekannte deutsche und amerikanische Teste angewandt wurden. Bei der Mehrzahl der Patienten kam es auch zu elektroencephalographischen, otologischen und ophthalmologischen Untersuchungen. Die Patienten gehörten zu den in der perinatalen Pathologie häufig vorkommenden folgenden Gruppen: Steißlage, operative Entbindung (Kaiserschnitt, Zangenentbindung), asphyxische Neugeborene, Frühgeborene, pränatal dystrophisch Geborene, nach mütterlicher Gestose Geborene, Kinder von rauchenden Müttern, bei Störungen der Plazentareife, Postmaturität, Kinder von diabetischen Müttern, übergewichtige Neugeborene, Hyperbilirubinämie. Hier sei bemerkt, daß man eine kleinere Gruppe, die der eine intrauterine Infektion erlittenen Kinder vermißt. Bei jedem Fall findet man die mütterliche Anamnese, die obstetrischen Angaben, Komplikationen.

Die Resultate wurden aufgrund der meistens im 1., 3. und 6. Jahr vorgenommenen Nachuntersuchungen analysiert, didaktisch zusammengefaßt und mit den entsprechenden Literaturdaten unterstützt.

Das große Verdienst der Arbeit besteht darin, daß es sich um prospektive Entwicklungsuntersuchungen handelt. Es wird auch bewiesen, daß durch eine verbesserte perinatale Versorgung nicht nur die Mortalität, sondern auch die Zahl der Zerebralparesen und geistig-neurologisch Geschädigten, ferner die Schädigungen der Sinnesorgane herabgesetzt werden kann. Der Nachteil einer solchen gründlichen und langfristigen Studie besteht wiederum darin, daß die Ergebnisse nur lange nach dem perinatalen Ereignis in Erscheinung treten. Dies sollte aber die Anregung zu ähnlichen musterhaft durchgeführten kontinuierlichen Kontrolluntersuchungen keinesfalls beeinträchtigen. Hervorzuheben seien noch die im Anhang auffindbaren, gut geplanten 17 Tabellen, die die Resultate zahlenmäßig demonstrieren.

Das vorbildlich verfaßte Buch ist Geburtshelfern, Neonatologen, Pädiatern, Pathologen, Neurologen, Psychologen und Heilpädagogen zu empfehlen.

G KORÁNYI

Chlamydial disease. Edited by S. DAROUGAR. British Medical Bulletin, Vol. 39. No. 2. Churchill-Livingstone, London 1983. Price £ 10.00

This issue of British Medical Bulletin is a concise and excellent review on microbiology, immunology and clinical features of diseases caused by *Chlamydia trachomatis*. Pioneers and international authorities of Chlamydia research are represented in its 18 chapters.

The first chapter is a fine review on the developmental phases of *C. trachomatis* and the morphological and biochemical characteristics of the elementary and reticular bodies. Energy parasitism of the microorganism and its clinical and diagnostic consequences are discussed. Detailed data on the immunochemistry of carbohydrate, lipid and protein antigens of *C. trachomatis* and their relationship to the microimmunofluorescent test are offered.

A most clinically minded chapter deals in detail with the clinical course, prognosis and therapeutic principles of trachoma; also, the most recent WHO classification of staging is described.

The chapter on chlamydial infections in newborns and infants begins with a description of conjunctivitis, the most conspicuous and early manifestation. It appears that differential diagnosis of neonatal conjunctivitis is rather difficult in the presence of silver salt prophylaxis. The symptoms of inflammation caused by *N. gonorrhoeae*, *C. trachomatis* and *H. influenzae* are indistinguishable. The newborn baby contracts the infection in the infested birth canal; the rate of transmission of *C. trachomatis* from mothers affected by chlamydial cervicitis varies from 28 to 75% according to various publications. The incidence of chlamydial conjunctivitis was found between 4 and 32% by various authors. The length of the incubation period is 3—13 days, in many a case the first symptom sets in after discharge from the maternity hospital. Local, and quite often inadequate, antibiotic treatment may prolong the incubation period or result in transition to the chronic form of the disease. In most cases the disease is of benign nature, characterised by spontaneous remissions for years and acute exacerbations. Cicatrisation is a rare complication. The authors propose abandonment of Credé's prophylaxis mainly because it is ineffective in preventing *N. gonorrhoeae* infection; it should be replaced by instant microbiological diagnosis and adequate systemic erythromycin treatment. Neonatal conjunctivitis is an alarming signal for maternal (and also paternal) genital infection and neonatal chlamydial pneumonia.

It has not been fully clarified how *C. trachomatis* colonizes the infant's nasopharynx, ears, rectum and vagina, causing pharyngitis, pneumonia or otitis media. The most important manifestation is pneumonia, a condition having been known as abacterial, pertussoid and eosinophilic pneumonia since 1941. *C. trachomatis* as the sole infective agent of these types of pneumonia has been questioned; cytomegalovirus, eventually ureaplasma or still unknown viruses have been incriminated as additional infective agents. Although this form of pneumonia has a low mortality rate, it may pave the way in neglected cases to chronic respiratory infections in adolescents.

The authors warn against a diagnosis of chlamydial pneumonia set up on the basis of serology only. Maternal antibodies and seroconversion elicited by conjunctivitis may here interfere. Therefore, serology should be complemented by culturing from the suspected cavity and conjunctiva of the infant. They also stress the fact that *C. trachomatis* can be transmitted sexually and treatment of both parents is indispensable.

The subsequent chapters delineate the broad spectrum of diseases caused by *C. trachomatis*. A special chapter is devoted to psittacosis caused by *C. psittaci*. Also a good summary on humoral and cellular immune responses following chlamydial

infections is offered. The role of late cellular immune response in some disorders is subject of intensive research. It may be hoped that progress in immunology of these diseases will once lead to production of an effective vaccine void of adverse effects; this would be very useful in preventing trachoma, still an enormous problem in developing countries.

In summary, the issue concisely sums up up-to-date knowledge on chlamydial infections. There are some disproportionalities, e.g. as many as four pages deal with the rather obscure, remote and uncertain relationship between chlamydias and Crohn's disease while their adverse effect on human reproduction, their role in infertility, miscarriage, premature and stillbirth is put aside after a few paragraphs. It is true that chlamydial infection plays here an indirect and unclear part, still, these problems would have deserved more attention.

Z VAJDA

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Evaluation of counselling for pregnant women exposed to potentially hazardous environmental factors

A. Czeizel, Juliet Métneki

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Pregnancy outcomes of 546 women seeking advice for exposure to suspected dangerous environmental factors during pregnancy were analysed. Induced abortion was recommended to 58 women, and this advice was followed by 55 of them. An additional 24 pregnancies were interrupted. The rates of fetal death (spontaneous abortion, stillbirth and infant death) in the non-terminated pregnancies corresponded to national figures. The proportion of birth defects among the liveborn infants of women exposed to hazardous physical, chemical, microbial and maternal factors was 5/67 (7.5%), 11/120 (9.2%), 6/158 (3.8%) and 1/22 (4.5%), respectively. These figures did not differ significantly from the expected ones based on the Hungarian registered and estimated figures. Furthermore, a causal relation between the environmental factors and the defects could be excluded in all but one case in which there was exposure to a high dose of oestrogen.

Five thousand seven hundred and forty women, alone or accompanied by their male partner, came to our Genetic Counselling Clinic between 1973 and 1980. One thousand seven hundred and seventy-four of the women (30.9%) were already pregnant at consultation and 546 (9.5%) asked for advice owing to exposure, during a planned pregnancy, to environmental factors that were suspected to be teratogenic. The majority of these counsellees were referred to us by the medical staff providing them with prenatal care. This paper reports on follow-up of pregnancy outcomes in women seeking advice for exposure during pregnancy to environmental factors suspected of being dangerous.

Material and Method

A questionnaire was sent to the 546 women who visited our Genetic Counselling Clinic in the period 1973—1980 because of exposure to potentially hazardous environmental factors during pregnancy. (The questionnaire was mailed about 9 months after the visit.) In 52 cases (9.5%) the address was wrong or had been changed. Counsellees who did not answer the first time, were asked again to fill out the questionnaire. Seventeen women did not respond, 3.4% of the counsellees with known address. Sixty-nine pregnant women who were lost had no newborns with registered congenital anomalies in the Hungarian Congenital Malformation Register (1) in the year following the visit. (This Registry has the personal data of index children and their parents.) Eventually 477 women returned completed questionnaires (Table I). Occasionally some

TABLE I

Material and distribution of pregnancy outcomes

Material	No		Per cent
Total sample	546		
Could not contact	52	69	12.6
No response	17		
Study sample			
Pregnancy outcome known	477		
Aborted (on advice)	55	79	16.6
Aborted (own decision)	24		
Fetal death	31		6.5
Livebirth	367		76.9
Infant death	5		1.4
Birth defect	23		6.3
Congenital anomaly	20		5.4
Severe congenital abnormality	10		2.7

pregnant women were exposed to more than one potential teratogen (e.g. an infection and a drug); in these cases only the seemingly more important factor was taken into consideration. When more than one drug was taken, each was evaluated separately (Table V). Available medical records (e.g. relating to X-ray or disease) or serological examinations (in infections) were used to confirm the reported exposure and the dose and time of any possible effect. In the case of exposure to chemicals, evaluation was based on the report of the women themselves.

As a second step the outcomes of the pregnancies were evaluated (Table I). Seventy-nine induced and 27 spontaneous abortions and one ectopic pregnancy were accepted on the basis of the questionnaires. Unfortunately, no fetuses were autopsied after termination of pregnancy or spontaneous abortion. A detailed necropsy record was requested and evaluated in 3 cases of stillbirth and 5 cases of infant death. The parents of 23 infants with birth defects were requested to return to our Genetic Counselling Clinic with their children. The affected children were checked thoroughly by our consultant experts. Four families who could not come were visited in their homes. The condition of 339 children declared healthy by the parents was checked on the basis of data requested from competent district paediatricians. The medical information received in 308 cases (90.9%) and the reported data did not change the distribution of major congenital anomalies. Finally, the data of the Hungarian Congenital Malformation Register were checked.

RESULTS

Induced abortion was recommended to 58 of the 546 women for the following reasons: proven rubella, varicella, and other infections in 32 cases (Table VII), chemicals (a drug or alcohol) in 11 cases (Table IV), mater-

TABLE II

Pregnancy outcome of women exposed to physical environmental factors and psychological stress during pregnancy

Physical factors	Number of pregnants	Method of diagnosis	Pregnancy outcome unknown	Induced abortion		Fetal death	Evaluated live births	Birth defect	
				indicated	done			No	per cent
Diagnostic X-ray (mainly abdominal)	82	Medical documentation	8	0	5	8	61	5	8.2
Therapeutic X-ray (malignant disease)	4	Medical documentation	0	4	4 (4)*	0	0	0	0
Mechanical trauma (mainly abdominal)	4	History	1	0	1	0	2	0	0
IUD	7	Medical documentation	0	0	1	3	3	0	0
Psychological stress	2	History	0	0	1	0	1	0	0
Total	99	—	9	4	12 (4)*	11	67	5	7.5

* The number of indicated induced abortions is shown in brackets

nal disease and its therapy in 11 cases (Table VIII) and radiotherapy in 4 cases (Table II). Our advice was followed by 55 women. However, an additional 24 women had their pregnancy interrupted; therefore the total figure of induced abortions was 79 cases (Table I).

For *spontaneous* abortion the reported birth rate of 6.8% was lower than the registered Hungarian value (13%). This may be an ascertainment bias since the recorded peak of spontaneous abortion occurs in about the 8th week of gestation in Hungary, and the majority of our counsellees had passed this time. The stillbirth figure, 0.8%, corresponded to the national value (0.8%). No anomalies were observed among the stillborns.

Out of 367 livebirths, five died within the infant period; the rate (1.36%) was lower than the national figure in the period studied (2.9%). The cause of death in these five cases was the very low birth weight: 800 g, 850 g, 900 g, 1180 g (with RDS) and 1450 g (in a twin), respectively. No congenital anomalies were observed in these autopsied dead infants. In 4 cases the reason for their visit to the Genetic Counselling Clinic was a suspected but unconfirmed rubella infection and in one case a mononucleosis.

TABLE III

Birth defects in newborns of women exposed to diagnostic X-rays

Defect	Current view of aetiology	Localisation of X-rays	Time of X-rays during pregnancy
Bilateral retinoblastoma	Gametic mutation	Gastric	4th week
Trisomy 13	Gametic non-disjunction	Cervical	4th week
Spina bifida cystica	Multifactorial	Chest	2nd week
Congenital inguinal hernia	Multifactorial	Cholecystography	4 times in 11th week
Congenital inguinal hernia	Multifactorial	Chest	15th week

In the following sections the various categories of environmental factors and their relation to birth defects observed in livebirths will be analysed.

Among the physical factors (Table II) diagnostic X-ray examinations were predominant (82.8%). Our estimate of the dose to which the fetus was exposed did not reach the threshold of teratological significant doses accepted in Hungary (10 rads) in any case, thus we recommended to maintain the pregnancies. Nevertheless 5 pregnancies were terminated. Among the 61 live-borns birth defects occurred in five (8.2%). The details of these cases are presented in Table III; when all factors are considered a causal relation between the X-ray exposure and the defects seems highly improbable.

TABLE IV

Pregnancy outcome of women exposed to chemicals during pregnancy

Chemical factors	Number of pregnant women	Method of diagnosis	Pregnancy outcome unknown	Induced abortion		Fetal death	Evaluated live births	Birth defect	
				indicated	done			No	per cent
Alcoholic beverage	5	History	1	1	1 (1)*	0	3	1	(33.3)
Contraceptive pill	75	History	10	0	0	1	64	3	(4.7)
Drug	84	History	12	10	16 (9)*	8	48	7	14.6
Occupational exposition (radiation 2, infection 1, chemical 3)	6	History: 4 Medical documentation: 2	1	0	0	0	5	0	0
Total	170	—	24	11	17 (10)*	9	120	11	9.2

* The number of indicated induced abortions is shown in brackets

One hundred and seventy pregnant women visited us because of exposure to suspected teratogenic chemicals (Table IV). Among the 5 women who consumed alcohol during pregnancy, one could be regarded as an alcohol addict, and for her termination of pregnancy was suggested and performed. Of the remaining 4 women, 3 indulged occasionally in large amounts of alcohol and one consumed moderate amounts of alcohol several times before the pregnancy was evident. These women wanted their pregnancies and they were encouraged to continue them. The women who mentioned moderate alcohol consumption gave birth to a 2800 g boy with tracheal stenosis. Seventy-five women took contraceptive pills during the periconceptional period. Among the 64 liveborn babies who were evaluated, three had defects: a 2500 g girl with intrauterine growth retardation showed a significant somatic retardation in her later postnatal life, too; a boy with predisposition for dislocation of the hip, and a girl with haemangioma. Another 84 pregnant women took 191 different drugs (Table V). In 10 cases termination of pregnancy was recommended (Table VI): 9 women followed this advice. An additional 7 women also had an induced abortion. Among 48 livebirths, evaluated birth defects occurred in 7 (14.6%). Three mothers of these children had received an oestrogen-progesterone combination. One, who had been given 20 ampoules of Limovanil® (oestradiol benzoate 2.5 mg and progesterone 12.5 mg) for the purpose of abortion gave birth to a boy

TABLE V

Pregnancy outcome of women exposed to drugs

Drug	Number of pregnant women	Number of birth defect
Analgetics	13	0
Antihistaminics	16	0
Anticonvulsants	3	0
Anti-inflammatory	24	1*
Antimicrobials	18	2*
Sedatives	53	1
Tranquilizers	6	0
Cardiotonics, vasodilatators	10	1
Sex hormones	30	3
Other hormones	16	0
Others	2	0
Total	191	8

* The same child

TABLE VI

Data of pregnant women to whom termination of pregnancy was recommended on the basis of drug consumption

Case number	Drug	Amount	Time (week)	Remarks
368/1978	chloramphenicol metronidazole	2 g/day 1 g/day	4—5th	Threatened abortion Kidney X-rays Nephrolithiasis
774/1978	diazepam chlordiazepoxide barbital	400 mg once 400 mg once 40 g once	4th	Suicide attempt
1120/1978	nitrazepam phenobarbital oestradiol progesterone	1 mg/day 200 mg/day 75 mg/day 150 mg/day	0—8th 4—6th	60 years old diabetic husband Cholecystography and ventricular X-ray 15 times. Attempted abortion
1232/1978	meprobamate diazepam valeric acid phenobarbital chlordiazepoxide	0.6 g/once 15 mg/once 3 g once 3 g once 1.5 g once	0—8th 6th	Epilepsy Suicide attempt
1561/1979	valeric acid phenobarbital	5 g once 5 g once	14th	Suicide attempt
1850/1979	oxytetracycline codeine unknown others	1620 mg/day 60 mg/day "many"	1—2nd	Symptoms of poisoning
1948/1979	phenytoin	300 mg/day	0—8th	Epilepsy, operated for arachalnoide cyst
1094/1980	phenmetrazine HCl oxytetracycline diethylstilstilboestrol-diproprionate	100 mg/day 2160 mg/day 40 mg once	5—8th 8th	Pneumonia Attempted abortion
1110/1980	oestradiol progesterone	10 mg/day 50 mg/day	3—4th	Psoriasis, hormonal pregnancy test
1544/1980	oestradiol progesterone thioridazine trimipramine	30 mg/day 150 mg/day 125 mg/day 75 mg/day	3—4th 0—10th	Attempted abortion Severe depression. ABO incompatibility

with limb reduction (unilateral, terminal transverse type fingers), and the other two, who received Limovan® (ethynyl oestradiol 0.01 mg and pregneninolone 10 mg) as a hormonal pregnancy test, gave birth to a boy with predisposition for dislocation of the hip and a girl with haemangioma. Of the additional 4 cases, 3 had mild anomalies: congenital inguinal hernia (meprobamate), predisposition for dislocation of the hip (co-trimoxazole), and haemangioma (prednisolone, penamecillin, oxytetracycline, nalidixic acid and terbutaline). The women had used clinical doses of these drugs in the 2nd and 3rd months of pregnancy.

TABLE VII

Pregnancy outcome of women allegedly exposed to microbial factors during pregnancy

Microbial factors	Number of pregnant women	Method of diagnosis	Pregnancy outcome unknown	Induced abortion		Fetal death	Evaluated livebirth	Birth defect	
				indicated	done			No	per cent
Cytomegalovirus	3	CMV specified IgM: 1	0	1	2 (1)*	0	1	0	0
"Influenza"	19	History and clinical symptoms	0	0	1	0	18	0	0
Hepatitis	4	Clinical symptoms	0	0	0	1	3	1	(33.3)
Herpes genitalis	7	Clinical symptoms	1	0	1	0	5	1	(20.0)
Herpes simplex	12	Clinical symptoms	2	0	0	0	10	0	0
Syphilis	1	Serologically excluded	0	0	0	0	1	0	0
Mononucleosis	1	Serologically	0	0	0	0	1	0	0
Mumps	12	Serologically in 8 cases	2	1	3 (1)*	0	7	0	0
Rubellavirus exposition or/and disease	157	Serologically in 17 cases	25	17	19 (16)*	8	105	4	(3.8)
Toxoplasmosis	10	Serologically in 4 cases	1	4	4 (4)*	1	4	0	0
Vaccination (cholera)	1	History	0	0	0	0	1	0	0
Chickenpox	12	Clinical symptoms in 9 cases	0	9	9 (9)*	1	2	0	0
Total	239	—	31	32	39 (31)*	11	158	6	3.8

* The number of indicated induced abortions is shown in brackets

The 7th case, a girl with cleft lip and palate and lacrimal atresia was born to a woman who took ampicillin and acetylsalicylic acid during the 9th to 11th weeks of pregnancy.

Among the infectious factors to which 239 women were exposed, rubella was most prevalent (65.7%) (Table VII). Termination of pregnancy was recommended only for rubella infection with seroconversion and for chickenpox with clinical symptoms. (Previously, our position had been the same for cases of toxoplasmosis proven by serological methods.) For one case of serologically confirmed cytomegalovirus infection (a women who had previously given birth to a microcephalic mentally deficient child) and one case of mumps infection (social factors), the indication for induced abortion was based primarily on other factors. Among the 158 evaluated live-borns, birth defects occurred in 6. Four of them were delivered by rubella-protected women. Since these defects were Down syndrome, perinatal brain injury, syndactyly, and multiple abnormalities (auricular anomaly, bronchial stenosis, thymus hyperplasia, strabismus) that did not correspond to the congenital rubella syndrome, a cause-effect relation could be excluded. This was also the case for a possible relation between potential exposure of one woman to hepatitis virus (husband) during pregnancy and the congenital inguinal hernia in her son. A girl with aortic stenosis was born by Caesarean section to a woman with a genital herpes infection.

Thirty-eight women presented themselves because of potentially hazardous maternal factors (Table VIII). Termination of pregnancy was recommended in 11 cases for medical reasons, and was performed in 10. One woman with severe heart disease and thrombophlebitis who was treated with oral anticoagulants and other drugs did not want her pregnancy terminated despite our warning; she

TABLE VIII

Pregnancy outcome of women with potential hazardous material diseases during pregnancy

Material factors	Number of pregnant women	Pregnancy outcome unknown	Induced abortion		Fetal death	Evaluated livebirth	Birth defect	
			indicated	done			No	per cent
Tuberculosis	2	1	0	0	0	1	0	0
Haematologic	2	0	0	0	0	2	0	0
Diabetes mellitus	8	0	3	3 (3)*	0	5	0	0
Hepatic disease	4	2	0	0	0	2	0	0
Thyroid disease	10	2	2	2 (2)*	0	6	0	0
Heart disease	4	0	2	1 (1)*	0	3	1	(33.3)
Kidney disease	8	0	4	5 (4)*	0	3	0	0
Total	38	5	11	11 (10)*	0	22	1	(4.5)

* The number of indicated induced abortions is shown in brackets

gave birth to a child with multiple abnormalities including valvular heart defect (without specification) and anal atresia. One pregnant woman had an induced abortion against our advice. It is noteworthy that fetal death did not occur in any of the cases.

Discussion

The principles and some statistics of our "information-guidance type" genetic counselling have been published previously (2). According to the results presented here, after being warned, 95% of pregnant women exposed to hazardous environmental factors terminated their pregnancies owing to the high risk of the fetus. In 419 pregnant women the suspected hazard of environmental factors was not a real or a significant one, therefore we recommended them to maintain their pregnancies and in 94% of these cases our advice was followed.

Another important point is the correctness of counselling. During the study period, the Hungarian registered birth prevalence for congenital abnormalities was about 4% and the theoretically acceptable figure would be between 6—8% (5). The 365 pregnant women for whom pregnancy continuation was recommended and who have known pregnancy outcomes and gave birth to children, 20 (5.5%) had a malformed liveborn. This observed figure corresponds to the expected one of congenital abnormalities in the Hungarian population. The birth prevalence of severe congenital abnormalities was 2.7% and this also fits well with the expected 3% rate. Furthermore the observed rates of different types of pre- and postnatal mortality did not exceed the national figures in 419 pregnant women where the maintenance of pregnancy was suggested and the pregnancy outcomes were known.

Of course, it would be better to use the record linkage than to organize a follow-up study. At present, however, the efficacy of record linkage has not reached the adequate level. On the one hand new congenital anomalies were not found. On the other hand, only seven congenital anomalies were registered from 20 known cases (35%). The completeness of reporting is better in the severe congenital anomalies, because out of 10, seven were notified.

According to Hungarian regulations, a 10% risk of severe and untreatable congenital anomalies without the possibility of prenatal diagnosis may be a medical indication for induced abortion before the 12th week of gestation. Such a risk could be anticipated in therapeutic X-ray of malignant diseases; in pregnant women with IUD in utero (though the teratogenic risk has not been confirmed);in alcoholic pregnant women who consumed large amounts of alcohol during pregnancy; in six drug groups such as androgens; antifolic acid derivatives;cytostatics; synthetic oestrogens; certain anticonvulsants like hydantoin, trimethadione and valproic acid derivatives; and dicou-

marol derivatives; rubella infection with seroconversion and chickenpox with clinical symptoms (though recent studies have demonstrated a fetal risk under 10%). The use of contraceptive pills during the periconceptional period entails only less than 1% risk increase if any (6, 8, 4). The oestrogens may increase the risk of limb reduction malformations threefold, from 0.04% to 0.12% (4). Thus a cause-effect relation could not be excluded in the case in which Limovanil® was administered to induce abortion. Thus the question may be raised whether or not the genetic advice given to the women exposed to Limovanil® was wrong. We believe that we can safely deny this, because the increase of specific risk (at most 0.1%) is individually negligible.

Of the 58 women to whom termination was recommended, three did not follow our advice. One aborted spontaneously (the 1561/1979 case in Table VI); one gave birth to a child with multiple congenital abnormalities (the case was mentioned among the women exposed to maternal factors); and one with rubella virus infection proved by seroconversion in the 8th—10th weeks of pregnancy, had a normal baby.

The efficacy of "official" advice concerning the potentially hazardous environmental factors during pregnancy seems to be good. The random risk, however, is a permanent Damocles' sword for the counsellor. It partly explains the defensive attitude of clinicians in the judgement of potential teratogens. (In Hungary Law protects the counsellor versus the rare occurrence of random risk.) Another problem is the sketchy teratological knowledge of physicians. Finally, in Hungary the restriction of the previous liberal Abortion Law in 1974 also modified the consideration of potentially hazardous environmental factors during pregnancy. The rate of induced abortion for medical indication increased from 2.7% in 1970—1973 to 15.9% in 1979—1980. Analysis of this significant increase showed that the reason for about 40% of these cases was not well grounded from the teratological point of view and that clinicians have great difficulty in evaluating the significance of human teratogenic exposures (3). The difference means the loss of about 4000 planned pregnancies per year.

Conclusion

Follow-up of women seeking advice at a genetic counselling clinic because of exposure to potentially hazardous environmental factors during pregnancy is important because it provides an evaluation of the counselling and yields information on the effect of environmental factors that is difficult to gather. Our data support the recent epidemiological observations which suggest that the majority of potentially hazardous environmental factors are not true teratogens (7). These observations might lessen the fears of pregnant women

concerning the possible dangerous consequences of potential teratogens and this may be a great help because all pregnant women are exposed to some environmental factors.

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Screening of children with high familiar risk of arteriosclerosis

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Serum total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C) and total triglyceride levels were determined in children with high risk arteriosclerotic family history.

Significantly higher TC and lower HDL-C levels were found in children whose parents' first arteriosclerotic sign had appeared before 40 years of age. There were no similar significant alterations observed in children whose parents' first arteriosclerotic symptom appeared after the forties. Screening therefore seems to be necessary in the offsprings of patients if the first sign of arteriosclerosis has been detected before 40 years of age.

Numerous papers were published during the last 15 years about efforts to prevent the development of arteriosclerosis by the screening of children with enhanced familiar risk [2, 9, 15, 16]. It has been shown by retrospective studies that results of cord blood lipid measurements have no prognostic importance [6, 8, 12, 18]; screening of older children with high risk family history seemed to be a more useful programme [6, 13, 14, 20].

One of the risk factors is the arteriosclerosis of the parents. A stronger effect of genetic predisposition than that of both environmental and nutritional factors was found in families of patients whose first arteriosclerotic symptom appeared in early age [4]. Early appearance of the first sign of arteriosclerosis may fall between 40 to 60 years [3, 10, 13, 14, 20, 22]. The serum total cholesterol level (TC) was higher in children of these families than in the control ones [11, 13] but it was the consequence of the increased serum high density lipoprotein cholesterol (HDL-C) level in some cases [11, 13]. The high risk of the latter children seemed to be doubtful according to the negative correlation observed between the serum HDL-C content and the development of coronary heart disease (CHD) [5, 7, 17, 19].

The estimation of serum TC and HDL-C levels seems to be a useful method of screening. With searching for the age when the appearance of the first arteriosclerotic symptom of parents occurs early enough for a reliable study of genetic effects. The 40 years

of age of the parents seemed to be a useful limit in the present study.

Material and Method

The study population consisted of 78 children 2—18 years of age whose parents had CHD proven by coronary arteriography or by acute transmural myocardial infarction. Group 1 consisted of 22 children whose parents' illness was observed before their 40th year. Group 2 consisted of 56 children whose parents' first arteriosclerotic symptoms appeared after 40 years of age. The age-matched control group consisted of 23 (2—18 year old) healthy children without any high risk family history.

After an overnight fast, the blood of children was sampled from the cubital vein. Serum TC and total triglyceride (TT) levels were measured by Boehringer tests. After precipitation of the serum by polyethyleneglycol 6000, the cholesterol content of the supernatant was measured by the Boehringer CHOD-PAP test according to the serum HDL-C measurement method described by Allen et al [1]. Other aliquots of serum were precipitated by sodium duodecylsulphate, centrifuged and the cholesterol content of the lower phase was determined by the Boehringer CHOD-PAP test according to the method described by Wilson and Spiger [21] who calculated the serum very low density lipoprotein cholesterol values (VLDL-C) from the difference between the serum TC level and the cholesterol content of this lower phase.

Statistical analysis was performed by Student's t test and the χ^2 test.

Results

The mean TC level of children whose parents' CHD was first observed before their forties (Group 1) was significantly higher than that of controls. The increase of the mean TC level of children whose parents' CHD appeared later was not significant. There was no difference in the mean serum TT levels (Fig. 1).

The mean serum HDL-C level in Group 1 was significantly lower than that in the control group. The serum HDL-C level of children in Group 2 was also lower, but the difference was not significant. There was no difference in the VLDL-C values (Fig. 2).

The significant difference observed in the serum HDL-C level of children

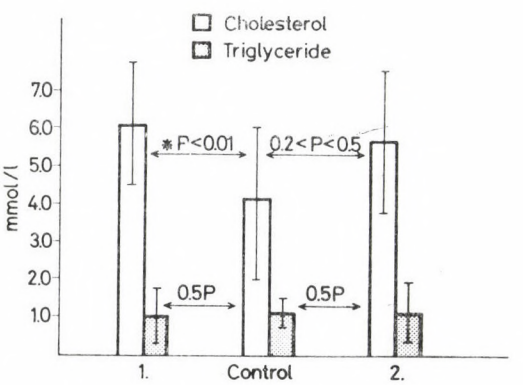

Fig. 1. Serum TC and TT levels of children in the different groups

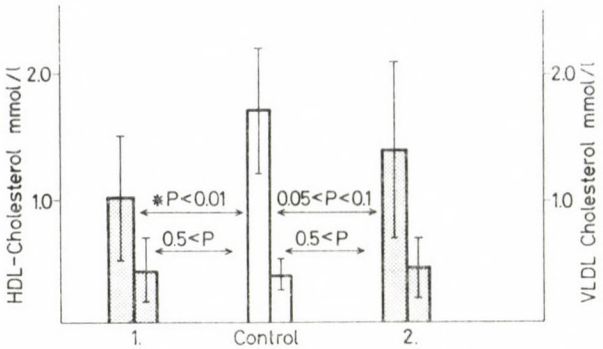

FIG. 2. Serum HDL-C (column 1)- and VLDL-C (column 2) levels of children in the different groups. Test groups are represented by dotted columns

TABLE I

Serum TC and HDL-C content of control population

	Unit	n	Mean	S.D.	Min.	Max.	Percentiles				
							10%	25%	50%	75%	90%
TC	mmol/l	23	4.2	2.1	1.7	7.6	2.1	3.2	4.0	4.8	6.5
HDL-C	mmol/l	23	1.6	0.4	0.6	2.8	0.75	1.3	1.65	2.05	2.4

in Group 1 was remarkable in the age group under ten years. The standard deviation was very high in the group over ten years (Fig. 3).

The incidence of extremely high serum TC (above 90 centiles) and the extremely low serum HDL-C (below 10 centiles) values showed that the frequency of extremely high TC or low HDL values was significantly higher in Group 1 than in the control group. Similar significant differences were not observed in children of Group 2 (Fig. 4).

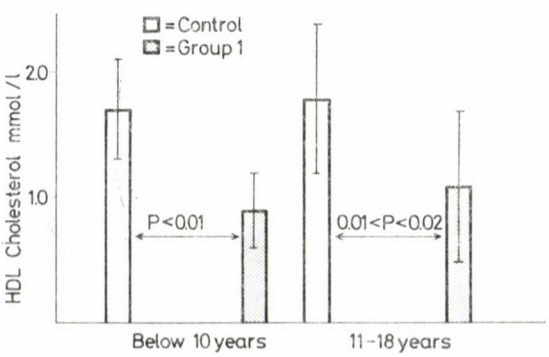

FIG. 3. Serum HDL-C values in group 1 according to the age of children

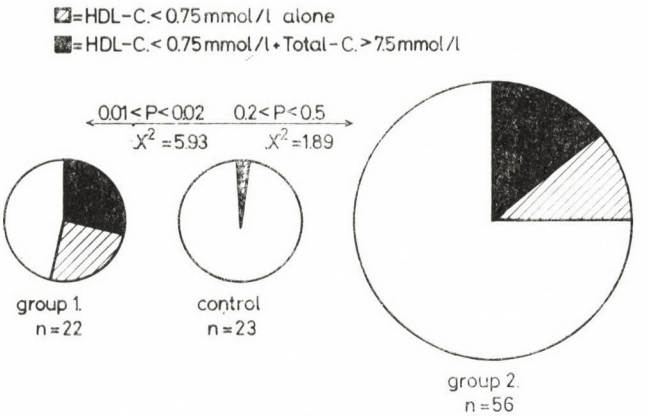

FIG. 4. Frequency of extremely low HDL-C values and/or the very high TC values in the serum of children in the different groups

DISCUSSION

Significant alterations were observed in the serum TC and HDL-C levels of children whose parents' CHD had appeared under 40 years of age. More than half of these children had extremely low serum HDL-C levels with or without an extremely high serum TC level. There were no similar significant alterations in children whose parents' CHD appeared after their forties. The genetic effect on the serum HDL-C level observed by other authors [4] may explain these phenomena.

According to the results, the appearance of CHD under 40 years of age very often shows the high risk of arteriosclerosis in the offspring. The very low serum HDL-C values observed together with normal serum TC values suggest the great importance of estimating the serum HDL-C level in these cases.

The results suggest that screening and intervention are necessary in the offspring of patients if the first sign of CHD is detected under 40 years of age.

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Multiple sclerosis in childhood: long term katamnestic investigations

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Sixteen children with multiple sclerosis, 14 with remitting and 2 with progressive course, and their follow up for 4—16 (mean, 9 years) are reported. The disease manifested in eight children at the age of 1.5—9 years, while in the other eight at the age of 12—14 years. The cases were acute, but had a stronger inclination to remission than in adulthood. In addition to the "classical" symptoms known in adult, multiple sclerosis, papilloedema and acute increased intracranial pressure were observed in nine children. Dehydrating treatment was life saving in these cases. Opsoclonus occurred in two patients, and two children had convulsions, one of whom later developed chronic epilepsy. The CSF was pathological in all the cases. Oligoclonal gamma-globulin subfraction was found in three out of the five patients tested. Three patients died. Histological examination was carried out in two of them; it revealed severe perivenous demyelinisation due to inflammation.

Cortisone treatment occasionally with azathioprine reduced the duration of exacerbations, but could not prevent renewed ones and caused severe side-effects.

Multiple sclerosis is a rare but not exceptional condition in children. The differences between cases in children and adults are age-dependent.

Leukoencephalomyelitis (LEM) is one of the most frequent diseases of the nervous system in adulthood. It is on the other hand still being disputed, how often it begins in childhood, to what extent do childhood cases tend to relapse, and whether or not they can directly progress into multiple sclerosis (MS). Most examinations are retrospective[1], trying to find out if parainfectious complications in the nervous system following an infection or vaccination, transient amblyopia, double vision, etc., had occurred in childhood, perhaps even decades before onset of the MS-process. So MS developed in 1/3 of the cases within 10—15 years [25] after manifestation of optic neuritis of unknown origin. Cohen et al [11] observed that 17 out of 60 cases of optic neuritis turned later into MS. Observations beginning actually in childhood are less frequent.

[1]Papers with retrospective data have not been taken into consideration in this study

Materials and Methods

Clarification of the questions mentioned above required prospective studies, so of the 4900 inpatients of our department in the 15 years 1964—1978 we followed up 16 children in whom recurring or progressive leukoencephalomyelitis had been diagnosed. The follow up lasted for 4—16 years. Our classification was carried out using the modified criteria of Bauer [1]. Cases diagnosed since the beginning of 1979 were excluded from the present study on account of the short catamnestic period. Symptoms and laboratory data are summarized in Tables I and II. Three patients died at the age of 8, 16 and 19 years, respectively. Onset of symptoms occurred between 1 1/2 years and 14 years of age in the present material (14 years is the upper age limit for admission to our hospital). Our patients have been classified into two groups on basis of their age at the first appearence of their symptoms. In Group I consisting of 8 cases, the symptoms started between 1 1/2 and 9 years of age, in Group II (also 8 cases) between 12 and 14 years. Fourteen children were observed during the appearance of their first symptoms or soon thereafter, while in two cases the first examination took place several years after the initial period.

The maternal grandmother of Case 4 died of MS at the age of 38 years. The first appearence of symptoms as well as later exacerbations were often associated with febrile upper respiratory disease (5 cases). The Paul-Bunnel reaction was positive in 3 children. Case 3 received phenytoin following febrile convulsions.

Damage of the diverse tract systems could be diagnosed on the basis of focal signs (visual, pyramidal, spinocerebellar system, sensory pathways). Psychic symptoms occurred in most cases.

Results

The characteristics of the two groups will be discussed separately.

A stormy onset was characteristic of almost every member of the younger age group. Acute paraplegia, tetraplegia, or hemiplegia were the most frequent initial symptoms, and paraplegia associated with paradoxical ischuria in Case 4. The disease of four children (Cases 2, 5, 7 and 8) started with papilloedema and headaches. In Case 6, papilloedema appeared later. With the exception of this case, no defective vision occurred simultaneously with swelling of the optic disk. Altogether three children (Cases 1, 3 and 6) developed transient amblyopia or amaurosis with decoloration of the optic disk. Rough, irregular, conjugated twitchings (opsoclonus) of the bulbs, independent of the eyes' position, were observed in two children (Cases 2 and 8), which recurred during exacerbations. Epileptic complication was observed in Case 1. Moderate mental retardation was observed in three children (Cases 1, 2 and 7).

Seven cases showed a fluctuating course, and the same symptoms returned repeatedly in four of them. Only Case 7 became chronic following the second exacerbation having had also acute initial symptoms. Three patients (Cases 4, 5 and 8) were in remission for 7—9 years. The symptoms of Cases 1 and 2 returned in short intervals. The number of exacerbations ranged from 2 to 10. Seven patients have already reached adolescence. Case 3 died at the age of 8 years after a 4-year course of remissions and exacerbations.

Case No 1. T. E., a girl born in August, 1965, after normal pre- and perinatal events had developed well up to the age of 1 1/2 years. She had been admitted in February, 1967, on account of weakness of the left limb and paralysis of the right side of the face. The most important laboratory findings in the CSF had been 298/3 cells (70% lymphocytes, 30% lymphoreticular cells), 29% protein, mastix: 22210—0.

Her next attack took place in 1968. In the following 15 years we observed a total of 9 exacerbations including the one in 1968. They showed a great variety of neurological symptoms: ataxia, transient amaurosis, hemianopsia, scotomas, pareses. She had again to be admitted in February, 1978, on account of pollakisuria, ischuria paradoxa, while in March on account of severe headaches and vomiting (signs of increased intracranial pressure).

Recurrence of the complaints was always followed by remission lasting from a few months to 2 years. Steroids were administered permanently, cytostatica temporarily. In 1972, 5 years after onset of LEM, generalised convulsions appeared, which were associated a year later with myoclonic absences.

At the last admission (4—13th October, 1978) she was a well developed girl with Cushingoid appearance. Decolorated optic disks with nystagmus of medium amplitude and frequency could be observed on either side. The speech was scanning, the gait paretico-atactic. Psychically she was aggressive and practically demented.

In the EEG a right temporal spike focus with a mirror focus on the left side appeared in July, 1974, two years after onset of the attacks. Secondary generalised convulsive activity with a temporal start could be found in later records. The CSF was tested on 11 occasions. The cell count varied between 12/3 and 82/3, while the protein content ranged from 11 to 40 mg/dl. The mastix curve was always normal (with the exception of the first puncture). CSF IgG was 3.2 mg/dl (February, 1978), while the gamma subfraction was characteristic of oligoclonal gammopathy. Electrophoresis of serum was normal (Dr. Kerényi).

All other laboratory results were normal. LE-cell, anti-H and latex were repeatedly negative. Immunoelectrophoresis showed a strong IgM precipitation line (1974).

In the last years, periodical deterioration of her condition made repeated treatment in an adult neurological ward necessary.

From the 8 patients of Group II, 7 showed remissions, and 1 was gradually progressive (Case 16). Only one of them was in durable remission in the last five years, exacerbations of the others returned frequently, and approached symptomatically the cases beginning in adulthood (age 20—27 years in December, 1982). Papilloedema was observed in a single patient (Case 15) as opposed to 5 cases in the younger group. Scotoma, transient amblyopia or amaurosis were observed in four adolescents, and decoloration of the optic disk in the same four patients (Cases 9, 10, 14 and 15).

Lesion of the pyramidal tract as well as of the spinocerebellar system was observed in every patient, except three in Group I (see Table I). It was probably due to the higher age of these patients, that five of them complained of paresthesia, as opposed to just one patient in Group I. Organic psychosyndrome developed in all but two (Cases 10 and 16) of the adolescents. Two patients died from Group II. The history of one of them was as follows.

TABLE I

Clinical data

No.	Name	Sex	Date of birth	Triggering cause	Age at onset yrs	Duration yrs	No of relapses	Papill-oedema	Sco-toma
1	E. T.	F	08. 1965	None	1 1/2	15 1/2	9	—	+
2	S. B.	F	05. 1969	Infectious mono-nucleosis	4	9	6	+	—
3	Cs. B.	M	07. 1960	Phenytoin	4	4✠	5	—	+
4	R. F.	F	12. 1968	Grippe	4	9 1/2	2	—	—
5	S. D.	M	10. 1960	None	6	16	2	+	—
6	A. B.	F	07. 1972	Grippe	6	4	3	+	+
7	C. D.	F	06. 1965	Grippe	8	9	2	+	—
8	I. K.	F	06. 1962	Infectious mono-nucleosis	9	11	2	+	—
9	G. M.	M	06. 1960	Grippe	12	4✠	12	—	+
10	J. K.	M	05. 1958	None	12	12	4	—	—
11	J. L.	M	06. 1955	None	12	15	9	—	+
12	P. B.	M	09. 1962	Grippe	12	8	8	—	—
13	Z. B.	M	06. 1962	Infectious mono-nucleosis	13	7	7	—	—
14	E. S.	F	02. 1953	None	13	6✠	8	—	+
15	I. N.	F	05. 1962	None	13	7	5	+	+
16	M. S.	F	10. 1961	None	14	7	1	—	—

Urinary retention occurred in Cases 1 and 4, opsoclonus in 2 and 8, and apraxia on the right side in Case 7

Case 9, G. M. a male patient born in August, 1960 had had temporary impairment of vision and pareses in April, 1972. Four further exacerbations had appeared in the same year (transient amblyopia, decoloration of papillas, pareses, ataxia), each one following a mild upper respiratory tract infection. Signs of brain stem herniation: anisocoria, tetraparesis, adversive convulsive attacks, coma could be observed; consciousness had returned after dehydration and i.v. administration of cortisone. After one month only minimal residual symptoms could be detected. The patient received continuously dexamethasone in large doses; upon every attempt to decrease the dose, the LEM process exacerberated. He was treated with cytostatics from January, 1974, without success. In May, 1975, we were forced to stop steroids and cytostatics on account of thrombocytopenic bleedings. He was admitted with severe tetraparesis and respiratory failure to the intensive ward in November, 1975. There, he developed amaurosis. After temporary improvement he died in May, 1976.

The ESR was regularly high (33—73 mm/h). The number of thrombocytes dropped to 22 000 during skin bleedings. The CSF was analysed 6 times. The cell count was 42—174 (lymphocytes and

Clinical symptoms						Course			Treatment	
Amaurosis	Decolor. pap.	Paresis of eye muscles	Pyramidal signs	Paraesthesia	Cerebellar symptoms	"Organic" psychic symptoms	Remitting	Chronic	Cortisone	Cytostatics
+	+	—	++	+	+++	+	+	—	+	+
—	—	++	+	—	+++	+	+	—	+	—
+	+	—	+++	—	—	+	+	—	—	—
—	—	—	+++	—	+	+	+	—	+	—
—	—	—	+	—	—	—	+	—	+	—
+	+	+	++	—	+	temp. disturbed	+	—	+	—
—	—	—	++	—	—	+	—	+	+	+
—	—	++	+	—	+++	—	+	—	+	—
+	+	—	++	+	+	+	+	—	+	+
—	—	+	++	+	+	—	+	—	+	—
+	+	—	++	—	+	+	+	—	+	—
—	—	—	+	+	+	+	+	—	—	—
—	—	+	+	—	+	+	+	—	+	—
ambl.	+	+	+++	+	+++	+	+	—	+	—
ambl.	+	—	++	—	+	+	+	—	+	—
—	—	—	+++	+	+++	—	—	+	+	+

M — male
F — female

lymphoreticular cells) in the first two years. In October, 1974, there was 148 mg/dl protein without leukocytosis. The lymphocyte migration index (LMI) was 0.69 and 0.70 in May, 1974, and May, 1975, respectively (normal value, 0.8—1.0). In the brain there was a severe inflammatory loss of perivenous white substance in the lower brain stem. Neither fresh, nor old plaques could be found.

Group I and II showed similar laboratory findings. The CSF during exacerbations was pathological (Table II). The cell count remained under 20/3 only in two patients, it exceeded 100/3 in 9 cases and 1000/3 in 1 patient; lymphocytes, lymphoreticular cells, a few plasma cells, and in some cases a few granulocytes were found.

Total protein values exceeding 30 mg/dl were considered pathological. The values of two patients (Cases 8 and 14) were below this limit, but the other data (cell count and/or colloid-curve) were pathological in these patients, too. Mild or pro-

nounced precipitation on the left side of the colloid-curve was missing in three patients only (Cases 8, 11 and 16). Since January, 1978, the CSF of five patients was analysed for oligoclonal gamma globulin. Three were pathological (Cases 1, 2 and 12), while two (Cases 6 and 7) were normal (see Table II). The lymphocyte migration index was pathological in seven patients (Cases 2, 4, 5, 6, 7, 9 and 10). It was normal in two patients and not investigated in the other 7 ones. LE-cell was positive in three out of ten analyses. Pneumoencephalography was carried out in 8 children, angiography in 5, with negative results.

The EEG was pathological in 11 children. Convulsive signs were recorded in Cases 1, 2 and 6. One patient (Case 1), receives continuous anticonvulsive treatment. The EEG was normal in three patients, and no EEG records were taken in two.

Discussion

On the basis of their clinical course and laboratory data at the first exacerbation, in most patients acute LEM had been diagnosed but the following attacks were considered signs of multiple sclerosis.

Among the causes inducing LEM, the role of common infections of the respiratory tract is widely known; we found this in the history of five of our cases. Infectious mononucleosis occurred in the history of three out of 16 children, which was a considerably higher proportion than the 1—3%, found in the literature [37, 47]. The triggering effect of diphenylhydantoin in autoimmune processes (Case 3) is well-known.

Most exacerbations, whether with early or late onset, showed a stormy course corresponding to the clinical picture of an acute inflammatory process accompanied by inflammatory changes of the CSF, differently from most cases of adult MS. The symptoms of our Case 9 resembled those of Devic's syndrome (optic neuromyelitis) six months before death due to acute amaurosis and tetraparesis [42].

The symptoms of the early group were to some extent monotonous, the same symptoms repeated themselves during the exacerbations in four cases. Most of the remissions were pronounced, even in Case 3, whose severe exacerbations had improved considerably. MS proceeds in young patients in acute or subacute series followed by more or less complete remissions. This might be explained by the nature of the histological alterations: in both cases in which a histological examination was carried out, we only found an inflammatory process [34].

Mild papilloedema was observed in five early and one adolescent cases, i.e. in more than 1/3 of the patients. The swelling was probably due to the greater water content of the brain tissue. The same may be the explanation for the repeated headaches accompanied by vomiting in Case 1, the upper brain stem herniation of Case 9, as well as for the oblongata herniation in Cases 3 and 9. It is

known that acute LEM may run its course in the form of a pseudotumour cerebri. It is less accepted, that this may also occur as a complication of later exacerbations [21]. Recognition of this complication is important, because elimination of the oedematous component by osmo- and oncotherapy may actually be life-saving. We have solved in this way the upper brain stem herniation of Case 9 three years prior to death. Even dehydration could not stop the terminal caudal brain stem symptoms in Cases 3 and 9 on account of the extremely severe oedema [34].

Opsoclonus, which occurred in Cases 2 and 8, is considered a disturbance of cerebellar function [5, 14]. This symptom is rare in childhood MS, and hardly occurs in adults. Other symptoms of our patients are regularly found in adult MS, so we shall refrain from their detailed analysis.

Out of the laboratory data, an increased ESR and a higher serum gamma globulin simultaneously with the appearance of neurological symptoms indicated an aspecific infection and proved the reaction of the humoral immune system. The positive LE-cell phenomenon found in three patients pointed towards a cellular immunopathological process [36], and so did the pathological LMI observed in seven patients [39].

The CSF showed inflammatory changes in every patient. A cell count lower than 10/3 was only encountered in Case 12, but this patient was admitted 4 years after onset of the disease, in remission 6 weeks after the end of an exacerbation. His other records were, however, pathological (Table II).

The pathological nature of the mastix reaction indicates an increase In the gamma fraction of the CSF. intracerebral IgG synthesis was first demonstrated in 1958 [17]; it can be observed regularly in prolonged viral infections and chronic inflammations of the central nervous system, such as MS.

The subfractions characteristic of the disease can be shown by electrophoresis [23, 26, 27, 29]; they may, moreover, be found also in the plaques of autopsied brains [35]. We found them in the CSF of three out of the five patients so examined (Cases 1, 2 and 12). One of them (Case 12) was the patient observed during remission, whose CSF cell-count was not increased.

Thus the clinical symptoms and laboratory data seemed to prove the presence of MS and in the majority this was confirmed by the remissions and exacerbations. Thus, contrary to general opinion, MS may start in childhood. Out of the 19 acute cases of childhood LEM observed by van Bogaert [3], 5 turned into MS during the following 18 years.

The eight cases in our Group I had all begun before adolescence. The disease is "an extreme rarity" in children below the age of 9 [33]. Those cases of Ford [16] which progressed to MS, were all older than 9 years. Out of the 8 patients below the age of 15 years of Gall et al [19], two were 7 and 8 years old, respec-

TABLE II

Laboratory Results

No.	Name	ESR mm/h	Cell/3	CSF prot. mg/dl	Mastix	Oligocl. γ glob.	LE cell	LMI	Paul-Bunnell	PEG	Angiography	E E G	Histology
1	E. T.	23	52	40	111110—0	+	neg.	n.d.	neg.	n.d.	n.d.	Temporal focus sec. gener.	
2	S. B.	75	320	30	21110—0	+	neg.	0.62	1:46	n.d.	n.d.	Right frontal spikes	
3	Cs. B.	10	30	55	21110—0	n.d.	n.d.	n.d.	n.d.	Normal	Normal	Diffuse, slowing	Perivenous encephalitis
4	R. F.	6	170	35	11110—0	n.d.	neg.	0.66	neg.	n.d.	n.d.	Diffuse, slowing	
5	S. D.	5	1168	128	23454220—0	n.d.	+	0.70	neg.	n.d.	n.d.	Normal	
6	A. B.	15	59	36	22210—0	neg.	neg.	n.d.	1:12	Normal	n.d.	Left tempero-basal slowing and spikes	
7	C. D.	15	20	31	110—0	neg.	+	0.74	neg.	Normal	n.d.	Irregular	
8	I. K.	25	130	24	10—0	n.d.	neg.	0.80	1:96	n.d.	n.d.	Slowing	
9	G. M.	33	174	154	211110—0	n.d.	neg.	0.69	neg.	Normal	Normal	Slowing	Perivenous encephalitis
10	J. K.	8	280	68	34554432100	n.d.	neg.	0.68	neg.	n.d.	Normal	n.d.	
11	J. L.	8	13	31	10—0	n.d.	n.d.	n.d.	n.d.	Normal	n.d.	Normal	
12	P. B.	4	8	43	1222110—0	+	n.d.	n.d.	n.d.	Normal	n.d.	n.d.	
13	Z. B.	33	48	40	1222210—0	n.d.	neg.	n.d.	1:48	n.d.	n.d.	Irregular	
14	E. S.	8	28	18	12210—0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	Normal	
15	I. N.	9	90	90	233210—0	n.d.	n.d.	0.90	n.d.	Normal	Normal	Left sharp waves	
16	M. S.	5	200	46	10—0	n.d.	+	0.84	neg.	Normal	Normal	Mild anomaly	

n.d. — not done
neg. — negative

tively, the rest was older. From the 711 MS cases of Carter [9], 9 were 10—15 years old. Out of the 7 childhood cases of Low and Carter [30] four had at least two exacerbations, the youngest one of these being 4 years old (and not two as Schneider et al [42] have written) while the others were 6, 7 and 8 years old. The 6-year-old girl patient of Kamio et al [24] resembled very closely our acutely remitting cases, with her 6 exacerbations within 2 years. The case of Brandt et al [8] started at the age of 2 years, and that of Bejar and Zeiger [2] also at that time but they failed to describe whether they first saw the patient then or only at the age of 16 years and whether the first 2 episodes they took from the history or they followed the case during 14 years.

The laboratory data are not characteristic of MS. Concerning the patient of Nobel [38] who died at the age of 2 1/2, we must point out that in their very short report nothing is said about the beginning of the gradually progressing case. Histologically, demyelinisation centres (Weigert preparation) appeared in the cortex and the white substance of the brain. Infiltration of the blood vessels was minimal, granular cells could only be found along the blood vessels. The histological examination was carried out by Marburg. In his opinion: "The former (i.e. multiple sclerosis) starts mostly in childhood... it appears that mostly in close connection with childhood infections". He mentioned the remitting course and even the occurrence of Charcot's triad in childhood. The published case cannot, however, be evaluated with any certainty because of the inadequate description and the lack of figures.

Our first patient, with onset at 1 1/2 years of age, was the youngest case ever reported. She is now 17 years old and has approached the adult type, despite the fact that her illness earlier was characterized by an acute course and great inclination to remissions, like all the patients in Group I.

In connection with our first patient, we must deal with the relationship between MS and epilepsy. According to some authors [20, 25] epilepsy is rare in MS; one must consider a mere coincidence in view of the frequency of the two diseases [16]. Isler [22] however thinks that epilepsy is not exceptional in adult MS. Different workers reported the frequency of occurrence of convulsions in patients with MS to be from 10.8% [18] to 1.1% [32]. The smallest and highest number of patients with MS examined from this aspect was 74 and 2400, respectively. The convulsions manifest themselves in several cases together with acute MS exacerbations, exceptionally in the form of status epilepticus [7]. When the patients died due to the basic disorder or epilepsy, fresh cortico-subcortical plaques were found, corresponding to the convulsive focus [4, 6, 7, 10]. The proportion of epileptic convulsions was 2—3% in the MS material of these workers.

Pathological EEG records were

reported by Boudin et al [4] in 33.6% and Czopf et al [12] in 64% of their cases. The corresponding number in another study [28] was 34% or 47.8% depending on the duration of the disease. Higher percentages such as 75% [18] have also been published. A pathological EEG does not, of course, necessarily mean convulsive activity in the brain.

Due to the small number of our patients, calculation of percentages would be pointless. EEG was recorded in 13 patients, taking 2—17 records in each case. It was pathological in 10 patients, convulsive activity was found in three (see Table II). Clinical epilepsy developed in one patient only (Case 1), in this case it has continued to be an important factor of the clinical events for nearly one and a half decades.

On the basis of our data, MS cannot be considered epileptogenic. Pathological EEG or even a spike focus may develop upon the basis of an organic lesion; an epileptic process is, however, rare. Occasional attacks are more frequent (Case 9).

The history of our 16 patients proves that onset of MS in childhood does not mean an unequivocally better prognosis compared with cases starting in adulthood. While inclination to remission is greater, malignant processes are not a rarity in children. Immediate life danger is great in acute exacerbations on account of the increased disposition to oedemas and to severe inflammatory reactions. In some chronic cases, the exacerbations become more and more frequent and permanent residual symptoms may develop [45].

In the two cases examined histologically, we failed to find any sign of earlier attacks and in particular of any plaque formation; we rather observed acute perivenous encephalitis. This observation corresponds to our clinical experience: acute exacerbations indicated acute inflammatory processes according to the findings in the CSF. A detailed analysis of the pathological findings lies outside the scope of the present work. We would only point out that it had been stated more than 30 years ago that perivenous encephalitis may permanently retain its histological independence [3].

As far as the therapy is concerned, most of our patients were given steroids, some of them also azathioprine. The acute symptoms disappeared quickly on cortisone. This fact is important on account of the acute course the disease takes in children. The mechanism of the quick remission has not been clarified [43, 46]. The role of remyelinisation — also proven in human subjects [41] — is insignificant in MS [44]. The maintenance cortisone dosage failed, however, to prevent recurrences, as emphasized also by Ellison and Myers [15]. They experienced the most lasting results by permanently administering azathioprine, but this entails numerous risks. We also met severe complications arising from steroid-azathioprine treatment in our Case 9. Plasmapheresis [13] has not been carried out in our cases.

On the basis of the above data it may be stated that some cases of LEM proceed with exacerbations and remissions already in childhood. Most of the individual relapses resemble single acute EM but they approach the clinical picture of chronic MS, or "progress" into it in many cases after a longer course. The clinical picture of MS is rare but not exceptional in children. It may start soon after infancy as shown by our Case 1. If the possibility of its occurrence is kept in mind, correct diagnosis is ensured by careful analysis of the symptoms complemented by laboratory tests. This may spare children the unnecessary contrast examinations, which were carried out in nearly 2/3 of our cases. The spread of computer tomography has solved this problem.

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Surgical treatment of renovascular hypertension in children and adolescents

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Renovascular disorders are rather rare in children and adolescents but have severe consequences due to complicating hypertension. Six cases successfully treated by surgery are described. The importance of early diagnosis and vascular correction is stressed; normalization of blood pressure has been achieved in every case.

The most frequent cause of "surgical" hypertension in childhood and adolescence is the typical form of coarctation of the isthmic section of the aorta. The next in rank are vascular abnormalities of the kidney or those leading to impaired renal circulation. The latter comprise atypical coarctation of the descending or abdominal aorta and disorders leading to stenosis of the renal artery.

Patients

Six patients with renovascular hypertension have been operated on during the last six years. Three of them were younger than 14 years while the others were adolescents between 14 and 18 years of age.

Table I summarizes the principal preoperative data.

Case 1. A girl of 14 years was found by screening in the school to have a blood pressure of 180/120 mm Hg. She was admitted for evaluation of this condition. At admission she had a normal appearance and was normally developed. Arterial pulsation was weak all over both lower limbs, over the abdominal aorta a holosystolic murmur could be heard. The murmur could be followed over both iliac arteries. Laboratory tests revealed only slight hypokalaemia. All renal functions were normal, the size of kidneys was also normal.

Catheter angiography and direct blood pressure measurements were carried out. The angiography revealed multiple arterial supply of both kidneys. The artery supplying the upper pole of the left kidney was occluded on a proximal section of about 15 mm. The abdominal aorta showed a funnel-shaped stenosis at the level of the renal arteries, the minimum diameter being 6 mm. The pelvic arteries below the bifurcation were of normal size (Figure 1). There was a blood pressure difference of 40 mm Hg between the suprastenotic and infrastenotic parts.

Since the lower extremities showed unimpaired arterial supply even during loading, we desisted from surgical treatment of the aortic abnormality. After infracolic approach indirect reimplantation of the left upper polar artery was carried out by help of a graft taken from the left saphenous vein. The patient's hypertension normalized during the early

TABLE I

Serial number of patient	Age, years	Gender	RR (mm Hg)	Diagnosis
1	14	F	180/120	Coarctation of abdominal aorta. Aorta angusta. Bilateral multiple renal arteries. Occlusion of left superior renal polar artery.
2	12	M	190/120	Bilateral multiple renal arteries. Stenosis of right renal arteries.
3	13	M	180/110	Stenosis of right renal artery. Hypoplasia of left kidney. Hydroureter and pyelonephritis on left side.
4	16	F	170/110	Stenosis of left renal artery. Occlusion of left subclavian artery. Takayashu's disease.
5	16	M	200/140	Occlusion of left renal artery. Double right renal artery. Hyperbilirubinaemia.
6	17	M	190/130	Stenosis and ventroposition of right renal artery. Multiple renal arteries on left side.

postoperative period. The surgical wounds exhibited primary healing, and the patient was discharged on the eighth day after surgery. She has been checked regularly during the past five years, and she has been free of complaints.

Case 2. A 12 years old boy, his hypertension was detected by school screening. He had been complaining of frequent headaches and irritability. He was admitted to our department. Protracted excretion of the radioopaque material by the right kidney and slight parenchymal loss in the same kidney suggested renovascular origin of the hypertension. Arteriography revealed each three renal arteries on both sides arising in ventroposition. All three arteries of the right kidney exhibited initial stenosis

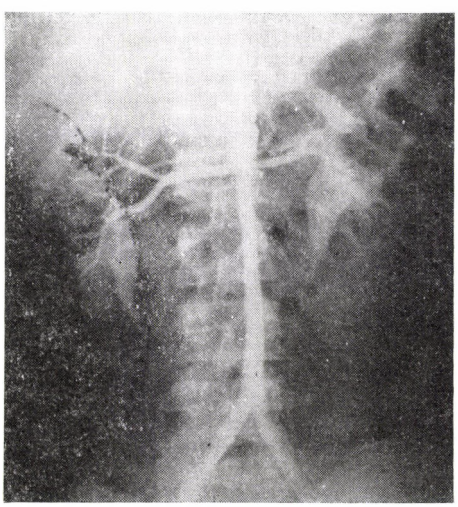

FIG. 1. Case 1. Aorta angusta and multiple renal arteries. Occlusion in a length of 15 mm of the artery supplying the upper pole of the left kidney

with poststenotic dilatation. Isotope nephrography showed a 30% functional share of the right kidney. Total kidney functions were unaffected. Slight secondary hypokalaemia was present. We decided to perform surgical reconstruction.

The arteries of the right kidney were approached supracolically after mobilization of the duodenum. All three arteries were ligated at the stenotic orificial level and indirect antecaval reimplantation was carried out using an autologous saphena graft shaping a new tripartite hilus. Some hours after surgery the patient's blood pressure stabilized at a value of 120/70 mm Hg. The postoperative period was uneventful. He has been followed for two years; he is free of complaints and is engaged in sports. Isotope renography showed reequalization of the function of the two kidneys.

Case 3. This 13 years old boy had been treated for urinary tract infection and hypertension in another hospital. The pyuria having been cured, urography and renovasography were carried out. These revealed hypoplasia of the left kidney which was supplied by a double renal artery. Excretion was unimpaired. The dilated left ureter was tortuous and at the vesicoureteral junction stenosed. The orificial section of the right renal artery exhibited a 80% narrowing. Percutaneous transluminal angioplasty was performed which resulted in a transitory moderation of the hypertension of 180/130 mm Hg. When the blood pressure had returned to the previous high values, angiography was again carried out and this showed persistence of the stenosis seen previously on the right renal artery. The patient was referred to us for surgery.

Since the functional isotope tests showed a 40% share of the left kidney within the total function, we decided to retain the left kidney. The right renal artery was exposed infracolically. After mobilization of the inferior vena cava and the left renal vein, aortorenal arteriotomy and dilatatory plastics using a Gore-tex patch were performed. After surgery the patient's blood pressure decreased only moderately; this was explained first by parenchymal damage of the left kidney. Control by Angiotron angiography, however, unequivocally demonstrated a stenosis of the reconstructed blood vessel section (Figure 2). Two weeks later surgery was repeated. The right renal artery was approached supracolically from the right side. Indirect

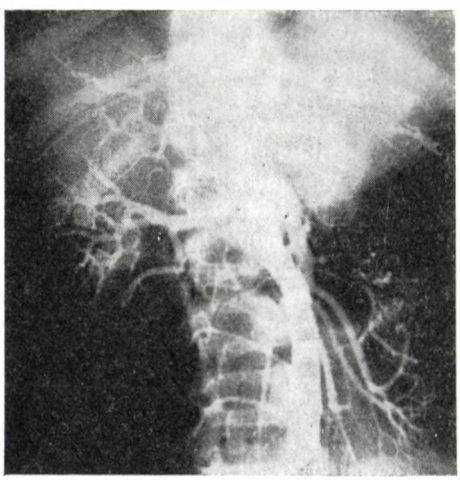

FIG. 2. Case 3. Abdominal summation aortography. Stenosis of right renal artery and hypoplasia of left kidney

reimplantation was carried out utilizing an arterial graft taken from the infrarenal part (internal iliac artery) of the aorta. This was followed by normalization of the blood pressure, only minimal doses of antihypertensive drugs were necessary. The surgical wounds exhibited healing by first intention. A control isotope renography performed two months later showed a 25% increase in the perfusion of the right kidney. Angiotron angiography confirmed ideal anatomical conditions (Figure 3). The patient's blood pressure could be kept normal with 0.5 mg prazosine twice daily. Now, cessation of antihypertensive treatment is being weighed and an ureteroplasty is planned.

Case 4. A girl of 16 years was admitted because of hardly palpable arterial pulsation on the left arm and a blood pressure of 170/110 mm Hg on the right arm. She was normally developed, had slight hirsutism, and a systolic murmur could be heard above the left supraclavicular fossa and the abdominal aorta. Endocrine hypertension was excluded by detailed laboratory studies. Angiography was indicated, the characteristic localization of the vascular malformations suggested Takayashu's disease. The left subclavian artery was found to be occluded as far as its second third; its distal section was supplied via the thyreocervical trunk (Figure 4). The remaining supraaortic trunks showed normal anatomy. A mild, sand-glass shaped stenosis was observed on the abdominal aorta at the level of the renal arteries. A stenosis exceeding 60% was demonstrated after the orifice of the left renal artery in a length of 10 mm (Figure 5). Since there was no functional or nutritive damage on the left upper extremity, surgical treatment of the renal artery stenosis, which had maintained the hypertension, was decided. During surgery under total heparinization "axillary" aorto-arteriotomy was carried out, after local endarteriectomy free circulation to the orifice of the renal artery was secured by using dilatatory plastics with a Gore-tex patch which was applied so as to correct the stenosis of the aorta as well.

The postoperative course was uneventful, the patient's blood pressure returned to normal values on the first postoperative day. Histology revealed vascular changes characteristic of Takayashu's disease. One year later the patient had a normal blood pressure. Now she has been free of complaints for four years, there has been no

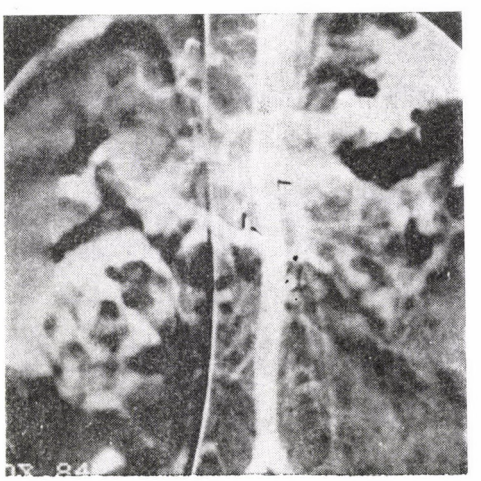

FIG. 3. Case 3. Postoperative angiogram demonstrates arterial autograft of good function on right side. Photograph made by subtraction technique

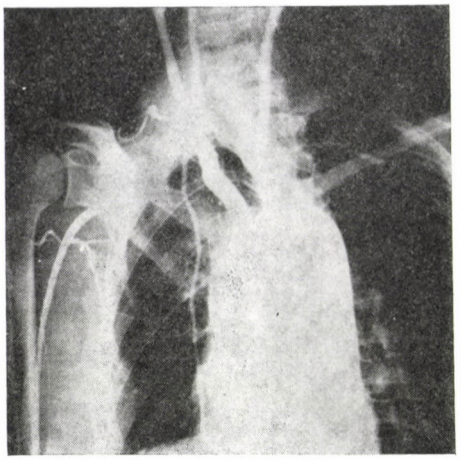

FIG. 4. Case 4. Takayashu's arteriitis occludes the intrathoracal section of the left subclavian artery, the other supraaortic arteries are unaffected

progress in her supraaortic condition, her blood pressure is now 130/80 mm Hg.

Case 5. A boy of 16 years had been found to have a blood pressure of 200/140 mm Hg. Conservative treatment had been attempted, this had resulted in a slight decrease in his hypertension. He had been admitted for further evaluation to another hospital. At admission he had had pronounced hypokalaemia and slightly elevated serum bilirubin. The latter had been explained as a result of mild congenital enzymopathy. By urography, bilateral excretion had been demonstrated but transport of the contrast material was protracted on the left side. There had been no difference in renal size. Aortography had revealed postorificial occlusion of the left renal artery in a length of 10 mm, the normal distal part was supplied through

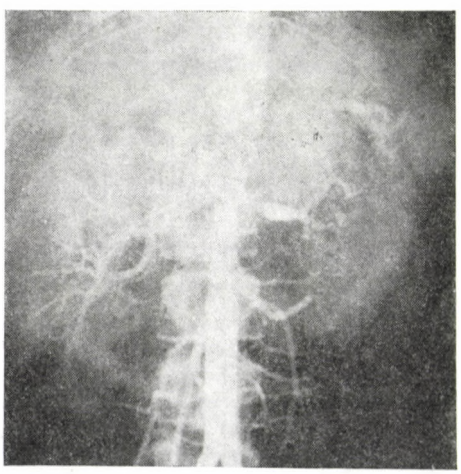

FIG. 5. Case 4. Takayashu's arteriitis. Abdominal aortogram shows the stenotic orifice of the renal artery and the slight, sand-glass shaped stenosis of the abdominal aorta

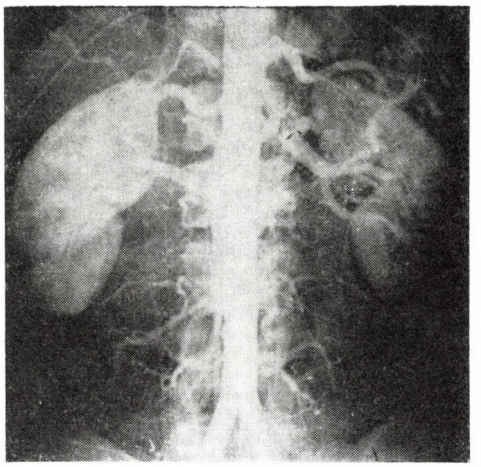

FIG. 6. Case 5. Occlusion of left renal artery, refilling of the poststenotic section through the rich collateral network

a rich arterial collateral plexus around the pelvis. There was a double right renal artery (Figure 6).

The patient was transferred to our department for surgical correction. Our findings were in full agreement with those obtained earlier. By upper-median laparotomy the left renal artery was prepared infracolically and direct aortorenal reimplantation was carried out. The patient's blood pressure returned to normal values within a few hours. His postoperative course was uneventful. He now has been free of symptoms and complaints for one and a half years.

Case 6. In a 17 years old male patient hypertension had been detected at a routine school examination one and a half years earlier. Urography, radiorenography, plasma renin activity of selected renal vein

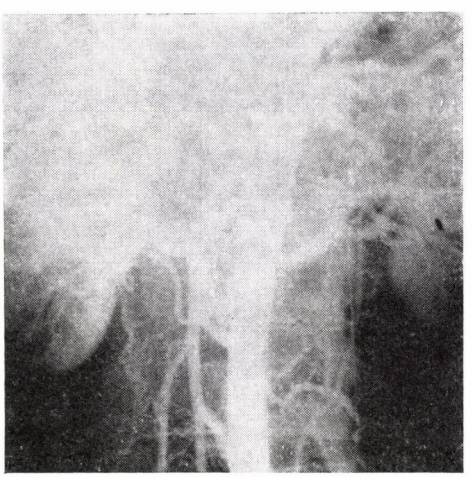

FIG. 7. Case 6. Renovasogram showing paraaortic stenosis of right renal artery

blood and aortography unequivocally pointed to hypoperfusion of the right kidney. There was a 90% stenosis on the right renal artery in ventroposition, at 1 cm after the orifice the stenotic blood-vessel divided into two arteries, crossing each other and exhibiting slight post-stenotic dilatation. The left kidney was supplied by multiple renal arteries. The kidneys were about the same size, but their functional share was 30% and 70%, respectively (Figure 7).

At surgery, the right renal artery was approached supracolically. After ligation of the stenotic common trunk antecaval indirect reimplantation was carried out, utilizing an autologous saphena graft. End-to-end anastomosis was made with the upper pole artery and side-to-end anastomosis with the artery supplying the lower pole. The patient's blood pressure returned to normal values within a few hours. After an uneventful postoperative period he was discharged on the eighth postoperative day. Now he has been in excellent health for one year, he is normotensive and capable of continuing his original studies. There is no functional difference between his two kidneys.

Table II sums up further data of our patients.

Discussion

Renovascular disease is rare in childhood and adolescence but it is a severe condition because of the high risk of hypertensive damage [2, 6]. Its exact incidence in these age groups is difficult to estimate because systematic screening has only been established in recent years [7, 17, 19, 21]. Within the highly selected renovascular material of our Department the share of paediatric cases is 4%.

There are some differences in pathology between childhood and adult cases. In children affected by renovascular hypertension 50—60% of the abnormalities are of congenital origin [4]. Atypical coarctation, multiplicity of the renal artery, ventroposition of its orifice are often accompanied by stenosis [13]. In the material of Stanley and Fry [29] orificial stenosis makes up 50% of all cases. In some of their cases the abnormality was accompanied by

Table II

Serial number of patient	Renin quotient	Type of surgery	Postoperative blood-pressure, mm Hg	Duration of follow-up
1	—	Left indirect aortorenal reimplantation, venous graft	130/70	5 years
2	—	Right antecaval aortorenal indirect reimplantation, hilar microreconstruction	120/70	2 years
3	1.43	I. Right orificial Gore-tex patch plastics II. Right antecaval indirect reimplantation, autologous arterial graft	160/100 120/80	3 months
4	1.3	Aortorenal Gore-tex patch plastics	120/70	4 years
5	—	Left direct reimplantation	120/70	1 year
6	1.8	Right indirect reimplantation, hilar microreconstruction	120/80	1.5 years

neurofibromatosis or abdominal coarctation of the aorta.

Inflammatory vascular changes occur much less frequently. In our material one patient was affected by Takayashu's arteritis. In the largest paediatric material there were three cases, one of them of grave outcome [31]. Even less frequently can fibromuscular hypertension be observed, although alterations affecting distal sections of the renal artery and attributed to a local weakness of the vascular wall may exhibit similar histology. Stenosis of sclerotic origin does not occur in these age groups.

It seems that hypoperfusion causes less parenchymal damage in children and adolescents, therefore 0.5—1.0 cm reductions in renal length should be considered pathological. The diagnostic value of urography is rather restricted since functional deficits can only be shown in advanced cases. In our cases renal arterial occlusion did not lead to appreciable changes in size or function. Isotope studies — perfusion and scintigraphy — allow a more refined approach.

Plasma renin activity was determined in half of our cases since only positive results are of diagnostic value. There is no close relationship between the renin quotient and the success of surgical treatment [16, 23].

The most decisive method is angiography, also in children. Indication for surgical treatment should be based on clinical data and angiography [3]. For visualising a short, orificial stenosis, oblique projections may be indispensable. Demonstration of a collateral plexus may be a valuable indirect sign, its visualisation may be facilitated by pharmacoangiography.

In respect of surgical techniques there are no basic differences between operations on children and adults [12, 15, 20, 22]. Surgery is performed under complete heparinisation. Indirect or direct reimplantation of the renal artery is the most suitable method, by-pass techniques and autotransplantation are less commonly used [11, 14, 25]. Similarly, segment resection, endarteriectomy and patchplastics are not often applied. In case of multiple renal arteries and small size, microsurgery is indicated. In our material in situ solutions were preferred. Some authors favour ex situ methods [32].

For reconstruction of the renal artery we usually apply reimplantation and saphena grafting but in small children autografts taken from the internal iliac artery can best be used. The small dimensions may necessitate knot technique; this is favourable also for the growing tissue [25, 27].

In children and adolescents the otherwise widely used transluminal angioplasty is less suitable because of anatomical variations and localisations. The intense tissue reaction of the young organism is a further discouraging factor [5, 9, 31].

In centres with angiosurgical experience the results are good. Reconstruction gradually extrudes nephrectomy [29], the rate of complete recovery is above 90%. In our own

material blood pressure normalised in all cases soon after surgery. In one case a relapse of hypertension occurred; repeated angiography proved to be helpful in early recorrection [10, 24].

Isotope diagnostics seems to be most suitable for follow-up. Late angiography can be used for checking the condition of the implant and may reveal subclinical alterations.

Early diagnosis and adequate treatment of renovascular hypertension of children and adolescents are based on well-organised screening programme and an efficient surgical background.

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High-frequency oscillatory ventilation (HFOV) in the treatment of neonatal respiratory disturbances: Case reports of two infants

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The technique of high-frequency oscillatory ventilation (HFOV) was successfully used in a preterm infant with severe hyaline membrane disease and in a term neonate presenting with intrauterine pneumonia and associated severe pneumomediastinum. None of the infants could adequately be ventilated by conventional ventilation; both of them deteriorated owing to severe hypoxaemia and hypercapnia. In the preterm infant with HMD a rapid and progressive improvement of oxygenation had been observed immediately after the beginning of HFOV, and he was successfully weaned off the ventilator after 71 hours on HFOV. His recovery was uncomplicated and definitive. In the term neonate presenting with IUP and associated severe PM, an improvement in oxygenation was detected, whereas the retention of $paCO_2$ remained unaltered. On leaving the MAP unchanged but doubling the flow rate, $paCO_2$ and arterial pH also normalised. No sign of PM was seen on the X-ray picture 17.5 hours after the start of HFOV. This patient was weaned off the ventilator after 29 hours on HFOV and his recovery was also uncomplicated.

It is believed that recovery of the PM was secondary to the low MAP and to the higher arterial pO_2 levels, and that HFOV may also have a direct role in the treatment of preexisting air leaks and perhaps also in their prevention. In our patients HFOV resulted in a definitive recovery, while no improvement had occurred on using conventional ventilation. To determine the exact mechanism of action, the clear cut fields of indications and the possible side effects of HFOV, further investigations are needed.

Abbreviations

BE	=	base excess
BPM	=	breath per minute
CPAP	=	continuous positive airway pressure
EEP	=	end expiratory pressure
FiO_2	=	fraction of inspired oxygen
HFOV	=	high-frequency oscillatory ventilation
HMD	=	hyaline membrane disease
IUP	=	intrauterine pneumonia
MAP	=	mean airway pressure
pH_a	=	arterial pH value
PIE	=	pulmonary interstitial emphysema
PIP	=	peak inspiratory pressure
PM	=	pneumomediastinum
$paCO_2$	=	arterial tension of carbon dioxide (kPa)
paO_2	=	arterial tension of oxygen (kPa)
Vol	=	volume
WBC	=	white blood cell

Data on the use of HFOV in the treatment of neonatal respiratory disturbances are scanty [1—3]. Marchak et al [3] investigated the effect of HFOV in eight preterm neonates with severe hyaline membrane disease (HMD), who required mechanical ventilation. The patients had been put on HFOV for periods of time ranging from 67 to 233 minutes (mean, 147 minutes), then they were returned to their conventional ventilator. Oxygenation improved during HFOV and the improvement correlated directly with the increases in mean airway pressure.

Frantz et al [1] used the HFOV technique in ten preterm neonates with severe HMD and in five infants presenting with pulmonary interstitial emphysema (PIE). The infants with severe HMD were studied for approximately one hour during the first two days of life on HFOV. Blood-gas values obtained during the one-hour period on HFOV were maintained at an acceptable range and they were similar to those obtained during conventional ventilation. Four of the five preterm infants with PIE were ventilated on HFOV for two days, while one neonate received HFOV for 26 days. HFOV was completely successful in two cases, while no improvement could be achieved in two other infants. The neonate on long term HFOV who had been ventilated by conventional ventilation for 12 days, developed bronchopulmonary dysplasia (BDP), but improvement occurred after 26 days on HFOV.

Recently, we have reported on our experience with HFOV in ten neonates presenting with severe HMD or intrauterine pneumonia (IUP) [2]. The infants were on HFOV for a mean period of 9.2 hours and then they were returned to their conventional ventilator. We observed a significant improvement in the blood--gas parameters of nine patients. The nonresponding infant had bilateral intraventricular haemorrhage and was running into an irreversible hypotensive shock.

In the present study we report on two infants who were unsuccessfully ventilated on conventional ventilators, but with the use of HFOV their blood-gas parameters and clinical condition showed a rapid improvement and finally they were successfully weaned off the ventilator. Moreover, definitive recovery was achieved, both in the preterm infant with severe HMD, and in the term neonate with IUP and pneumomediastinum (PM), without any signs of handicap at the control examinations carried out two and three months later.

Material and Methods

High frequency volume displacements were generated by a special chamber with elastic side-walls. Volume displacement could be altered by positioning the eccentric came along a scale on the diving disk. The distal end of the chamber was fixed, while the proximal one was driven by an electric motor with a constant frequency of 17 Hz. A connector with two opposite side-ports was interposed between endotracheal tube and the tubing from the oscillator.

Through one port nebulized and warmed fresh gas was introduced at a rate of 8—40 l/min from an air/oxygen blender. Excess fresh gas and CO_2 which had diffused out of the lung, were released through the second connector port. This port was connected to a plastic tube which was 20 mm in diameter and 3 m in length. In order to be able to administer smaller or larger flow rates without influencing mean airway pressure, the lumen at the distal end of the tube was variable, i.e. it could be tightened or enlarged. Mean airway pressure (MAP) was measured 30 mm proximal to the endotracheal connecting port with the aid of PT 100 MEV transducer and EPM 100 electric manometer (PTK, Hungary). With the use of the devices we could measure not only the mean pressure but also the amplitudes of oscillation at a frequency of 17 Hz.

In order to keep the relative humidity and temperature of the inhaled gas at appropriate levels (85 $\pm$ 5% and 36 $\pm$ $\pm$ 1°C, respectively), a special nebulizer unit was devised. Bacterial filters (Bourns Adult Main-Flow Filter 51000—01054, Bear Medical Systems, Inc.) were used to avoid bacterial invasion.

The schematic diagram of our high frequency device is shown in Figure 1.

We measured the pressure at three different points of the circuit in a container of impedance similar to that of the infant's respiratory system ventilated with HFO through a 3.0 mm endotracheal tube. The three measuring points were, 30 mm proximal to the junction of the endotracheal tube (1st measuring point) and the distal end of the endotracheal tube, where pressure was measured with (3rd measuring point) and without (2nd measuring point) a 5 French umbilical artery catheter passed through the endotracheal tube. The amplitudes of oscillation of different measuring points showed a good correlation to each other.

The correlations between the amplitudes of the 1st and 3rd measuring points observed at different baseline pressure are shown in Table I.

With the use of these correlations, after having measured the amplitudes of oscillation at the 1st measuring point, one could estimate the magnitude of oscillation even at the 3rd measuring point.

According to the results of our measurements, the amplitudes of oscillation were practically not influenced by alterations of the flow rate. Moreover a good correlation was found between the volume and amplitude of oscillation (Figs. 2, 3, 4).

The values in Table I and those of

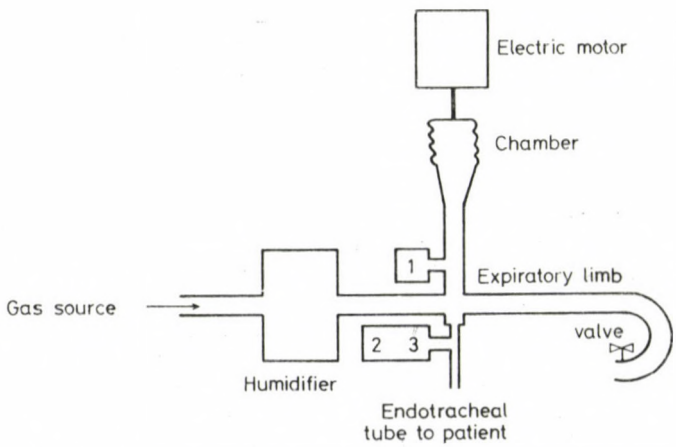


FIG. 1. Schematic diagram of our high frequency device

TABLE I

Amplitudes of oscillation measured at 1st and 3rd measuring points

Mean pressure cm H_2O	r	Regression equations
0	0.99	$A_3 = 0.19 \times A_2 + 1.25$
10	0.92	$A_3 = 0.18 \times A_2 + 3.29$
20	0.92	$A_3 = 0.21 \times A_2 + 3.12$

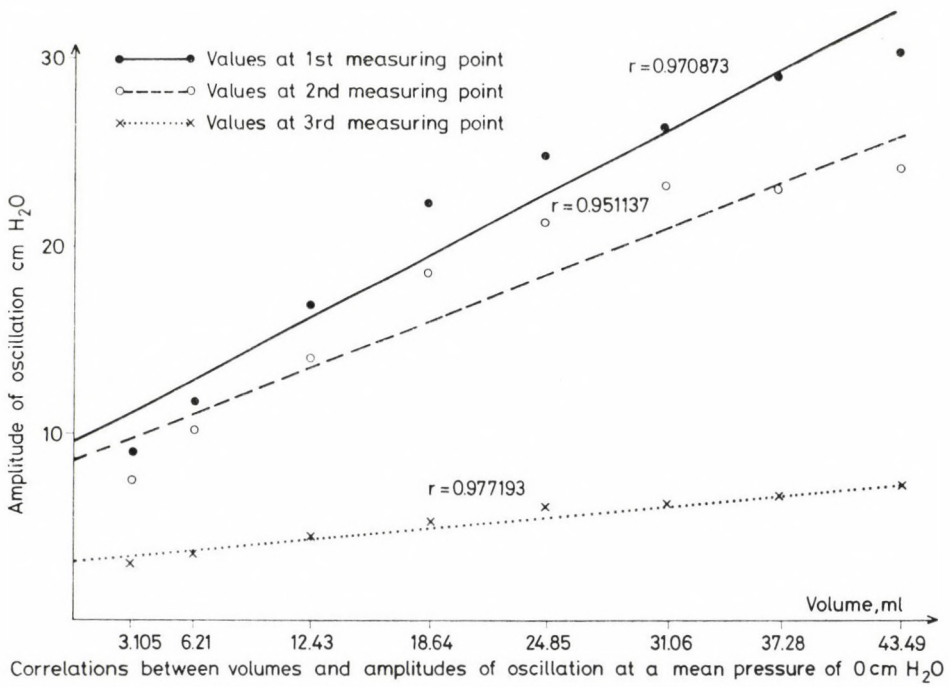


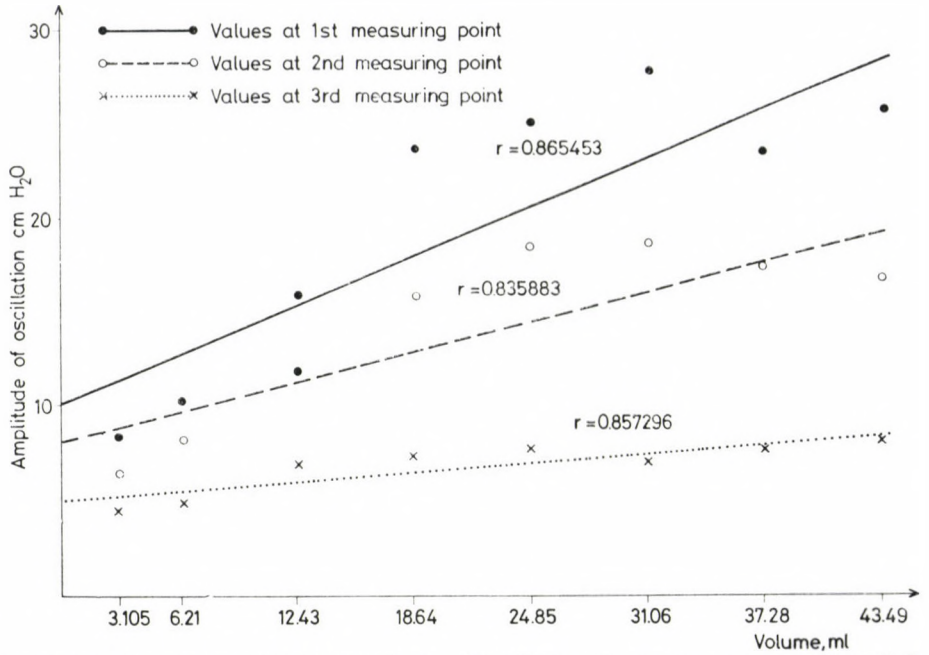
FIG. 2.

Figs 2, 3 and 4 represent the average of the amplitudes of oscillation observed a flow rates of 10, 20, 30 and 40 l/min. If mean pressure was 0 cm H_2O the measurements were also performed at a flow rate of 0 l/min.

The study was carried out with the approval of the University Scientific Committee, and informed parental consents were also obtained.

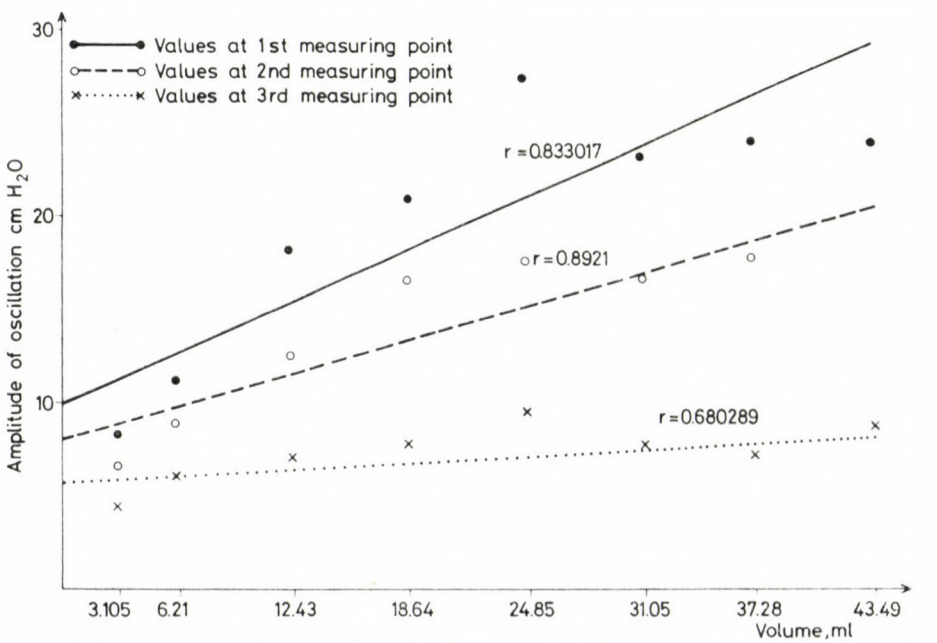
CASE REPORTS

Case 1. This boy was the third child of a mother who had two normal children. He was born in the 32nd gestational week after premature rupture of the membranes with a birthweight of 1750 grams. Apgar scores were 5/7. Because of severe respiratory distress he was transferred to our unit at the postnatal age of 1 hour and



Correlations between volumes and amplitudes of oscillation at a mean pressure of 10 cm H-O

FIG. 3.



Correlations between volumes and amplitudes of oscillation at a mean pressure of 20 cm H_2O

FIG. 4.

20 minutes. He displayed the typical clinical picture of severe HMD.

No signs of injury to the central nervous system could be detected. Chest X-rays showed granularity with a slightly more wet appearance than usual and bilateral air bronchograms with small lungs. Mild cardiomegaly was also present. HMD (stage 3) with pulmonary fluid was diagnosed (Figure 5).

The blood-gas values were, pH_a: 7.05, PaO_2: 1.99 kPa, $PaCO_2$: 5.98 kPa, BE: −19 mmol/l. Nasal CPAP therapy (flow rate: 6 l/min, EEP: 4 cmH_2O, FiO_2: 1.0) resulted only in slight and transitional improvement in the baby's condition. Oxygenation could not be significantly improved (blood-gas values 1 hour after the start of CPAP therapy were, pH_a: 7.28, PaO_2: 3.85 kPa, $PaCO_2$: 6.78 kPa, BE: −4 mmol/l) and at a postnatal age of 8 hours apnoeas occurred. The neonate was put on a conventional ventilator (Bourns LS 104—150) and was ventilated with the following parameters: FiO_2: 1.0, BPM: 40, I : E = 1 : 1, Vol.: 70 ml, Flow rate: 125 ml/sec, PIP = 26 cmH_2O, EEP= = 4 cmH_2O, MAP = 15 cmH_2O. After a mild improvement the infant's condition began to deteriorate. Transcutaneous pO_2 value declined and remained under 3.9 kPa despite different ventilator settings and the administration of tolazoline. Blood-gas values obtained at 12 hours postnatal age were, pH_a: 7.45, PaO_2: 3.05 kPa, $PaCO_2$: 4.12 kPa, BE: −1.5 mmol/l. Since transcutaneous pO_2 value showed a further decrease, at the postnatal age of 12 hours and 20 minutes we put the infant on HFOV (volume: 19 ml, 17 Hz, FiO_2: 1.0, flow rate: 8 l/min. MAP: 16 cmH_2O). After the initiation of HFOV a rapid and significant increase in transcutaneous pO_2 was observed (Figure 6), and a few minutes later the FiO_2 had to be lowered.

Fifteen minutes after the introduction of HFOV, FiO_2 was only 0.55, while arterial blood-gas parameters were as follows: pHa: 7.35, PaO_2: 16.89 kPa, $PaCO_2$: 6.38 kPa, BE: 0 mmol/l. We observed a significant fall in spontaneous breathing on HFOV: in about 10 minutes it decreased to 4—5 breaths per minute. For the fol-

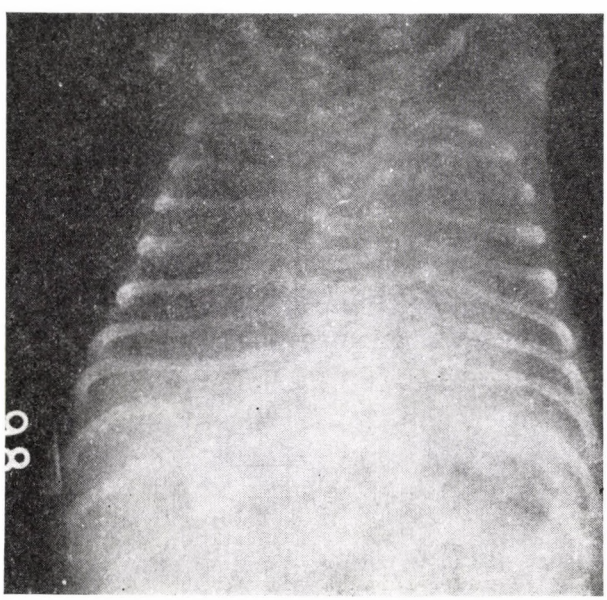


FIG. 5. Case 1 Chest X-rays at 1.5 hours of life

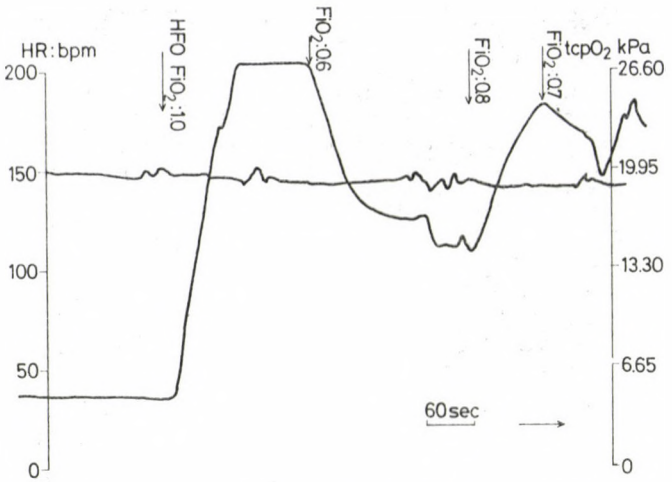


FIG. 6. Case 1. $TcpO_2$ and heart rate curves a few minutes before and after the beginning of HFOV

lowing 4 hours the infant was ventilated with the above parameters. Blood-gas values at the end of this period were, pH: 7.29, PaO_2: 17.82 kPa, $PaCO_2$: 6.58 kPa, BE: −4 mmol/l. To eliminate carbon dioxide-retention, flow rate was increased to 11 l/min, while with regard to the slightly higher arterial oxygen tension, MAP was simultaneously decreased to 13 cmH_2O. The normalization of $PaCO_2$ and a practically unaltered PaO_2 were observed at the control examination twenty minutes later (PaO_2: 17.68; $PaCO_2$: 5.05 kPa).

These ventilatory parameters were maintained for the next 40 hours on HFOV. The values of arterial pH were between 7.38 and 7.43 while arterial pO_2 and pCO_2 tension fluctuated between 13.56 to 25.27 kPa, and 3.72 to 5.71 kPa, respectively. Prior to HFOV severe arterial hypotension had occurred as a consequence of hypoxaemia and HMD, and it was impossible to normalize blood pressure by volume replacement or by dopamine infusion (4 μg/kg/min). At this stage arterial blood pressure was already in the normal range (40/14 mm Hg), but could be maintained only with infusion of 4 μg/kg/min of dopamine.

At the end of this period PaO_2 increased over 26.6 kPa. FiO_2 was reduced to 0.40, and HFOV was continued for another 30 hours with the same parameters. Weaning from HFOV was then started: the oscillator was switched off, while flow rate, MAP and FiO_2 were left unchanged. Spontaneous breathing returned in 3 minutes and soon became regular with a frequency of 40—45 breaths per minute. After 21 hours and 15 minutes the patient was extubated and ventilated by nasal sprongs with the following CPAP parameters: flow rate: 5 l/min, FiO_2: 0.7, EEP: +2 cmH_2O. Chest X-rays following extubation in Fig. 7.

On nasal CPAP, further improvement occurred in the patient's condition, and he maintained normal blood-gas values. After 4 days it was possible to wean him off the nasal CPAP and following one week postintensive care he was discharged in good condition.

Case 2. This boy was a second child born at term after a normal pregnancy and delivery. Apgar scores were 9/6/8 and birthweight 3150 g. Respiratory distress occurred shortly after birth and the baby was transferred to our unit at 4 hours.

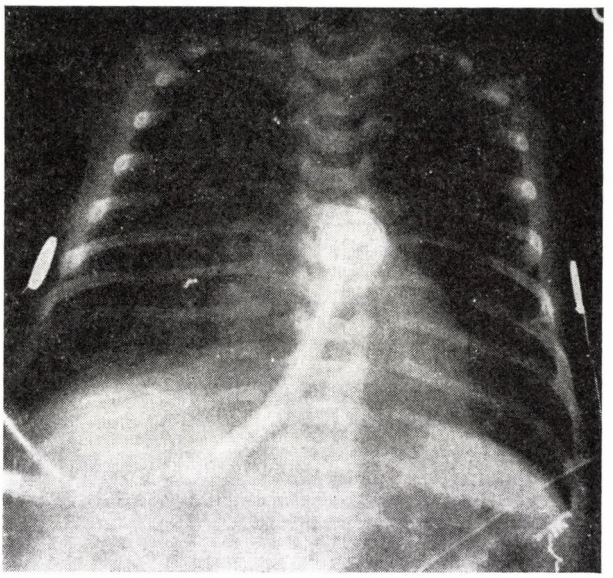


FIG. 7. Case 1. Chest X-rays following extubation. Alveolar aeration, granulatority disappeared almost entirely, the lungs became larger and radiolucent. Some parahilar streakiness can still be seen on the right. The heart is not enlarged

Examination showed severe respiratory distress with a Silvermann-Anderson score of 9. Peripheral pulses and arterial blood pressure were normal and there was no murmur audible over the heart. There was no hepatomegaly and no sign of a central nervous system lesion. Arterial blood gases under headbox in oxygen were pH: 7.10, PaO_2: 5.05 kPa, $PaCO_2$: 8.11 kPa and BE: −13 mmol/l. WBC count was 11.4 G/l; the gastric aspirate was full of white blood cells and Gram positive cocci. Chest X-rays showed a typical picture of intrauterine pneumonia with wet lung. Later bacteriological examinations revealed a streptococcal infection. Following nasal CPAP therapy (flow rate: 5 l/min, FiO_2: 0.7, EEP: +3 cmH_2O) and antimicrobial treatment (penicillin, oxacillin, gentamycin) a significant improvement occurred in the clinical condition and the arterial blood gas status (pH: 7.41, pO_2: 7.98 kPa, pCO_2: 4.65 kPa, BE: −2 mmol/l).

At 45 hours the neonate's clinical condition deteriorated with the reappearance of severe dyspnoea and tachypnoea. Since arterial pO_2 had subsided and remained low despite an FiO_2 of 1.0, the baby was put on a conventional ventilator (Loosco Amsterdam Infant Ventilator MK 2), and was ventilated with the following parameters: flow rate 5 l/min, FiO_2: 1.0, BPM: 40, PIP: 30 cmH_2O, EEP: 5 cmH_2O, I : E = 1 : 1. After a transitional improvement a further deterioration occurred both in the clinical status and in the blood gas parameters. Fig. 8 shows the patient's chest X-rays at 62 hours of age.

Beside severe interstitial infiltrates a significant pneumomediastinum could be visualised. In order to improve oxygenation and in this way enhance nitrogen-washout, tolazoline was also administered and hyperventilation was attempted.

Since no improvement occurred, the neonate was put on HFOV at the postnatal age of 62 hours and 25 minutes and was ventilated with the following parameters: 17 Hz, volume: 26 ml, FiO_2: 1.0,

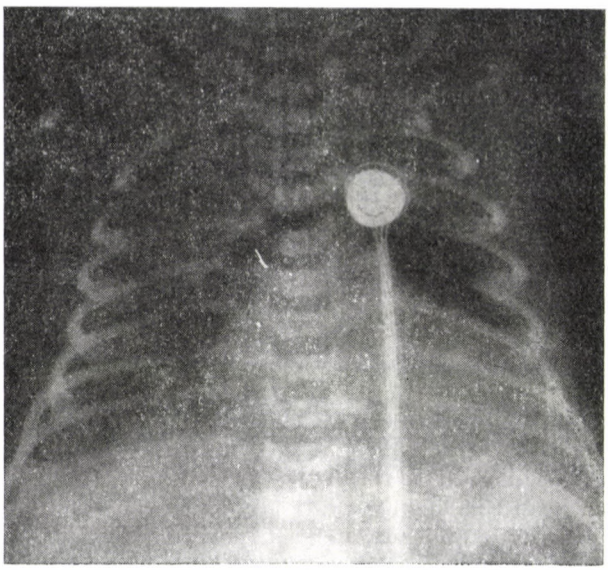


FIG. 8. Case 2. Chest X-rays at 62 hours of life: a voluminous collection of air can be seen around the heart. The air is elevating the thymic lobes. The heart is pushed downward and the air blocks the great vessels. Dense, hazy infiltrates with superimposed pulmonary oedema in both lungs

TABLE II

TABLE II. Case 2. Blood-gas parameters prior to and after HFOV "t/min" represents time intervals in minutes that elapsed prior to (—) and following (+) the beginning of HFOV

HFOV +100 min +250 min
FiO_2: 1.0
Flow rate: 20 l/min ····· 40 l/min ····· →
MAP: 11 cmH_2O ·····
Vol: 26 ml ····· 9 cm H_2O ····· →

pH	7.35	7.33	7.23	6.92	6.99	7.01	7.13	7.20	7.32
PaO_2 (kPa)	9.04	6.51	5.71	3.32	9.84	17.55	19.81	20.74	19.28
$PaCO_2$ (kPa)	5.32	5.18	7.98	9.31	7.18	11.3	6.25	7.31	5.58
Total CO_2 mmol/l plasma	22.5	21.0	25.5	15.0	13.5	22.0	16.0	21.8	22.0
Actual bicarbonate mmol/plasma	21.5	20.0	24.0	13.5	12.3	20.2	15.0	20.5	21.0
B. E. mmol/l blood	—3.5	—5.0	—4.5	—21.8	—20.0	—14,0	—14,0	—8.0	—4.2
t/min/	—215	—175	—50	—5	+15	+95	+168	+330	+780

flow rate 20 l/min, MAP: 11 cmH_2O. The blood gas parameters prior to and after HFOV are summarized in Table II.

Following HFOV, oxygenation improved significantly, while carbon dioxide retention remained practically unchanged. Doubling the flow rate resulted in normalization of $PaCO_2$, while by enlarging the lumen at the end of the low pass filter MAP could be maintained at a constant level. 780 minutes after the start of HFOV, arterial pH raised over 7.30, and PaO_2 and $PaCO_2$ values were between 21.28—28.32 and 5.28—7.15 kPa, respectively. Following HFOV a remarkable decrease in spontaneous breathing occurred. This fall, however, was not as great as that of the preterm infant with HMD.

Chest X-rays performed 17.5 hours later are seen in Fig. 9.

After 34 hours on HFOV with a gradual decrease in FiO_2 to 0.60 and in MAP to 7 cmH_2O, and otherwise unchanged parameters the oscillator was finally switched off. Since the clinical condition and blood gas parameters remained normal, 9 hours and 40 minutes later the infant was extubated and successfully weaned off the ventilator. He required 4 days postintensive care with oxygen supplementation, and on the next week he was discharged.

With the appropriate nebulization and heating of the inhaled gas, no side effects were seen in our patients. Moreover, when seen two or three months after discharge, none of the infants had any sign of RLF. They had normal chest X-rays, and their somatomental development was appropriate for both gestational and postnatal age.

Discussion

Studies on HFOV have demonstrated that HFO is not only capable of ensuring gas exchange in the infant with RDS, but may offer better possibilities for oxygenation than conventional ventilation in the preterm infant with HMD. Since it was suc-

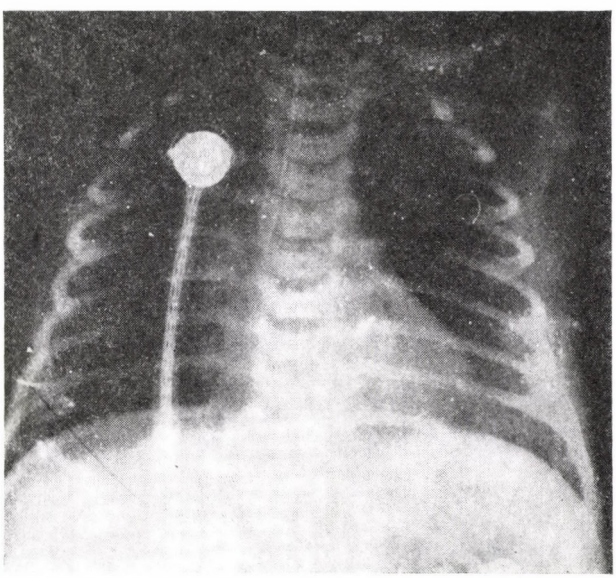


Fig. 9. Case 2. Chest X-rays at 17.5 hours after the beginning of HFOV. Pneumomediastinum and pulmonary oedema disappeared, the lungs are radiolucent

cessfully applied in some cases of PIE, with the use of HFOV the incidence and gravity of pulmonary barotraumas may be lowered better than by conventional ventilation.

Our patient with severe HMD could not adequately be ventilated by conventional means, since the arterial pO_2 values remained under 30 mm Hg in spite of any change in ventilator settings and the administration of tolazoline. With the use of HFOV a rapid and excessive improvement in the oxygenation occurred, and a few minutes later it was possible to lower the FiO_2. The infant was ventilated with HFOV for 70 hours and 50 minutes, and he could easily be weaned off the ventilator. In the other infant presenting with IUP and severe PM, on HFOV a rapid improvement occurred in the blood-gas parameters, in the clinical condition and in the radiological findings. No signs of PM could be seen on the X-ray picture 17.5 hours after the beginning of HFOV therapy. It was thought that in this case at least three factors must have been involved in the rapid recovery, the low airway pressure, the higher arterial pO_2 value and an eventual direct effect of HFOV. In this case the CO_2 retention could only be eliminated by doubling the flow rate, while the MAP was kept constant by elarging the diameter of the lumen at the distal end of the low pass filter.

The importance of nebulization, warming and sterility of the inhaled gas must be stressed, especially when high rates of flow are necessary to avoid postextubation atelectasis. To determine its exact mechanism of action, the correct area of indications and the possible side effect of HFOV need further investigations. It seems, however, encouraging that in our patients with life-threatening respiratory disturbances HFOV resulted in definitive recovery, while no improvement could be achieved by conventional ventilation.

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Absence of responses in energy metabolism and respiratory quotient to carnitine infusion in premature infants

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Plasma levels of total, free and acylcarnitine, as well as oxygen consumption and respiratory quotient were determined in premature infants maintained at neutral temperature. The effects on these parameters of intravenous infusion of 24 mg/kg/day carnitine were studied. Total, free and acylcarnitine increased and the acyl/free carnitine ratio decreased significantly during the four-hour study period. Resting heat production and respiratory quotient remained practically unchanged throughout the study period, indicating that in the face of carnitine sufficiency exogenous carnitine did not influence whole body heat production and substrate utilization pattern in premature infants. Further examinations in carnitine depleted infants will be required to clarify the regulatory role of carnitine in neonatal fatty acid metabolism and non-shivering thermogenesis.

Interesting findings and observations concerning the effects of carnitine on lipolysis and energy generation in white adipose tissue [6, 7] raised the question as to the possible capacity of exogenous carnitine to influence total body heat production in premature infants with and without carnitine deficiency. The exploration of this aspect may help in understanding the functional role of carnitine in postnatal fat and energy metabolism. This study examines the effect of intravenously infused carnitine on total oxygen consumption and RQ in premature infants exhibiting a satisfactory plasma carnitine level.

Material and Methods

Ten premature infants fed on human milk and without serious postnatal complications were admitted to the study. They were single births with a mean gestational age of 34.4 weeks (range, 33—36 weeks) and mean birthweight of 1961 g (range, 1660—2200 g). The study was performed at 3.8 postnatal days (range, 2—5 day). The caloric and volume intake prior to the study averaged 86 kcal/kg/day and 136 ml/kg/day, respectively.

The design of the study was as follows. Two hours after the last feed carnitine with a quarter physiological NaCl solution was infused at a rate of 24 mg/kg/day during a 4 hour-period. Blood samples were withdrawn before and by the end of the second and fourth hour of carnitine infusion. The samples were placed in heparinized test tubes and after centri-

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fugation stored at −20°C until laboratory analysis. Free and esterified carnitine were measured radiochemically [4] with some modification [14]. Total carnitine refers to the sum of esterified and free carnitine.

Oxygen consumption and CO_2 production were measured using the Kipp diaferometer, which allowed a continuous measurement of respiratory gas exchange and of the respiratory quotient [12]. Readings were made every minute, except when reference was made to room air. The studies were performed within the zone of thermoneutrality. Energy metabolism was expressed as kcal and kJ/kg/day. Each value of heat production and RQ represented the average 30 minute intervals throughout the study period.

Results

Fig. 1 shows the plasma carnitine values prior to and during carnitine infusion. It is seen that the significant increase in total, free and acylcarnitine concentration was associated with a significant fall of the acyl/free carnitine ratio by the end of the four-hour infusion period indicating a much larger increase in the plasma level of free than of esterified carnitine.

In Fig. 2 are seen the average values of resting heat production and respiratory quotient at neutral tem-

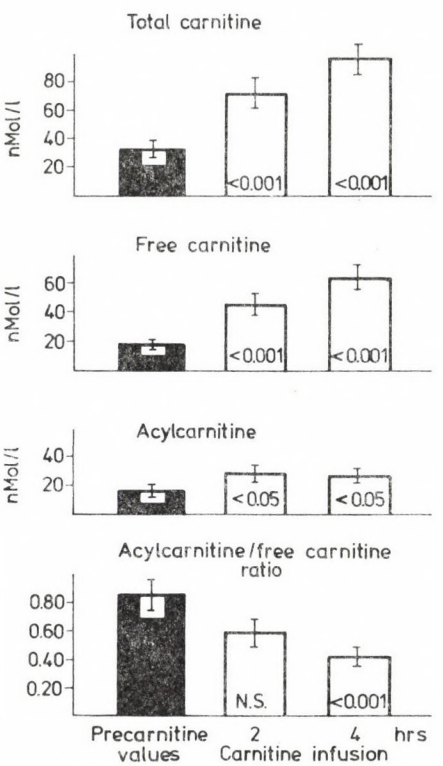

Fig. 1. Mean plasma total free acylcarnitine and acylcarnitine/free carnitine ratio prior to and during carnitine infusion

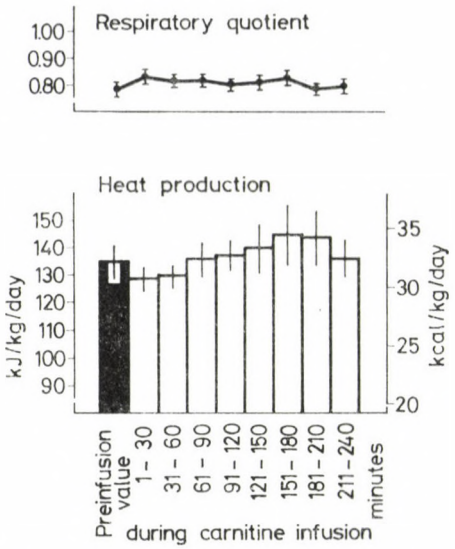

FIG. 2. Mean heat production and respiratory quotient calculated for 30 minute periods before and during carnitine infusion

perature calculated for periods of 30 minutes, and also that no significant changes occurred in these metabolic parameters during carnitine infusion.

DISCUSSION

It is well known that after birth a marked increase in fatty acid oxidation occurs, reflected by a large fall in the respiratory quotient [2, 18]. As a result, the energy metabolism of the unfed newborn is dominated by fat utilization, about 80—85% of energy is produced by this pathway [5]. It is, in fact, this metabolic transition which has brought into focus the functional role of carnitine for optimum oxidation of fatty acids in the neonatal period. So far no examinations aimed at the effect of exogenous carnitine on heat production in the newborn infant have been reported. The findings of an increase in core and skin temperature in response to carnitine administration in newborn rabbits [16] fit in with the in vitro observations, that carnitine stimulated lipolysis and oxygen consumption in the human white adipose tissue [7]. However, a direct proof of the capability of exogenous carnitine to enhance non-shivering thermogenesis as suggested by Hahn and Skala [3] is still lacking. Therefore, further work will be required to clarify the regulatory role of carnitine in neonatal fatty acid metabolism and non-shivering thermogenesis. This has led us to examine heat production and RQ during carnitine infusion in premature infants. The results were negative, no significant change could be observed in oxygen consumption

and respiratory quotient in response to carnitine.

Further understanding of the suggested role of carnitine in a better energy generation by fat metabolism will come from the exploration of its influence on heat production and substrate utilization in carnitine depleted infants maintained at and below the neutral temperature. The relationship between carnitine deficiency and cold induced thermogenesis appears to be of particular interest. Plasma carnitine and substrate measurements in themselves cannot answer some important questions arising from a number of investigations [8, 9, 10, 11, 15, 17]. What does, for example, represent the low serum level of carnitine during intravenous fat alimentation? Does it indicate deficiency and hence an impaired fatty acid oxidation or is it simply due to the increased fatty acid utilization? It is conceivable that in different conditions associated with an increased fat metabolism, a low plasma carnitine level may develop without depletion in tissues (heart, skeletal muscle, brown fat) oxidizing fatty acids as a major source of energy. It would also be necessary to know what degree of deficiency matters in energy generation and does exogenous carnitine exert the same regulatory function in fatty acid oxidation as suggested for the endogenously produced carnitine.

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Transplantation of stem cells of embryonic liver in a patient with severe combined immunodeficiency

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A patient had severe combined immunodeficiency syndrome and x-chromosomal recessive heredity. Since the parents and siblings were not suitable as HLA-compatible bone marrow donors, stem cells from embryonic liver were transplanted intravenously in 3 stages (6×10^6; 3.5×10^6, and 9×10^7). Transplantation was tolerated well; there were no signs of a graft-versus-host reaction. Examination of the immunological condition after transplanation showed evidence of T-cell reconstitution, immunohistochemistry revealed beginning immune globulin production. The child died at the age of 5 months due to respiratory failure.

Severe combined immunodeficiency (SCID) is a partly genetically determined lethal T and B cell defect which was originally thought to be caused by deficiency of lymphatic stem cells. Today SCID designates a heterogeneous group of congenital T-B-cell defects of differing pathogenesis. Apart from the conventional "Swiss type" of SCID with autosomal recessive heredity, there is a type with x-chromosomal recessive inheritance.

The preferred therapy for SCID is allogeneic bone marrow transplantation from an HLA-identical, MLC-compatible sibling donor. This method has so far given the best results with regard to long lasting and complete immunological reconstitution [1, 2, 3]. Recently there has been a number of papers reporting on successful bone marrow transplantation from related or non-related donors who were not fully identical [11, 12, 19], but in these cases a graft-versus-host reaction is to be expected.

As an alternative treatment for SCID patients for whom histocompatible bone marrow donors are not available, transplantation of vital embryonic liver has been proposed [4, 5, 8, 20]. We have carried out transplantation of stem cells of embryonic liver in an infant suffering from SCID with x-chromosomal recessive heredity, at the age of 12 weeks. The present paper reports on this therapeutic approach.

Genealogy

Under the authors' care is a family from which over two generations a total of 11 male descendants originated. Of these, 8 had died in early

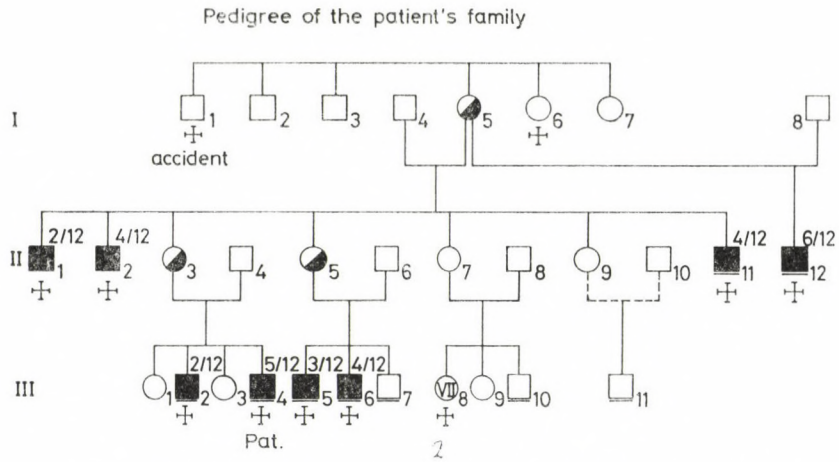

FIG. 1. Genealogical tree of the family afflicted with SCID

infancy (Fig. 1). The grandmother (I 4) of the family had had 3 brothers and 2 sisters who had all reached adult age.

The marriage of I 4 had produced a total of 7 children, 3 boys and 4 girls, plus a boy born out of wedlock (II 11). The boys had died between 2 and 6 months of age.

In the meantime, 3 daughters of I 4 have married (II 3, II 5, II 7). The oldest daughter (II 3) first gave birth to a healthy girl, followed by a boy (III 2) who was the first for whom the diagnosis "Severe combined immunodeficiency" was put forward and verified in immunological and histological terms. He died from generalized cytomegalovirus infection and giant-cell pneumonia. The third child of II 3 is a clinically healthy girl.

The second daughter (II 5) of I 4 has so far had 3 boys, of whom 2 died in our hospital due to immunodeficiency. In the third boy (III 7) from this family immunodeficiency was excluded both immunologically and histologically. He is developing normally. Healthy boys have been born to II 7 and II 9 whose transmission status therefore remains unclear. On the whole, there can be no doubt about the x-chromosomal heredity of SCID in this family as it has affected only boys and, in addition, half brothers from different fathers in one generation.

Report of a Case

Another boy (III 4) was born in this family in March 1981 who was admitted to our hospital at the age of 4 weeks. SCID had been diagnosed because of a reduced and disproportionate transformation response of peripheral lymphocytes to non-specific T and, later on also to B cell mitogens, as well as of a continuous decrease in serum immunoglobulins after the 4th week of life.

Histological examination of an extirpated lymph node revealed an immature structure without characteristic B and T

regions and without secondary and tertiary follicles. Immunohistochemistry showed no IgA and IgG production, while IgM, $\varkappa$ and λ were detected in small amounts. Other findings which supported the diagnosis were negative skin test reactions, the absence of a thymic shadow and the absence of plasma cells in the bone marrow. ADA activity in plasma and erythrocytes was normal. At the age of 12 weeks stem cells of embryonic liver were transplanted three times during a period of 12 days (Table I). The patient received a total of 1×10^8 cells (i.e. 2×10^7 cells per kg of body weight) intravenously. A maximum of 90 min elapsed between removal and the intravenous application of stem cells. Transplantation was tolerated well, and subsequently there was no clinical or morphological (skin excision) evidence of any graft-versus-host reaction. The patient died 8 weeks after transplantation, at the age of 5 months, due to respiratory failure. A few days earlier 100 ml human gamma globulin had been given intravenously.

Autopsy revealed a SCID and a dysplastic thymus anlage without lymphocytes, generalized cytomegalovirus infection and marked hyaline membranes in the lung where immune complexes were detected by means of immunohistochemistry.

Methods

HLA typing was done according to a modified NIH technique using the lymphocytotoxic test on Terasaki plates.

Lymphocyte transformation test (LTT). 1×10^6 mononuclear cells from heparinized blood (free of phenol) which had been enriched by glass adhesion were cultivated in Parker's medium with antibiotics and 20% autologous plasma at 37°C for between 3 and 7 days, in the absence (control) or presence of different mitogens: phytohaemagglutinin (PHA) 20 μl/ml, concanavalin A (Con A) 20 μg/ml, pokeweed mitogen (PWM) 20 μl/ml (3 and 7 days, and lipopolysaccharide from *E. coli* B) 44 0111 (LPS). Evaluation was carried out morphologically by counting of transformed cells.

Mixed lymphocyte culture (MLC). In view of the expected non-reactivity of the potential recipient, the response of the potential donor toward the cells of the recipient was tested, with the donor cells acting as the responding cells and the recipient (patient's) cells as the stimulating cells. The lymphocytes of the potential donor and the potential recipient were separated from heparinized blood using Ficoll-Paque, and suspensions of 1×10^9 lymphocytes/l in RPMI 1640 medium were prepared adding Hepes, L-glutamine, antibiotics and 20% inactivated AB serum. The responding cells were subjected to no further treatment. Stimulating cells were incubated with mitomycin C (Serva) 25 μg/10^7 cells for 30 min at 37°C. After washing they were readjusted to 1×10^9 lymphocytes/l. Equal aliquots of both responding and stimulating cell suspensions were pipetted on round-bottom plates (100 μl = 1×10^5 cells each), gassed with CO_2, incubated for 6 days at 37°C

Table I

Transplantation of stem cells from embryonic liver

Uterus — TE	Weeks of gestation	Transplanted cells
Uterus myomatosus	8	6×10^6
Carcinoma in situ	6	3.5×10^6
Carcinoma in situ	12	9×10^7

and labelled 16 hours before the termination of cultivation with 37 KgBq/well ^{3}H-thymidine (spec. activity 333 KBg/mmol). The cells were harvested using a Flow cell harvester and radioactivity was measured in LKB liquid scintillation counter.

Immunoglobulin determination was done according to Mancini using conventional LC plates (Behring Corp).

Stem cell preparation (Table I). Indication for extirpation of the uterus during pregnancy was in one case a myoma, and in two others in-situ carcinoma in 40/42-year-old women who wanted no further children. After removal of the uterus the entire amniotic sac was removed under sterile conditions and the liver dissected in a laminar air box. The liver was cut into small pieces with scissors, suspended in the medium and passed through cannulas up to S 20. The medium consisted of an electrolyte solution containing infesol, tromethamol, calcium gluconate, cyanocobalamine, folic acid, streptomycin and penicillin. Cell counts were estimated and one vitality test and several sterility tests were performed.

Results

HLA typing showed an antigen difference in the A-locus between the father and the patient, and uncertain homozygosis in the B-locus. MLC gave a threshold index and therefore no full compatibility for the D-locus. The other family members had only one compatible haplotype each, so that a suitable donor for allogeneic bone marrow transplantation was not available.

At no time was the patient suffering from lymphopenia. The number of lymphocytes was always above 2.0 Gpt/l. Transplantation of stem cells from the embryonic liver was followed by a marked increase with a maximum of 6.8 Gpt/l 6 weeks after transplantation (Fig. 2). The LTT during the 4th week of life showed slightly reduced T cell functions of peripheral lymphocytes. At the same time an extreme increase in the detection of B cell properties and spontaneous activation of lymphocytes was observed. In the 10th global T cell function, measured by PHA response, was reduced (PHA transformation 0.23, standard 0.79), although certain T cell subpopulations and especially suppressor and helper cells, continued to be stimulated well (Con A, PWM three-day value). There was a slight increase of B cells in the circulation which could be autonomously stimulated by LPS. Shortly before and one week after liver cell transplantation almost no T or B cells could be stimulated, except for a T cell subpopulation stimulated by PWM after 3 days. On the other hand, subnormal T-cell PHA transformation was verified 6 weeks after transplantation, with the Con A transformation (suppressor cells) higher than the PHA transformation. This indicated an incomplete but clear T cell reconstitution (Fig. 2). The concentration of immunglobulins in the serum which was still normal at the age of 4 weeks, continuously dropped despite the transplantation of stem cells of embryonic liver and substitution with human gamma globulin.

The serum IgM content had been in the lower standard range before

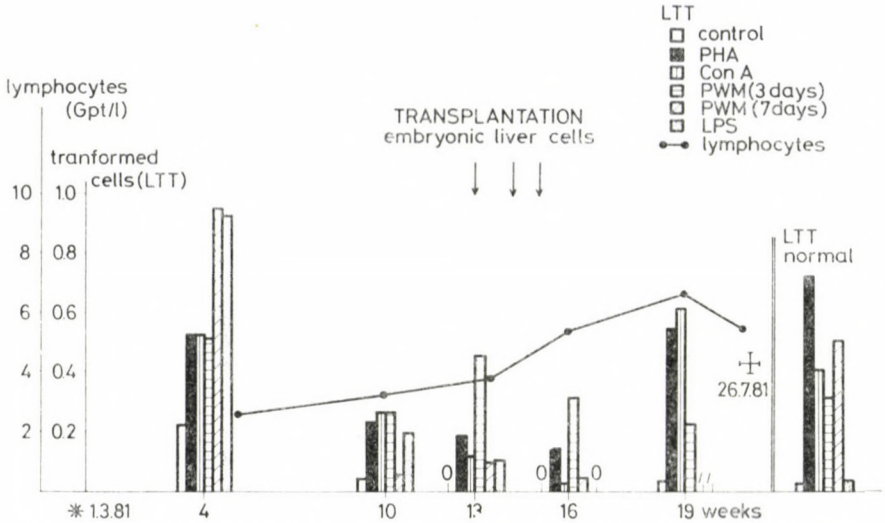

FIG. 2. Absolute lymphocyte number and results of the lymphocyte transformation test before and after transplanation of stem cells from embryonic liver

transplantation and rose very slightly 5 weeks after transplantation. The immunohistochemical staining (PAP technique) of cytoplasmic IgM, IgD, kappa and lambda in the lymph node, spleen and appendix (IgG in isolated cases) also pointed to an initial immunoglobulin secretion of lymphoplasmocytoid cells.

DISCUSSION

In view of the genealogy, the immunological, histological and immunohistochemical findings and the development of the disease, the diagnosis of SCID with x-chromosomal recessive heredity could not be doubted. No satisfactory explanation could be offered for the fact that clearly functional T cells were found at the age of 4 weeks (PHA, Con A and PWM transformation in the LTT, Fig. 2). This may have been caused by the intrauterine transmission of maternal cells since maternal lymphocytes which pass the placental barrier may persist in children suffering from SCID and contribute to the development of chimerism without a graft-versus-host reaction [13, 17, 18]. Similarly, antigenic stimulation, for example by vaccines, may induce lymphoproliferation in cases of SCID [1].

In our patient bone marrow transplantation was impossible because there was no HLA identity and no MLC compatibility of the other family members. In addition, no one was ready for bone marrow donation. We decided to transplant stem cells from embryonic liver in view of the fact that thymus transplantation alone had brought only short [16], and thymus factors no, success whatever

[7, 22]. Until the 12th week of gestation the embryonic liver contains no mitogen-reactive lymphocytes [14] and is therefore suitable for allogeneic transplantation without regard to histocompatibility antigens. The development of graft-versus-host disease with fetal liver over 12-weeks remains a theoretical consideration, especially if the applied cell-mass is so low as in our case. Liver cell transplantation is designed to give the recipient a population of lymphatic precursor cells which have only minimal potency for triggering a graft-versus-host reaction. The qualitative function of these cells concerning their suitability for transplantation and proliferation seems to differ widely, depending on the stage of gestation and the particular case, and therefore no correlation between the number of cells transplanted and the therapeutic success seems to exist [12]. In animal experiments approximately 2×10^8 cells per kg of body weight have been required to restore the immunocompetence and to develop a donor cell population [9]. However, stem cells from embryonic liver of this order of magnitude can be obtained only from fetuses older than 16 weeks where serious graft-versus-host reactions are to be expected. Successful transplantation or auto-restauration [21] has been achieved in humans with fewer cells [4, 20].

We have transplanted a total of 2×10^7 embryonic liver cells from the 6th, 8th and 12th week of pregnancy (calculated from the last menstruation) in three sessions, over a period of 12 days (Table I). The haemopoietic cell composition of the 6-week liver remarkably differs from that of the 12-week one, including differences in lymphocyte and lymphoid cell content [10].

The functional reconstitution of the immune system after liver cell transplantation develops gradually and takes several months [9]. No significant population of donor cells has so far been detected earlier than 6—10 weeks after transplantation [14]. Our patient showed a marked T cell mitogen response of the peripheral lymphocytes and a simultaneous rise in the absolute number of lymphocytes as early as 6 weeks after transplantation (Fig. 2). No increase in the serum immunoglobulins was found, with the exception of a slight rise in IgM (Fig. 3). IgM has also been detected immunohistochemically in the form of cytoplasmic IgM in lymphocytes of lymph nodes, spleen and gut. On the other hand, IgG-IgA secretion was not clearly demonstrable by the time the patient died at the age of 21 weeks, 8 weeks after transplantation.

At least some of the SCID patients suffer from a primary alteration in differentiation of the epithelial thymus anlage so that the hormone output of the thymus is impaired and the bone marrow stem cell does not develop into a differentiated T cell. In most cases, therefore, complete immunological reconstitution has been achieved only after simultaneous or subsequent thymus trans-

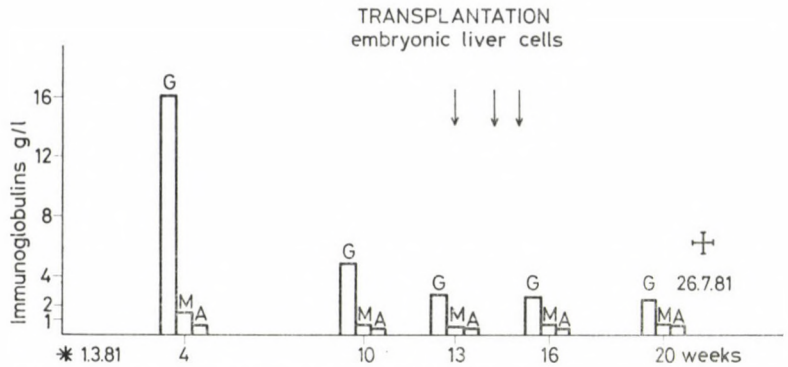

FIG. 3. Immunglobulins (g/L) before and after transplantation of stem cells from embryonic liver

plantation [15] or grafting of cultivated thymus epithelium [6]. The results were particularly favourable in cases where the lymphocytes of SCID patients after co-cultivation with normal thymus epithelium had differentiated into functional T lymphocytes [6].

Transplantation of stem cells from embryonic liver does not influence thymus function, nor the rise ot the thymic hormone level [14] and, in our patients, did not induce homing of the patient's thymus with T lymphocytes. Unfortunately, in our case the time of observation from transplantation to death was not long enough for an assessment of whether the stem cells from embryonic liver alone would have caused full and lasting immunological reconstitution. Moreover, we were not able to document true transplantation, and that is why auto-restauration could not be excluded [21].

According to our clinical observations, the intravenous application of human gamma globulin seems to have initiated the fatal phase of the disease. It is possible that the human gamma globulin produced a local immunocomplex reaction. The antibodies of the human gamma globulin preparation may have reacted with bacterial as well as antigens of the patient and led to massive excretion of immune complexes which induced the alteration in pulmonary diffusion. Breakdown of these immune complexes was obviously impossible because of a reduced capacity for phagocytosis of the macrophage system due to the generalized cytomegalovirus infection.

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Metabolic alkalosis with hypertonic dehydration in a patient with diarrhoea and magnesium oxide ingestion

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An eight weeks old infant with diarrhoea and dehydration became markedly alkalotic after administration of magnesium oxide powder. The literature does not substantiate the premise that significant magnesium deficiency may occur during acute gastroenteritis in an otherwise healthy infant. Physicians should be warned about this form of therapy as a possible cause of metabolic alkalosis. The possibility of congenital alkalosis with diarrhoea should be considered in differential diagnosis.

The interest about metal minerals in human nutrition and in the treatment of many illnesses has increased in the last decades. Among them, magnesium is most important in the biological process. Magnesium is the fourth most abundant cation in the human body and, after potassium, it is the second in the intracellular water and soft tissues. The magnesium content of the human body is about 20 mmol/kg fat free body weight. Approximately half of the total body magnesium is contained in bone and the other half exists primarily in the intracellular water of soft tissues. The highest non-osseous magnesium content can be found in skeletal muscle and heart. In the blood the magnesium concentration is low: 0.75—1.25 mmol/l and, accordingly, only 1% of the total body magnesium is in the extracellular water. About one third of the plasma magnesium is protein bound, the major part of the remaining diffusible fraction is free ionised magnesium [20].

It is well-known that magnesium takes part in many enzyme systems, especially those which are connected with carbohydrate metabolism and transphosphorylation; besides it is involved in most reactions of lipid and protein metabolism. In the intracellular water of soft tissues, magnesium acts as a cofactor in a wide spectrum of enzymatic reactions.

The main dietary sources of magnesium are green vegetables, meat, grains and seafood. The daily requirement ranges between 0.15—0.25 mmol/kg body weight. The average

Abbreviations: CNS, central nervous system
GFR, glomerular filtration rate
RPF, renal plasma flow

adult ingests about 10—20 mmol a day in the diet and of this, approximately one-third is absorbed from the gastrointestinal tract. Experiments with radioactive magnesium showed that after ingestion of magnesium in a meal, can be detected within an hour. This may be due to absorption through the gastric mucosa but also to early passage into the duodenum. The rate of absorption reaches a steady level at 2—3 hours. Absorption occurs along the length of the small intestine, and clearly occurs in the upper as well as the lower small intestine. Radioactive magnesium is not absorbed from the rabbit large bowel although magnesium poisoning has been reported after an enema of $MgSO_4$ solution [9].

Magnesium is taken up by a carrier or channel but it may be taken up also by simple diffusion. In humans it is believed that most of the magnesium uptake occurs in the duodenum; in the ileum and colon less magnesium is absorbed than in the duodenum.

The absorption of magnesium is known to be affected by some factors such as the quantity of protein ingested and the presence of phytate [18]. Excess dietary magnesium is readily absorbed and the sulphur is excreted by the kidney. The kidney plays an important part in the regulation of the total body magnesium content, both by increasing the excretion when the intake is high, as in one of the experiments, and by reducing the excretion during periods of deficiency or low intake [10]. The variation in the urinary excretion of magnesium was produced without any significant change in the plasma concentration. The maximum renal response may be due to either raised or lowered magnesium absorption from the alimentary tract but also to a delay in the operation of the regulatory mechanism.

The regulation of magnesium metabolism depends on the parathyroid glands. Alterations in the blood concentration of magnesium affect the activity of the parathyroid and it has been demonstrated [5] that hypermagnesaemia suppresses the activity of these glands, although other hormones, such as the thyroid, insulin, adrenaline, mineralocorticoids and vitamin D_3, have also an effect on magnesium homeostasis.

This report describes an eight week old infant, who became markedly alkalotic as a result of the "therapeutic" administration of magnesium oxide powder for acute gastroenteritis. The baby's mother purchased the magnesium oxide in a drug-store after she read of alleged beneficial effects of the compound in cases of diarrhoea.

Report of a Case

An eight week old girl previously in good health, was admitted with diarrhoea of five days duration. The patient was a 3086 g product of a normal full term uncomplicated pregnancy and delivery. She had a normal neonatal course, there was no hyperbilirubinaemia. Prior to the onset of her present illness she had no

medical problems. All ten siblings were alive and well.

On admission the infant appeared to be in no distress, her skin turgor was fair, her mucous membranes were moderately dry, the anterior fontanel was below plane, the eyeballs were not sunken. Neurological examination demonstrated an infant with a healthy cry, poor suck, 2+ symmetrical deep tendon reflexes. Her rectal temperature was 39.4°C, respirations were 42 per minute, neither laboured nor shallow, apical pulse was 140 per minute and regular. The rest of her findings was normal.

Initial therapy was based on the clinical impression that the infant was five to ten per cent dehydrated. The initial rehydrating agent was 0.25% saline with 5% glucose with no added bicarbonate or lactate. After the initiation of therapy, our laboratory reported that the baby's electrolytes on admission were Na: 164 mmol/l, K: 5.1 mmol/l, Cl: 102 mmol/l, B. U. N.: 32.5 mmol/l, and glucose: 5.1 mmol/l.

Pending repeat electrolyte determinations, a heel-stick blood gas determination was made in arterialized capillary blood employing the Astrup method. It revealed a pH of 7.51, pCO_2 of 5.9 kPa, and a base excess of +11.0 mmol/l. The rehydrating solution was changed to 5% dextrose in 0.5% saline, and the therapy was continued.

At admission the patient's mother denied administering any medication to the infant during this illness. It was only after repeated questioning, necessitated by the unusual laboratory findings, that she told of having read in a book that she should administer magnesium oxide powder and so gave two tablespoons to her infant on each of two successive days. The last dose was said to have been administered 18 hours prior to admission.

Serum magnesium approximately 3.5 hours after the start of rehydration was 1.87 mmol/l (maximum normal value in our laboratory is 1.06 mmol/l); CSF magnesium was 1.11 mmol/l.

The infant was carefully observed over the next forty-eight hours. Thirteen hours after admission, the infant had a generalized convulsion with tonic-clonic movements. Seizure activity continued intermittently over the next three hours. Treatment included intravenous phenobarbital (10 mg/kg) followed by 10% paraldehyde infusion, 2 mg valium and 1.0 g calcium-gluconate. During the period of maximum seizure activity, arterial blood gas analysis revealed a pH of 7.35 with a pCO_2 of 70, the base excess was +9 mmol/l. Following the seizures the infant had no further complications. Her electrocardiogram was repeatedly normal. The BUN was normal which suggested normal renal function.

Prior to discharge, complete neurological evaluation including electroencephalogram was normal. Three months later the infant's further growth and development appeared normal.

Discussion

Our dilemma of explaining hypertonic dehydration with metabolic alkalosis left us two diagnostic considerations. First we considered congenital alkalosis with diarrhoea. This entity also known as familiar chloride diarrhoea was first described some thirty years ago [6, 11]. It is characterized by large amounts of watery diarrhoea that persist from the neonatal period. The stools have an excessively high chloride concentration and this is generally the key to diagnosis. Fecal chloride is always greater than 70 mmol/l [7], and after a few months of age it often stabilizes around 150 mmol/l [19]. Hypoelectrolytaemia develops rapidly and often secondary hypoaldosteronism is seen

[19]. Our patient's history, clinical findings and laboratory values which demonstrated hypertonicity seemed to make this an unlikely diagnosis. Additionally our patient produced no stools from shortly after admission until the next morning, at which time an assay was not deemed necessary.

Our diagnostic consideration was the administration of an exogenous alkalizing agent which was indeed the case. The administration of magnesium oxide to this infant resulted in substantial intestinal absorption of the compound, causing hypermagnesaemia and metabolic alkalosis. Since the mother would not give us the powder, we were unable to determine the relationship of this preparation (with possible added sodium chloride) to the child's hypernatraemia, but we ascribed the generalized seizures to the combination of hypernatraemia and metabolic alkalosis. It is difficult to incriminate the hypermagnesaemia since the magnesium ion is known to be CNS depressant via inhibition of nerve impulse conduction [15].

Hypermagnesaemia is rare beyond the neonatal period and with normal renal function. We have never before seen alkalosis in the face of otherwise uncomplicated hypertonic dehydration and diarrhoea. No intravenous alkalizing agent was given to the child.

The influence of modern food faddists and their publications must be constantly kept in mind. The importance of eliciting a thorough and rigorous history of "medications" is emphasized. While examining "health foods" books of the kind described by our patient, we came across the following statement: ". . .diarrhoea often causes such severe deficiences of magnesium that tremor, muscle spasm, and even convulsions occur. Despite the fact that magnesium is laxative, if given daily during diarrhoeas, it improves the appetite, speeds healing, increases the hours of sleep, and helps the baby to be more relaxed."

There appears at best to be a scant evidence in the literature of the development of symptomatic hypomagnesaemia in infants with acute gastroenteritis who previously have been well nourished. Paul and O'Brian [21] reported significant hypomagnesaemia in 47% of a group of Malay and Chinese children with acute gastroenteritis but state that "many of of these children showed signs of protein malnutrition". They further state that "the depression of the magnesium value did not correlate with the severity of the disease". Agarwal et al [11] reported on magnesium levels in the serum of 24 infants with acute gastroenteritis and could find hypomagnesaemia in only one who previously was well nourished.

Jones [14] states that "magnesium deficiency in well nourished infants after gastroenteritis of short duration is not commonly seen". He states that he only knows of three cases in the literature. Additionally it should be remembered that serum magnesium levels do not reflect the state of the body with respect to this

essentially intracellular cation [22].

In conclusion we are concerned about the implications of scientifically unfounded claims in the lay press, and specifically the dangers when misguided individuals attempt to medicate small infants with the newest "health food". We wish to emphasize these dangers as well as to report on one unusual manifestation of illness produced by them. Excessive oral intake of magnesium-containing drugs, antiacids or laxatives may result in hypermagnesic states, particularly in patients with renal or intestinal disease.

Hypermagnesaemia mainly affects the nervous system, suppressing the release of acetylcholine and blocking transmission at the neuromuscular junction, resulting in paralysis of the voluntary muscles. This effect, however, can only be observed at concentrations of about 4.1 mmol/l. Respiratory depression is the consequence of peripheral respiratory paralysis, a main contributor of mortality from hypermagnesaemia. Magnesium also affects the autonomous nervous system, diminishing the release of acetylcholine and thus blocking transmission at the sympathetic ganglia. In the bowel it inhibits synaptic-dependent mesenteric neurones. Magnesium-induced bowel hypomotility may cause meconium plug syndrome in newborns of magnesium treated toxic mothers.

There is a controversy in the CNS effect of this cation as it is believed to be depressant and anaesthetic. This is only true when magnesium is applied directly intrathecally or intraventricularly. At very high serum concentrations (5.3—6.1 mml/l) in spite of peripheral muscle paralysis, the patients were awake and cognizant of pain.

Magnesium is used as an anticonvulsant in toxaemic pregnancy. The mechanism of this effect is not well understood because peripheral administration results in a poor CNS level, suggesting that peripheral rather than central actions (on neuromuscular junctions, autonomous nervous system, etc.) may play a role in the anticonvulsant effect.

There is more evidence that the anaesthetic, anticonvulsant effects may derive from the adjuvant use of magnesium with other agents. CNS depression may be the secondary consequence of hypoxaemia and hypotension, known effects of hypermagnesaemia.

Increased magnesium levels may influence GFR and RPF of the normal kidney with variable results. In the rat kidney, sodium and water reabsorption decreased and a similar effect was observed in the intestinal absorption of sodium.

Excretion of excessive magnesium is usually accompanied by higher excretion of sodium, calcium, chloride and variable amounts of potassium. The incidence of hypermagnesaemia in acute renal failure is invariably high, even without excessive magnesium intake. It may be accompanied by acute anuria, acidosis, and hypermagnesaemia. In renal failure, hypermagnesaemia may result in brady-

cardia, electrocardiographic changes and hyperreflexia at a serum concentration of 4.1 mml/l. At higher serum levels, respiratory depression, coma and cardiac arrest may occur. Immediate but transient reversal of toxicity can be achieved with administration of calcium.

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An in vitro steroid sensitivity test: antibody-dependent cellular cytotoxicity (ADCC) reaction of peripheral lymphocytes in children with nephrotic syndrome

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A steroid effect is described that can be measured in vitro; this was determined by means of the antibody-dependent cellular cytotoxicity (ADCC) method. Examinations on 15 children with nephrotic syndrome revealed a significant correlation between the steroid sensitivity measured in vitro and the clinical sensitivity to prednisolone.

The in vitro measurement of steroid sensitivity yielded fast and reliable information on the effectivity of prednisolone treatment.

In the antibody-dependent cellular cytotoxicity (ADCC) test, an IgG antibody on a target cell serves as the recognition link for the Fc-IgG receptor carrying effector cell. Several cell types of different linkages have been shown to act as effectors: neutrophils, eosinophils, monocytes and lymphocytes. Whereas all the ADCC effectors, which have been named K cells, are Fc-IgG (+), only about half of the Fc-IgG (+) lymphocytes can function in ADCC [22] and these are probably high avidity EA rosette formers. The first evidence for a Null cell-mediated ADCC activity came from Wisloff and Froland [23] who showed that lysis was independent of B and T cells. Using various other methods for purification of the Null subset, Horwitz et al [7] isolated L lymphocytes which did not form rosettes with sheep erythrocytes and lacked membrane stable Ig but possessed an Fc receptor and had the capacity to kill antibody-coated target cells. Data from Takasugi's group have been interpreted to the effect that natural killer (NK) cells were indeed K cells, but "armed" with natural antibodies in vivo [9].

Many of the investigators exploring the relationship between K and ADCC effectors [2, 10] suggested that NK and ADCC effectors were probably the same. This was supported by recent investigations with monoclonal antibodies: anti-human Leu 7 (HNK-1) reacted with both NK and K cells [1]. It has been shown that these cells may bear T-cells and/or myelomonocytic markers and that they can be characterized morphologically as large granular lymphocytes (LGL) which are mostly present in the low-density Percoll fractions [19]. Inter-

feron treatment increased their actvity, while other mediators such as prostaglandins, steroids and cytophilic immunoglobulins inhibited their activity [11].

The ADCC resistance or sensitivity was studied earlier in renal transplant recipients with in vitro methylprednisolone, and it was found that the allograft survival time is correlated with the result of the above test, i.e. it reflected the in vivo steroidresistant or sensitive state [20, 15, 8, 4, 5].

We have performed an investigation of the ADCC reaction to assess the steroid sensitivity of children with nephrotic syndrome, a comparison being made with the clinical effect of steroid therapy.

Patients and Methods

ADCC activity was determined on 16 occasions in 15 children with nephrotic syndrome who were patients of the Nephrology Unit of the Department of Paediatrics, University Medical School, Szeged. The method detailed below was used. 13 healthy young blood donors served as controls.

Separation of effector cells

The effector lymphocytes were isolated on a Ficoll Uromiro gradient [3] after treatment of the whole blood with colloidal iron powder (GAF, USA), from 10 ml venous blood taken with heparin. The lymphocyte suspension was adjusted to 5×10^6/ml in RPMI 1640 culture medium containing 10% FCS.

Performance of ADCC reaction

Fresh, human „O" Rh (D) positive red blood cells were used as target cells. Human anti-D serum was adsorbed onto the cells [21] labelled with $^{51}Cr/Na_2\ ^{51}CrO_4$; 7—8 GBq/mg Cr; Amersham). The effector: target cell ratio was then adjusted to 20 : 1 or 10 : 1. Methylprednisolone was then added to the culture medium in a final concentration of 5—10 μg/ml, the cells were incubated at 37°C in a 5% CO_2 thermostat for 18 hours. The cytotoxicity was calculated from the activity of the supernatant by the formula

$$\text{cytotoxicity \%} = \frac{\text{test supernatant cpm} - \text{spontaneous cpm}}{\text{incorporated total activity cpm}} \times 100$$

The steroid sensitivity tests were carried out not only with the traditional effector excess cytotoxic reaction, but also with the target cell excess cytotoxic capacity test [6, 14].

Steroid sensitivity or resistance was given as a percentage of the inhibition of the ADCC reaction due to the steroid. The ranges were as follows.

<30%	ADDC inhibition: steroid resistant
30—50%	ADDC inhibition: moderately steroid-resistant
>50%	ADCC inhibition: steroid-sensitive

Results

The results of the ADCC inhibition test are given in Table I. In vitro steroid sensitivity was confirmed in every patient with nephrotic syndrome (NS) with a clinical prednisolone sensitivity of ++++. Special mention should be made of patient H. H., in whom the steroid sensitivity found clinically was +++, which

TABLE I

Results of ADCC test in children with nephrotic syndrome

Initial	Age in years	Sex	ADCC inhibition per cent	Sensitive	Moderately sensitive	Resistant	Renal biopsy diagnosis	Maximum proteinuria g/day	Prednisolone sensitivity	Treatment
B. M.	5	♀	45—64	+			MSGN	9.1	++++	Pr. Pr + Chl
K. Zs.	6	♂	33—41		+		(NS)	4.3	++	Pr
Sz. R.	10	♂	0			+	MSGN/MPGN	6.3	0	Pr, Pr + Chl
N. G.	9	♂	50—67	+			MSGN (Sch.HH)	3.26	++	Pr, Pr + Chl
B. Zs.	10	♀	41—50		+		MSGN	23.0	0	Pr, Pr + L. Pr + Chl + hep.
Gy. Z.	8	♂	50	+			MCNS	2.4	++++	Pr, Pr + Chl
Sz. É.	3	♀	50	+			MCNS/FSGN	2.5	++	Pr, Pr + Chl
B. Zs.	10	♀	33			+	MSGN	22.0	0	Pr + L + hep dipyridamide
U. P.	5	♂	36			+	MSGN	5.3	0	Pr + L + indomethacin
K. I.	7	♂	60—74	+			(NS)	3.0	++++	Pr
F. R.	6	♀	48—57	+			MCNS	4.0	+	Pr, Chl
N. E.	11	♀	35—44		+		MSGN (Sch.H)	4.5	0	Pr, Pr + Chl
H. H.	6	♀	15—30			+	MCNS	8.7	+++−+	Pr, Pr + Chl
P. E.	5	♀	53	+			MCNS	1.0	++++	Pr
V. A.	4	♂	34—56		+		(NS) (Sch.H)	3.2	++	Pr
F. P.	9	♂	9—27			+	(NS)	1.5	0	Pr

NS = nephrotic syndrome
Sch.H = Schönlein-Henoch
MCGN = minimal change glomerulonephritis
MSGN = mesangioproliferative glomerulonephritis
MPGN = membranoproliferative glomerulonephritis
FSGN = focal sclerotic glomerulonephritis
Pr = prednisolone
Chl = chlorambucil

TABLE II

Mathematical evaluation of ADCC inhibition test

Control		ADCC inhibition, per cent	Prednisolone sensitivity clinically (in vivo) 0 = 0 points + = 1 point ++++ = 4 points	Prednisolone sensitivity (in vitro) resistant = 0 points moderately sensitive = 1 point sensitive = 2 points
(n = 13)		(n = 16)	(n = 16)	(n = 16)
$\bar{X}$ =	61.07	41.37	1.68	1.12
S.D. ±	9.34	16.9	1.6	0.88
difference	$p < 0.001$	corr. coeff. = 0.71		corr. coeff. = 0.76
		$p < 0.01$		$p < 0.001$

had decreased to + by the time of the in vitro examination.

The change in percentage ADCC inhibition was studied in correlation with the morphological change in the kidney and with the clinical picture, i.e. with the sensitivity displayed to prednisolone treatment. The correlation coefficients were calculated by taking into consideration the number of NS attacks and the duration and effectivity of steroid treatment.

The ADCC inhibition test demonstrated that 7 nephrotic syndrome patients were steroid-sensitive, 4 cases were moderately sensitive, and 5 cases were steroid-resistant.

A significant correlation was found between the result of the in vitro steroid sensitivity test and clinical prednisolone sensitivity in the steroid-sensitive group, in the moderately sensitive group, and in the steroid-resistant group (Table II).

The in vitro steroid resistance exhibited a good correlation in the NS cases with a renal biopsy finding of MPGN or MSGN, involving a serious prognosis. Only in one MCNS patient was there a contradiction; this was H. H., where in vitro steroid resistance was observed in spite of the slight biopsy finding. From among the NS patients demonstrated to be steroid-sensitive in vitro, 2 patients with a renal biopsy finding of MSGN gave a partially contradictory correlation for the clinical picture and the sensitivity; for other 5 steroid-sensitive NS patients the MCNS biopsy finding correlated well with the result of the in vitro test.

2 of the 4 moderately steroid-sensitive NS cases belonged in the serious MSGN group.

CASE REPORTS

Brief accounts of our NS cases are provided below for the purpose of evaluation of the response given to prednisolone.

B. M. (born 18.02.1979), female. Diagnosis: MSGN. Maximum proteinuria without prednisolone: 9.1 g/day. All 5 recurrences responded well to prednisolone, but because of the frequent relapses, alternating prednisolone and chlorambucil treatment was administered for 6 weeks during the last recurrence. Proteinuria-free since combined treatment.

K. Zs. (born 18.01.1978), male. Diagnosis: NS. Bilateral renal hypoplasia, bilateral megaureter. Maximum proteinuria: 4.3 g/day. Renal biopsy: not informative. The proteinuria decreased substantially in response to prednisolone, though it still persists at 0.25—0.5 g/day.

Sz. R. (born 29.09.1973), male. Diagnosis: MSGN from first renal biopsy in April, 1980; MSGN/MPGN, from second renal biopsy in November, 1982. An exact classification was not possible, but at any event the state corresponded to immune complex nephritis. Maximum proteinuria: 6.3 g/day. Prednisolone alone was ineffective. Prednisolone supplemented with chlorambucil led to moderation of the proteinuria. After a biopsy in 1982, prednisolone administration was stopped. The proteinuria is moderate, at present it is 1.8—2.5 g/day.

N. G. (born 10.04.1975), male. Diagnosis: MSGN (Schönlein-Henoch). Maximum proteinuria: 3.26 g/day. Prednisolone alone only moderated the proteinuria; prednisolone combined with chlorambucil eliminated it.

B. Zs. (born 13.02.1974), female. Diagnosis: MSGN. Maximum proteinuria: 23 g/day. The proteinuria has proved resistant to therapeutic efforts. Treatments: Prednisolone; prednisolone + chlorambucil; prednisolone + chlorambucil + heparin; prednisolone + chlorambucil + heparin + dipyridamole; plasmapheresis on 4 occasions.

Gy. Z. (born 29.12.1972), male. Diagnosis: MCNS. Maximum proteinuria: 2.4 g/day. Responded well to prednisolone in all cases but, due to recurrences, chlorambucil too, was administered for 6 weeks.

Sz. É. (born 08.12.1982), female. Diagnosis: MCNS/FSGN. Maximum proteinuria: 2.5 g/day. During prednisolone treatment, the proteinuria was intermittent; accordingly, chlorambucil administration too was begun. Proteinuria currently, 1.5 g/day.

U. P. (born 19.05.1979), male. Diagnosis: MSGN. Maximum proteinuria: 5.3 g/day. Proved steroid-resistant clinically. The proteinuria responded only to prednisolone + chlorambucil + indomethacin treatment. Proteinuria currently, 0.5—1.0 g/day.

K. I. (7 years old), male. NS began 3 years ago, there have been 4 recurrences. Maximum proteinuria: 3.0 g/day. Renal biopsy was not performed. A rapid and good response to steroid in all cases.

F. R. (6 years old), female. Diagnosis: nephroso-nephritis, MCNS. Albuminuria initially accompanied by massive haematuria. Maximum proteinuria: 4.0 g/day. Had steroid treatment for 3 months, during which marked hypertension and obesity developed, and proteinuria decreased to 1 g/day. Subsequently only chlorambucil was administered. Renal biopsy confirmed MCNS.

N. E. (11 years old), female. Diagnosis: MSGN (Schönlein-Henoch). Maximum proteinuria: 4.5 g/day. The proteinuria was unchanged in response to prednisolone treatment, but decreased somewhat when prednisolone was supplemented with chlorambucil.

H. H. (born 04.04.1978), female. Diagnosis: MCNS. Maximum proteinuria: 8.7 g/day. Reacted well to steroid in all cases, though the fourth recurrence took place during inadequate alternating steroid treatment. Renal biopsy was performed during the fifth recurrence; finding: MCNS. Therapy: prednisolone + chlorambucil.

P. E. (born 25.10.1979), female. Diagnosis: MCNS. Maximum proteinuria: 1 g/day. Only prednisolone is administered.

V. A. (born 04.03.1980), male. Symptoms of NS began in March, 1984, in connection with Schönlein-Henoch nephropathy. Maximum proteinuria: 3.2 g/day. 60 mg/m² steroid treatment started on 27.04.1984. The proteinuria improved to 1.4 g/day, but steroid treatment did not eliminate it.

F. P. (born 29.01.1975), male. Admitted with suspicion of focal nephritis. Maximum proteinuria: 1.5 g/day. Participates in 60 mg/m² steroid therapy since 16.05.1984, but the proteinuria is unchanged.

Discussion

Our examinations revealed a significant correlation between the clinical effect of prednisolone treatment, i.e. the prednisolone sensitivity, and the degree of steroid sensitivity or resistance based on the percentage ADCC inhibition in children with NS. Mathematical evaluation showed that the inhibition values obtained with steroid in the blood donors used as controls were almost the same at different target: effector cell ratios both in the ADCC reaction and in the ADCC capacity test, and thus the percentage inhibition could be expressed as a concrete number. At the same time, the percentage inhibition values obtained at different target: effector cell ratios in the case of NS patients were partly different; accordingly, the percentage inhibitions obtained with steroid were given as ranges, which results in a better reflection of the general steroid sensitivity.

The in vitro value of steroid sensitivity or resistance was fairly constant in time (when control examinations were carried out in intervals of 2—3 months) and was well reproducible [14]. For this reason, sensitivity tests were performed only once in the present series of examinations. The only exception was B. Zs., a MSGN patient, in whom the test was repeated after an interval of 2 months.

As concerns the explanation of the steroid effect, one of our conceptions is that the in vitro and in vivo actions of the steroid may be connected with the number of steroid receptors on the surface of lymphocytes, or with the blocking of the receptors.

Szekeres et al (17) have demonstrated that the progesterone binding ability of lymphocytes is of informative value from the aspect of the outcome of pregnancy.

Investigations into the effects of monoclonal antibodies on in vitro immune functions showed that interleukin-2 (IL-2) played important roles in the manifestation of the suppressor and helper activities exerted by T lymphocytes [12]. It has also been demonstrated that the steroids exert their suppressive action during the interaction of interleukins and T lymphocytes [13]. At the International Transplantation Congress in Brighton, Rosenbert et al [15] described a selective effect of methylprednisolone, which was detected in the interaction of IL-2 and T lymphocytes. As a key role is attributed to the HLA-DR antigens in the effect of interleukins, this would explain the manifestation of both steroid sensitivity and steroid resistance in normal individuals. In the future, therefore, we plan to compare the HLA-DR types of healthy subjects with the steroid effect exhibited in vitro.

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Use of counter-immunoelectrophoresis for the detection of chlamydial antigens in serum

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An increasing number of reports on Chlamydia trachomatis pneumonia in infancy has recently been published in the literature. Demonstration of the aetiologic agent depends, however, on laborious culture procedures and serological techniques. Based on the observation of a cross-reaction between certain Acinetobacter species and Chlamydiae, the detection of chlamydial antigens in sera of 13 infants with pneumonia due to Chlamydia trachomatis was performed with antiserum to Acinetobacter by the counter-immunoelectrophoresis technique.

There has been an increasing number of recent reports on Chlamydia trachomatis pneumonia in infancy [1, 2, 5, 6, 7, 8, 10, 14, 20]. Despite a rather distinctive clinical syndrome, the diagnostic tests used for the detection of Chlamydia trachomatis depend on time-consuming and sophisticated procedures like recovery of the organism from the nasopharynx or tracheal secretion, and various serologic techniques. Encouraged by the reports on the detection of antigen in sera of patients with Pneumocystis carinii pneumonitis using the counter-immunoelectrophoresis technique [11, 13], we employed this method for a rapid diagnosis of chlamydial pneumonia in early infancy.

Based on the observation of a serological cross-reaction between Acinetobacter calcoaceticus subspecies anitratus and Chlamydia [3, 4], detection of chlamydial antigens in sera was performed with antiserum to Acinetobacter by counter-immunoelectrophoresis. After a preliminary report [17] we now present our advanced experiences of this technique in 13 infants with Chlamydia trachomatis pneumonia.

Material and Methods

Counter-immunoelectrophoresis (CIE). In our study CIE was performed with an immunoelectrophoresis kit (Instrumentation Laboratory, D-5303 Bornheim 2) containing a universal electrophoresis chamber, a glass slide ($225 \times 75 \times 3$ mm), sodium barbital buffer solution (pH 8.6; ionic strength 0.056), filter paper wicks, a power supply (Pherostat 273) and an agar gel puncher [15]. The glass slide was coated with 25 ml of 1% agarose (Agarose H; LKB Instrument GmbH, D-8032 Gräfelfing) dissolved in the buffer solution.

After cooling, parallel wells 3 mm in diameter were punched 7 mm apart (edge to edge), and the slide was placed in the centre of the chamber. Wells were filled with solution, using capillary pipettes. Antibody containing wells were placed at the anodal side and patients' neat sera (i.e., neither diluted nor concentrated) at the cathodal side. The agarose coated slide was attached by filter paper wisks to reservoirs containing buffer solution. The universal chamber attached to the power source, a constant current of 60 mA (at the power source) was applied for 80 min at room temperature. Slides were inspected unstained for precipitin lines without any additional aid. The production of rabbit hyperimmune serum to Acinetobacter calcoaceticus was carried out by one of us (H. B.); its efficacy for the detection of chlamydial antigens has been published [3, 4].

The potential usefulness of this antiserum for a precipitating reaction with chlamydial antigens in the setting of our CIE technique could be demonstrated using chlamydial group antigens (Institut Pasteur Production, Code 52471), prepared from infected yolk sacs [21]. These antigens served as a positive control during the CIE procedure with patients' sera.

Patients. The study included 48 selected infants admitted from January, 1982, to July, 1983. Their clinical diagnosis (physical findings and radiographic evidence) was either a "delayed" respiratory distress syndrome [18, 19] in older newborns (>one week) with concomitant purulent conjunctivitis and eosinophilia (>400/mm^3) in their blood count, or an afebrile pneumonia with a chronic cough (>one week) in young infants (<six months). The latter had sometimes a staccato cough similar to that with pertussis.

Additional blood samples were obtained from each child for blood cultures and CIE.

For the documentation of a chlamydial infection we determined anti-chlamydial antibody titres. This was also performed by CIE with sera collected from the patients on admission or at the time of the first suspicion of a chlamydial infection, respectively, and with sera after a clinical course of three to four weeks. The source of antigen was the same which had served as a positive control.

The mere qualitative detection of anti-chlamydial antibodies was demonstrated with the patients' undiluted serum samples. Then twofold dilutions of the patients' sera were run against the reference antigen. The highest serum dilution giving a visible precipitin reaction in the CIE procedure was considered the quantitative titre of anti-chlamydial antibodies.

Beside the determination of these titres in all 48 infants, we took secretions of the nasopharynx or trachea of the children with a positive reaction in the CIE procedure (patients' sera and Acinetobacter antiserum) for routine bacteriological cultures and for the demonstration of intracellular chlamydial inclusion bodies in tissue cultures (Mc Coy cells). The latter was done in a two step procedure: First we took the clinical specimen which was immediately frozen to minus 200°C by liquid nitrogen and then stored at this temperature until the final culture procedure with Mc Coy cells [9].

Thirteen newborns with the diagnosis of hyaline membrane disease or aspiration syndrome were selected as control patients.

Results

There were 13 positive results in the CIE procedure of the selected infants. Table I demonstrates the results of the children submitted to Mc Coy cell cultures. All blood cultures were negative for aerobic and anaerobic bacteria. All routine bacteriological cultures from nasopharynx and trachea secretions were negative for Acinetobacter species.

All other children had negative, non-diagnostic titres; there were 11 infants with a qualitative detection of antibodies, but no precipitin reaction with diluted samples.

According to the method applied, the quantitative titres of the anti-chlamydial antibodies did not differentiate between IgM and IgG antibodies. There was, however, an at least twofold increase between the first titres and those obtained three to four weeks later in all 13 children (by this means excluding passively transmitted maternal antibodies). The latter titres are those depicted in Table I.

TABLE I

Results of Mc Coy cell cultures and antigen detection by counter-immunoelectrophoresis in 13 children

Secretions	Culture results (Mc Coy cells)	Routine bacteriological cultures	Counter-immunoelectrophoresis serum	Anti-chlamydial antibody titre
Trachea	+	—	+	1 : 64
Trachea	+	—	+	1 : 64
Trachea	+	—	+	1 : 128
Nasopharynx	+	—	+	1 : 128
Nasopharynx	—	—	+	1 : 32
Nasopharynx	+	—	+	1 : 64
Trachea	—	—	+	1 : 128
Trachea	+	—	+	1 : 64
Nasopharynx	—	—	+	1 : 64
Trachea	+	—	+	1 : 128
Nasopharynx	+	—	+	1 : 64
Nasopharynx	+	—	+	1 : 32
Trachea	+	—	+	1 : 32

The cause for the three negative Mc Coy cell cultures with a positive CIE result could not be evaluated. They might have been due to some technical problem on the way from the patient to the final procedure in the laboratory.

There was no positive CIE result in the 13 control patients; they had no positive blood culture and all Mc Coy cell cultures were negative for chlamydial inclusion bodies. In eight control patients a qualitative detection of chlamydial antibodies was possible, but no precipitin reaction occurred with diluted serum samples.

DISCUSSION

Chlamydia trachomatis pneumonia has recently been described as a

distinctive syndrome characterized by a chronic, afebrile course, diffuse lung involvement, elevated serum immunoglobulins, and eosinophilia. Although these clinical findings apparently are indistinguishable from the pneumonia syndromes associated with other organisms like Cytomegalovirus, Pneumocystis carinii, and Ureaplasma urealyticum [14], diagnostic investigations to demonstrate a specific infectious agent should be made because of therapeutic and prognostic implications. Unfortunately, identification of a specific aetiologic agent depended on rather laborious culture procedures and serologic techniques until recently. CIE has shown promise not only in the early detection of bacterial antigens [12, 15, 16], but also in the recognition of parasitic agents like Pneumocystis carinii [11, 13].

Because culture and isolation of Chlamydia trachomatis are currently available in a few laboratories, diagnostic antisera against its elementary bodies seem difficult to obtain. The observation of a serologic cross-reaction between Acinetobacter calcoaceticus and Chlamydia [3, 4] encouraged us to use this more available hyperimmune serum to bacterial antigens in a CIE setting. Our study not only indicates that chlamydial pneumonias are associated with an antigenaemia of the aetiologic agent, but also demonstrates that a rapid detection of chlamydial antigen is possible in serum using the CIE technique. Based on the findings of this antigenaemia, future developments are to aim at the use of genuine anti-chlamydial antibodies avoiding the still necessary exclusion of a positive blood culture with Acinetobacter species. With the availability of monoclonal anti-chlamydial antibodies still further improvements will probably be obtained with the detection of chlamydial antigens.

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Nutrition of newborns small for gestational age with human milk lyophilisate enriched human milk during the first week of life

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In 24 very low birth weight infants appropriate or small for gestational age, the metabolic response to nutrition with human milk lyophilisate enriched human milk was estimated during the first week of life.

In contrast to newborns appropriate for gestational age, in newborns small for gestational age signs of metabolic overloading could be observed: increased urinary amino acid excretion and neonatal cholestasis. Both depended on the degree of fetal growth retardation.

It was concluded that nutrition with human milk lyophilisate enriched human milk cannot be recommended for very low birth weight infants during the first week of life. In newborns small for gestational age the metabolic situation has to be estimated before starting a high protein diet. If the level of total bile acids amounts to more than 30 μmol/l, protein intake should be increased carefully during the first week of life.

In the first days of life pooled human milk (HM) is sufficient for the enteral nutrition of newborns weighing at birth less than 1500 g. This is due to the low concentration of protein and energy substrates of pooled HM on the one hand and the limited capacity of the gastrointestinal tract on the other hand [4]. As parenteral nutrition is affected by metabolic imbalance, nosocomial infections and some other factors [5, 14], concentrated formulae [1, 9, 10, 18] or enriched HM [13, 17, 19] are desired for the nutrition of these newborns. Most of these recommendations have been given for very low birthweight infants independent of their intrauterine development [9, 10, 17, 18, 19].

In a previous study it has been shown that in newborns with a birthweight lower than 1500 g and severe fetal growth retardation the metabolic capacity is decreased during the first days of life [7]. In these newborns feeding with native HM led to increased cholestasis while in newborns weighing more than 1500 g with only mild fetal growth retardation the metabolic response to enteral feeding with native HM showed no difference as compared to newborns appropriate for gestational age (AGA) [6].

The present study had the aim to clarify whether very low birthweight infants with mild fetal growth retardation could be nourished with enriched HM without any risk during the first week of life.

Patients

Eleven premature AGA newborns fed human milk lyophilisate (HML) enriched HM (Group 1), five premature SGA newborns between the 5th and 10th percentile according to Lubchenco et al [16] fed HML enriched HM (Group 2), and eight premature SGA newborns with the same degree of fetal growth retardation (FGR) as in Group 2, fed native HM were studied. All of them were without signs of evident illness, especially without idiopathic respiratory distress syndrome, persistent fetal circulation, and bacterial infectious disease.

In Table I details of the infants studied are given.

Table I

Details of the infants studied

Group	1	2	3
Weight at birth, g	1396.4 (1270—1460)	1310.6 (1160—1420)	1385.0 (1250—1410)
Length at birth, cm	40.9 (36.5—43.0)	43.4 (37.5—45.0)	44.2 (39.5—45.5)
Gestational age, weeks	29.9 (29—31)	32.2 (31—33)	32.6 (32—33)
Intrauterine development	appropriate for GA	small for GA	small for GA
Nutrition	HM[1] + HML[2]	HM + HML	native HM
Number	11	5	8

[1] Human milk [2] Human milk lyophilisate

Methods

During the first week of life the following parameters were estimated daily: nitrogen intake (Kjeldahl method), urinary nitrogen losses (Kjeldahl method), alpha amino nitrogen excretion (ninhydrin method) and body weight. On the 2nd, 4th, 6th and 8th days, serum alpha nitrogen concentration and the acid base balance (Astrup method) were measured. On the 8th day of life the serum bile acid concentration (according to Senger et al [22]), and the amount of stools were also estimated.

To have comparable nitrogen intakes within the different groups, only milk from mothers delivering preterm babies was used for nutrition as well as for lyophilisation. In Table II the composition of native HM, and HM enriched with HML are given, and Table III shows the mean daily nitrogen intake in the different groups during the first week of life.

Within the first 8 hours of life it was decided whether or not a predominant enteral nutrition should be started. The volume of enteral feeding was 45 ml/kg BW as a maximum on the first day of life, and this was increased to 200 ml/kg BW by the 8th day of life. Depending on the clinical course, supplementary parenteral nutrition with nitrogen free solutions was given during the first two days of life.

Enteral nutrition was realized by nasogastric tube two hourly.

For statistical analysis, Student's t test was applied.

TABLE II

Composition of native pooled human milk (HM) and human milk lyophilisate enriched human milk (HM + 6 g HML/dl) used in this study (M ± SD)

	GM	HM + 6 g HML/100 ml
Protein (g/l)	11.8±1.2	15.8±2.1
Fat (g/l)	41.4±4.9	55.4±6.1
Lactose (mmol/l)	174.2±16.2	242.6±20.8
Osmolality (mosmol/l)	291.6±11.2	384.9±19.6

TABLE III

Daily (M ± SD) nitrogen intake (mmol/kg BW/24 h) in the different groups during the first week of life

Day	Group 1	Group 2	Group 3
1	6.3±0.5	6.5±0.7	6.0±0.2
2	13.9±0.9	14.7±1.0	11.1±0.3
3	18.4±0.8	18.7±0.9	13.9±0.3
4	21.5±0.8	21.5±0.8	16.7±0.3
5	25.3±0.9	25.1±0.8	19.8±0.4
6	28.9±0.9	29.1±0.9	22.1±0.4
7	32.4±1.1	32.5±1.0	24.9±0.5
8	35.4±1.1	35.7±1.1	27.6±0.6

RESULTS

During the balance periods there were no signs of an enteral volume overloading. During the study three patients had to be excluded owing to disturbances in which predominant enteral nutrition was impossible. These patients were, one with persistent ductus arteriosus observed on the 3rd day of life, one with intracranial haemorrhage on the 2nd day of life, and one patient affected by *E. coli* infection on the 6th day of life. Disturbances of acid base balance were not observed in any infant during the study.

The renal nitrogen losses were not significantly different. Even if there was an increasing nitrogen excretion in SGA newborns fed HML enriched HM from the 6th day of life, the differences were not significant (Fig. 1). The slightly higher nitrogen excretion in this group was caused by a significantly higher urinary alpha amino nitrogen excretion from the 2nd day of life (Fig. 2). There were no significant differences between Group 1 and Group 3.

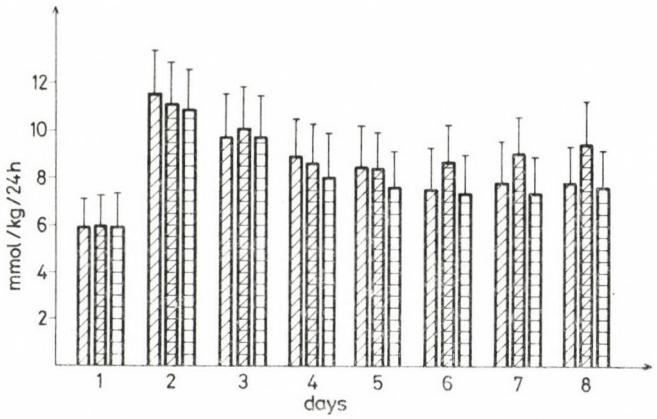

FIG. 1. AGA (▨) and SGA (▨) infants fed HM enriched with HML and SGA infants (▭) fed native pooled HM

The amount of stools in SGA newborns fed HML enriched HM was twice more than in AGA newborns fed HML enriched HM; they amounted to 28.9 ± 6.2 g/kg BW/24 h, and 13.2 ± 4.9 g/kg BW/24 h, respectively.

The significantly higher stool losses and the slightly increased renal nitrogen excretion in SGA newborns fed HML enriched HM led to a steadily increasing difference in the nitrogen balance in Group 1 (Fig. 3). From the 6th day of life this difference has become significant. In the same period, the differences between SGA newborns fed HML enriched HM and those fed native HM has become smaller day by day (Fig. 3).

In contrast to the very different urinary alpha amino nitrogen excretion (Fig. 2), the serum concentrations were not so different (Table IV). In both groups fed on HML enriched

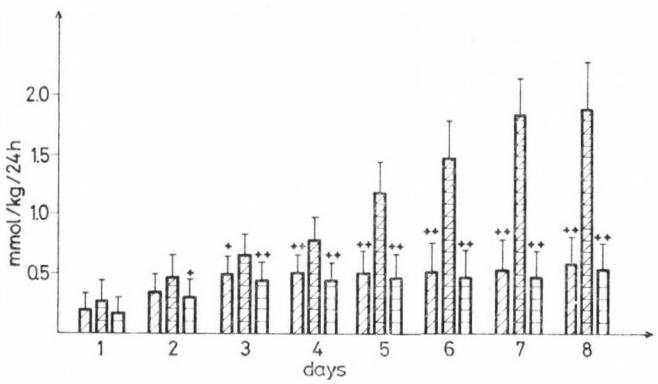

FIG. 2. Mean (M ± SD) daily renal amino acid elimination (mmol/kg BW) 24h in AGA (▨) and SGA (▨) infants fed HM enriched with HML and in SGA infants (▭) fed native pooled HM

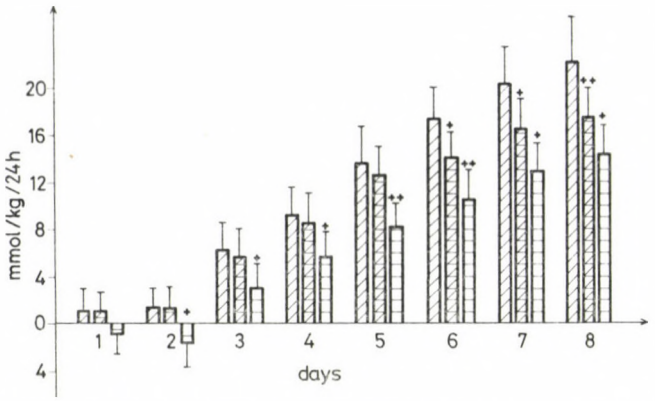

FIG. 3. Mean (M ± SD) daily nitrogen balance (mmolkg BW) (24h) in AGA (▨) and SGA (▨) infants fed HM enriched with HML, and SGA infants (□) fed native pooled HM

HM the serum alpha amino nitrogen concentrations were higher than in Group 3 in the second part of the observation period, while in SGA newborns fed native HM were the lowest con.entrations observed. The differences between Group 1 and Group 2 were significant only on the 8th day of life ($p < 0.01$), and between Group 2 and Group 3 on the 6th and 8th days of life ($p < 0.05$ and < 0.01, respectively).

The serum concentrations of total bile acids were remarkably high in SGA newborns fed HML enriched HM, on the 8th day of life (Fig. 4); there was a significant difference between the AGA and SGA newborns fed native HM ($p < 0.01$). In SGA newborns the higher protein intake led to a significant increase in serum total bile acid concentration ($p < 0.01$) and the values reached were unambignously pathological.

The highest postnatal weight loss was observed in Group 1 (Table V). The difference between Group 1 and Group 2 as well as between Group 2

TABLE IV

Serum alpha amino concentration (mmol/l) during the first week of life in the different groups (M ± SD)

Day	Group 1	Group 2	Group 3
2	3.39±0.51	3.49±0.57	3.47±0.65
4	3.38±0.63	3.71±0.71	3.11±0.59
6	3.26±0.59	3.92±0.81	2.97±0.60
8	3.22±0.62	4.36±0.78	2.62±0.64

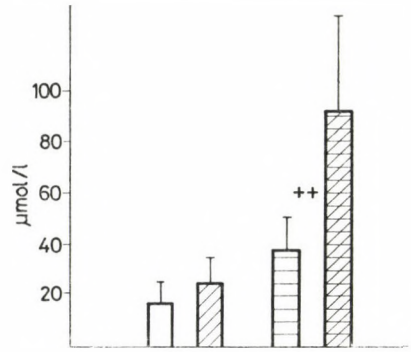

FIG. 4. Mean (M ± SD) serum concentration of bile acids in AGA infants fed native pooled HM (▭) as well as HM enriched with HML (▨) and SGA in infants also fed native pooled HM (▭) as well as HM enriched with HML (▨), on the 8th day of life

TABLE V

Body weight (M ± SD) in the different groups during the first week of life

Group	1	2	3
Maximum postnatal weight loss, per cent	−1.5 ±1.10	+0.6 ±0.69	−1.0 ±0.87
BW on the 8th day of life, in per cent of birth weight	+0.99 ±0.49	+1.21 ±0.63	+0.4 ±0.43

and Group 3 was significant ($p < 0.01$), whereas there was no difference in relative body weight between Group 1 and Group 2 on the 8th day of life, but relative body weight was significantly lower in SGA newborns fed native HM ($p < 0.05$).

DISCUSSION

The metabolic situation of SGA newborns was characterized, depending on the fetal growth retardation, by a reduced cellular mass caused by the reduced number of cells as well as the reduced cell volume, lower intracellular concentration of enzymes, and low insulin levels [2, 8, 15, 20]. All these result in a diminished functional capacity of the liver of such infants, leading to particular problems of nutrition during the first days of life. There is a discrepancy between the substrate deficiency caused by fetal malnutrition and a limited capacity for substrate utilization. As it has been shown in SGA newborns with severe fetal growth retardation, feeding native HM in the same amount as in the present study caused metabolic overloading [6, 7].

The present results demonstrate that in SGA newborns with mild to moderate fetal growth retardation, feeding of HML enriched HM may overcharge the metabolic capacity during the first week of life.

Two facts seem to be important: the insufficient utilization of reabsorbed amino acids and the increased cholestasis.

Because only HM was used in the present study, an inadequate amino acid supply could not be the cause of the high amino acid excretion in SGA newborns fed HML enriched HM [4, 20, 21]. The high serum concentration of bile acids points to an overcharged liver function [12, 22, 25]. The insufficient amino acid utilization shown by the urinary amino acid elimination increases with increasing protein intake.

On basis of the present results the question cannot be answered when the postnatally developing liver function permits a higher nitrogen intake. Most investigators started nitrogen balance studies when enteral feeding had reached a constant level, e.g. during the 2nd or 3rd week of life [11, 13, 19, 23]. This, together with the different degree of fetal growth retardation of the investigated newborns may be the most important reason for the fact that the limited metabolic capacity of SGA newborns has been described by only few investigators [7, 15, 20], while a high protein intake was recommended for all low birth weight infants [1, 9, 10, 13, 17, 18, 19].

The higher amount of stools of SGA newborns fed HML enriched HM than that of AGA newborns on the same diet must have also been due to the inadequate liver function and especially to the limited fat absorption [9, 12].

The higher osmolality of HML enriched HM (Table II) did not cause any clinical problem during the observation period, but in agreement with some other authors [3] we decided that 400 mosmol/l should be the upper limit from this point of view.

In SGA newborns as well as in AGA newborns, nitrogen retention can be improved by increasing the protein intake. The signs of a metabolic overloading in SGA newborns, especially the high serum concentration of bile acids, are, however, reasons to increase the protein intake very carefully in these infants during the first week of life. The serum concentration of bile acids seems to be a good marker to recognize this metabolic situation. In each case with a serum bile acid concentration of more than 30 μmol/l one hour after a meal, concentrated nutrients are certainly unfavourable. Thus, in SGA newborns concentrated HM or formulae cannot be recommended for very low birthweight infants. The influence of fetal growth retardation on metabolic capacity has to be estimated before starting a high protein diet.

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Book reviews

SCHINZEL A: *Catalogue of unbalanced chromosome aberrations* XX + 913 pages with 15 figures and 278 plates. Walter de Gruyter, Berlin—New York 1984. Price DM 298,—

I must admit in advance that this long awaited work had first disappointed me. After the nicely published Atlas des Maladies Chromosomiques by J. de Grouchy and C. Turleau (1982), the technical presentation of this book seemed to be rather modest — in contrast with its price. But in a short time I became more and more delighted with it. Intending now to review its value I can only repeat what has been written by W. Schmid in the foreword. Schinzel's monography fulfils my expectations, it is an opus of the highest value for clinical cytogeneticists. The invested tremendous work can be appreciated by colleagues who have sacrificed work and energy to keep up a complete literature collection — which became unnecessary at the moment of the appearance of this book. The long experience of Prof. Schinzel in clinical cytogenetics is reflected by the really careful and at the same time critical selection of case reports published during a period of 12 years when the banding techniques refined the detection of more and more different types of chromosome rearrangements. The literature is complete, the illustrations are rich and correctly selected. According to Schmid's statement, the main merit of the author is the special attention given to the grouping of partial duplications and deletions by the exact breakpoints and the concomitant partial aberrations. This point of view draws attention not only to the precise karyotype—phenotype analysis, but it is supported by the new results in chromosome mapping, too. On the other side, the detailed description of life expectancy, degree of somatic and mental retardation points out the importance of follow-up studies in clinical cytogenetics.

The description of the clinical characteristics of different aneuploidies (750 pages) is supplemented by 50 pages on basic knowledge of the modern techniques, the different types of rearrangements. Furthermore, an excellent review of the general clinical characteristics of chromosome aberrations can also be found. The rapid development in gene mapping is the cause that this part is less current, since the Los Angeles Conference brought out many new findings, probably during the book was in press. The monograph is supplemented by an Index of Malformations and Minor Anomalies, too. It is well known that such an index can never be complete and, in addition, this is the only part in the book where some inaccuracies occur.

One may state without exaggeration that this book is an important contribu-

tion to the further development of clinical cytogenetics. Giving an opportunity to look over the progress during the last decade, Schinzel's work will stimulate cytogeneticists who find pleasure in their work.

Magda Osztovics

Pädiatrie Band I Herausgegeben von *P.* Grossmann, W. Plenert. 527 Seiten mit 221 Abbildungen und 169 Tabellen. Georg Thieme, Leipzig 1984. Preis M 145,—

Die Verfasser haben ein den heutigen Erfordernissen entsprechendes, ausführliches Buch publiziert, das außer dem klassischen Kenntnismaterial auch die Grenzgebiete behandelt und so nicht nur ein Lehrbuch für Medizinstudenten ist, sondern auch die nötigen Informationen für die Weiterbildung zum Facharzt enthält. Von den geplanten 3 Bänden sind in dem vorliegenden ersten Band die Fragen der Entwicklung des Kindes und Jugendlichen, allgemeine Diagnostik und Therapie, Kinder- und Jugendgesundheitsschutz, klinische Genetik und Teratologie, ferner das Neugeborene, Ernährung im Wachstumalter und Stoffwechselstörungen erörtert. Im jedem Kapitel wird das Wissensmaterial, mit Tabellen und Abbildungen illustriert, mit dem Schrifttum ergänzt, eingehend und klar erläutert. Ähnliche Werke erscheinen in vielen Ländern und Sprachen, die alle einem aktuellen Bedürtnis des Fachgebietes und den nationalen Besonderheiten entsprechen. Alle haben eine Gemeinsamkeit, in dem sie einerseits ein Kompromiß von Lehrbuch und Nachschlagwerk darstellen und anderseits der integrativen Funktion, den Zusammenhalt der Kinderheilkunde dienen.

Durch das Kapitel über den Kinder- und Jugendgesundheitsschutz mit dem diesbezüglichen statistischen und gesetzlichen Angaben gewinnt man einen Einblick in die nationalen Eigenheiten dieses komplexen Systems in der DDR.

Zusammenfassend soll festgestellt werden, daß wir ein zeitgemäßes Buch der Kinderheilkunde in der Hand halten, das sich bei Medizinstudenten, jungen Fachärzten und Allgemeinärzten als eine wichtige Informationsquelle erweisen wird.

K. Schmidt

W.-R. Cario: *Die portale Hypertension im Kindesalter* 163 Seiten mit 36 Abbildungen und 20 Tabellen. Georg Thieme, Leipzig 1984. Preis M 46,—

Die Monographie gliedert sich in drei Teile. Der erste befaßt sich mit den Problemen der portalen Hypertension im allgemeinen. Nach einer ausführlichen Erläuterung der Pathoanatomie und Pathophysiologie wird die Diagnostik und Notfalldiagnostik behandelt. Zur Wahl der definitiven Therapie wird ein logisches diagnostisches Programm empfohlen. Bei der Notfalltherapie findet man die Maßnahmen, die bei einer Blutung des Ösophagus und Kardia vorzunehmen sind. Es ist bedauerlich, daß der besonders bei Kindern ausgezeichnete, dauerhafte Ergebnisse sichernden endoskopischen Sklerotisierung nur etwa 17 Zeilen gewidmet wurden.

In dem zweiten Teil werden die möglichen Auswirkungen der portosystematischen Anastomosen auf die systemische Hämodynamik, Leber und den Stoffwechsel besprochen und auf die frühen Zeichen, ferner die Prophylaxe hingewiesen.

Der dritte Teil faßt die Empfehlungen zur besseren Prophylaxe, Diagnostik, Therapie und Dispensairebetreuung zusammen.

Der Aufbau des Werkes ist logisch, klar und übersichtlich. Das etwa 670 Angaben (bis 1980) anführende Literaturverzeichnis soll als besonderer Wert hervorgehoben werden. Das kleine Buch ist Kinderärzten und Kinder- und Gefäßchirurgen zu empfehlen.

T. Verebély

A. WILLE: *Die Enkopresis im Kindes- und Jugendalter* X + 142 Seiten mit 2 Abbildungen und 54 Tabellen. Springer-Verlag, Berlin—Heidelberg—New York—Tokyo 1984. Preis DM 82,—

Das Buch ist Band 35 der Reihe Monographien aus dem Gesamtgebiete der Psychiatrie. Es befaßt sich mit einem für Psychologen und Kinderärzte sehr wichtigen Thema. Zwischen 1973—78 wurden 1857 Kranke untersucht, von denen 165 Kinder an Enkopresis litten (128 Knaben und 37 Mädchen). Das Durchschnittsalter war 7—9 Jahre (von 4 bis 16 Jahren).

Es wurde festgestellt, daß bei den Patienten, die die vollkommene Reinigkeit nicht erreichten, neben der Enkopresis sehr oft auch Enuresis bestand. Außer der Enuresis konnten auch andere Symptome beobachtet werden, besonders bei sekundärer Enkopresis, wie z. B. Schlafstörungen, Eßstörungen, Naschen. Bei einigen Probanden erschienen Persönlichkeitsstörungen wie Stehlen, Lügen usw. Bei 21% der Patienten wurde ein infantiles psychoorganisches Syndrom, bei 7% eine motorische und bei 14% eine sprachliche Störung gefunden. Der Intelligenztest zeigte folgendes Bild: bei 14% war der IQ zwischen 70 und 90, bei 58% zwischen 90 und 110 und bei 27% über 110.

Bis zum 5. Lebensjahr waren 22% der Patienten wenigstens einmal mehr als einen Monat, 12% einen Monat von ihrer Mutter getrennt. Ein Viertel der Enkopretiker waren unerwünschte Kinder und 11% waren unehelich geboren. Das dysharmonische elterliche Milieu spielte eine ausschlaggebende Rolle: Ehekonflikte, Scheidung, Stiefeltern, Geschwistereifersucht oder aber ein zu enges oder schlechtes Mutter—Eltern—Kinder Verhältnis. Bei den Eltern der Enkopretiker konnten neurotische Symptome oft verzeichnet werden.

Die Arbeit gliedert sich in 4 Teile.

I. Teil: Literaturübersicht der Enkopresis;

II. Teil: Drei vergleichende Untersuchungen:

1. Vergleich der enkopretischen Knaben und Mädchen mit dem ganzen Krankengut;

2. Vergleich von primären und sekundären Enkopretikern;

3. Vergleich der Enkopretiker mit Euretikern:

III. Teil: Katamnestische Untersuchungen. Es wird bestätigt, daß die Enkopresis als eine ernsthafte Störung des Kindes- und Jugendalters betrachtet werden muß. Es gab Fälle, bei denen eine 7jährige Behandlung erfolglos blieb.

IV. Teil: Die Therapiemöglichkeiten, besonders die individuelle Behandlung werden besprochen.

Für die Entstehung der Enkopresis waren folgende Faktoren verantwortlich:

1. Konstitutionelle späte Entwicklung;

2. Frühe Traumatisierung—Scheidung—Hilflosigkeit;

3. Familieneinfluß, — zu strenge Disziplin;

4. Auslösende Faktoren, wie Veränderungen in der Familie, Geburt von Geschwistern, Wechsel der Umgebung, usw.

5. Eigendynamik der Enkopresis: es handelt sich um einen Kreislauf. Das enkopretische Kind lenkt die Aufmerksamkeit auf sich, selbst durch erlittene Strafe, was von ihm als eine positive Erscheinung betrachtet wird, es verändert die Familienkonflikte, der Enkopretiker wird als Sündenbock betrachtet.

Es wird betont, daß bei der Entwicklung der Enkopresis alle fünf Faktoren, mit verschiedenem Gewicht, eine Rolle spielen.

Maria SZOKOLY

G. NISSEN, C. EGGERS, J. MARTINIUS: *Kinder- und jugendpsychiatrische Pharmakotherapie* XII + 370 Seiten mit 10 Abbildungen. Springer-Verlag, Berlin—Heidelberg—New York—Tokyo 1984. Preis DM 38,—

Das von drei in der Pharmakologie gut orientierten Klinikern verfaßte lückenfüllende Buch impressioniert den Leser mit seinem Reichtum an Informationen und

der Gedrungenheit des Stils. Es soll in der Lösung der so viel diskutierten und ständig wieder aufgeworfenen Frage Hilfe bieten: sollen wir Medikamente, und wenn ja, welche Medikamente in der Kinderpsychiatrie anwenden? Das Problem ist von mehreren Gesichtspunkten diskutiert, da die Lösung psychischer Probleme mit Pharmaka in der frühen Periode der Persönlichkeitsentwicklung noch weniger adequat sein dürfte, als später und da die bei Erwachsenen gut bewährten Psychopharmaka im Kindesalter manchmal ungewöhnte paradoxe Wirkungen entfalten. Dennoch gelangen diese Substanzen unvermeidlich auch in der pädiatrischen Praxis zu einer immer bedeutenderen Rolle. In Fällen von psychotherapeutischer Unzugänglichkeit, Heftigkeit der Symptome, Gespanntheit in Krisensituationen fühlt sich der Kinderpsychiater gezwungen, auch bei relative Indikation zum Medikament zu greifen. Bei Psychosen und organisch begründeten Symptomen handelt es sich in erster Reihe um eine pharmakologische Behandlung.

Die Autoren gehen davon aus, daß man Pharmaka lediglich mit der gründlichen Kenntnis derer Wirkungsmechanismus verwenden darf. So werden also auf den ersten 80 Seiten des Buches die allgemeinen pharmakologischen Grundlagen besprochen: Absorption der Medikamente, ihre Verteilung im Organismus, Biotransformation und Elimination, Fragen der Gewebeaffinität und des Angriffpunktes mit Hinsicht auf die verschiedenen Typen von Rezeptoren. Probleme der Kontrolle der Wirkung und ethische Beziehungen werden sodann erörtert. Der Adaptierung dieser Kenntnisse zum Kindes- und Jugendalter ist ein großer Teil gewidmet. Hier werden die alternativen und ergänzenden Behandlungen, wie Psychotherapie, Soziotherapie, Rolle der Heilpädagogik, Regeln für den Kontakt mit Eltern kurz und klar erläutert.

Das dritte Kapitel behandelt die einzelnen Gruppen der Psychopharmaka. Im vierten Kapitel werden die Indikationen angegeben und betont, daß jeweils solche Syndrome angeführt wurden, bei denen die Verabreichung von Psychopharmaka in Frage kommen kann. Mit der knappen, doch klaren Schilderung von Ätiologie, Symptomatologie und Behandlungsrichtlinien dürfte dieser Teil einem kleinen praktischen psychiatrischen Lehrbuch entsprechen. Die Gesichtspunkte sind eklektisch, was in diesem Themenkreis auch nicht anders sein kann.

Das letzte Kapitel befaßt sich mit den Symptomen der Vergiftungen. Der Band enthält abschließend ein Verzeichnis der auf dem deutschen Sprachgebiet zur Zeit angewandten Psychopharmaka.

G. Vikár

B. Meyer-Probst, H. Teichmann: *Risiken für die Persönlichkeitsentwicklung im Kindesalter*. 318 Seiten mit 118 Abbildungen und 112 Tabellen. Georg Thieme, Leipzig 1984. Preis M 98,—

Dieses Buch, eine Rostocker Längsschnittstudie, beschäftigt sich mit dem sog. sozial-biologischen Problem, d. h. mit dem Einfluß der gesellschaftlichen Faktoren auf die Persönlichkeitsentwicklung mit Rücksicht auf die biologischen Gegebenheiten des Menschen. Der Mensch hat eine biologische Natur und ist gleichzeitig ein gesellschaftliches Wesen. So wird auch das Krankheitsbild durch die sozialen Faktoren erweitert, modifiziert.

Das eine Ziel dieser Studie war, die Ursachen und Bedingungen interindividueller Differenzen und ihre prognostische Bedeutung aufzuzeigen am Beispiel der Entwicklung von Risikokindern. Die Bezeichnung „Risikokind" signalisiert eine erhöhte Gefahr für eine Entwicklungsstörung oder -schädigung. So ist also nicht jedes mit einem Risikofaktor belastete Kind ein Risikokind. Risikofaktoren sind sowohl biologische als auch psychosoziale Bedingungen, die allein oder in Verflechtung störend oder hemmend wirken können und die Entwicklungschancen beeinträchtigen.

Ferner untersucht diese Studie die Interaktion zwischen frühkindlichen zerebralen Belastungsfaktoren und den Umweltverhältnissen. Die frühkindliche Hirnschädigung als Ursache für Verhaltensanomalien wird von verschiedenen medizinischen Disziplinen untersucht; ihre determinierenden Faktoren von der Perinatologie und Neuropsychiatrie, die Kompensation von der Sonderpädagogik und die kausalen Zugangswege von der Neuropsychologie.

Die Längsschnittstudie verfolgt die Entwicklung von 294 Risiko- und Kontrollkindern aus Rostock seit der Geburt 1970/71. Bei den berücksichtigten Einflußfaktoren auf die Persönlichkeitsentwicklung unterscheiden sich die biologischen Risikofaktoren (die perinatalen Risiken mit den alimentären, infektiösen, toxischen, hypoxischen und traumatischen Einwirkungen während der 1—2 Lebensjahre) und die psychosozialen Risikofaktoren (z. B. depravierende Milieueinflüsse). Die eingesetzten psychologischen Methoden waren: Entwicklungskontrollverfahren, Intelligenztest für das Vorschulalter, Lerntest, Figur-Grund-Bilder, Sprechprobe, Konzentrationshandlungsverfahren, Enzephalopathie-Fragebogen, Erzieher-Fragebogen, Erziehungseinstellung-Fragebogen, schulärztlicher Siebtest. Die medizinischen Bezugsdaten stützen sich auf die Dokumentation der Perinatologen und Neurologen.

Diese Längsschnittmethode hat folgende Ergebnisse gebracht: Die biologischen und psychosozialen Risikofaktoren sind miteinander vielfältig vernetzt; biologische Risikofaktoren ziehen andere nach sich, und das gilt auch für die psychosozialen Faktoren; so bilden sich Risikoketten. Ihre Wirkung auf die psychische Entwicklung des Kindes ist von dem 2. Lebensjahr an zu ermessen. Auf die Risikofaktoren reagiert der Entwicklungsquotient am empfindlichsten im Alter von 2 Jahren, er bringt nicht nur die motorischen, sprachlichen und Denkleistungen zum Ausdruck, sondern auch das Niveau der Spieltätigkeit und Sozialanpassung. Im Vorschulalter ist der EQ instabil; ein gutes Ausgangsniveau begünstigt, ein schlechtes hemmt den Entwicklungsfortschritt.

Die Kleinkinder, die körperlich hinter der Altersnorm zurückbleiben, sind den psychosozialen und biologischen Risiken mehr ausgesetzt. Wenn man Mädchen und Jungen bei gleicher Risikobelastung vergleicht, ist festzustellen, daß die Mädchen besser entwickelt sind, in der frühen Kindheit eine bessere organismische Kompensationsfähigkeit haben und daher psychisch weniger gestört sind. Die biologischen und psychosozialen Einflußfakten verursachen das abweichende Verhalten ebenso wie die verschiedenen Leistungen. Unruhe, Ungeschicklichkeit, schlechte Konzentration und Ausdauer sind stärker biologisch determiniert; Intelligenz, Sprache und soziale Verhaltensweisen sind stärker milieuabhängig. Auch der körperliche Gesamteindruck (akzeleriert, altersgerecht, retardiert) steht mit der Risikobelastung im Zusammenhang. Die Entwicklung des Kindes wird auch vom Ausmaß der Risikobelastung bestimmt. Der Einfluß prä- und perinataler Komplikationen (biologische Faktoren) auf die geistige Entwicklung nimmt mit zunehmendem Alter ab, während die psychosozialen Belastungen ihren entwicklungshemmenden Einfluß beibehalten oder verstärken. Die biologischen und psychosozialen Risiken wirken analog. Das ist die Voraussetzung für ihre wechselseitigen Verstärkungseffekte. Die als Reaktion auftretenden Vorgänge von Kompensation und Dekompensation beruhen auf der Plastizität des Zentralnervensystems.

Die Aufhellung der Wirkungsweise von Risikofaktoren dient dem Ziel der Prävention und Kompensation; beides wird durch therapeutisches und pädagogisches Bemühen verwirklicht.

Klara GALLUS

W. Berger, V. Dietz, A. Hufschmidt, R. Jung, K.-J. Mauritz, D. Schmidtbleicher: *Haltung und Bewegung beim Menschen* X + 198 Seiten mit 70 Abbildungen und 2 Tabellen. Springer-Verlag, Berlin—Heidelberg—New York—Tokyo 1984. Preis: DM 98,—

Das Buch ist das ausgezeichnete Produkt einer achtjährigen Zusammenarbeit von Neurophysiologen, Neurologen und Sportphysiologen. Die Entwicklung der Haltung und Fortbewegung des Menschen, deren normale und pathologische Charakteristika wurden mittels modernen Verfahren untersucht. Die Ergebnisse und Folgerungen dieser Untersuchungen sind heute ganz aktuell, da die bewegungsarme Lebensweise und andere Zivilisationsschäden auch die Haltung beeinträchtigen. Auch erfordert die Entwicklung des Sportes, die Leistungssteigerung mit gleichzeitigem Gesundheitsschutz der Sportler und die gezielte Wahl der Belastbarkeit die genaue Kenntnis der Bewegungsphysiologie und der Auswirkungen der verschiedenen Trainingsarten. Zu alldem bietet das Buch wertvolle Hilfe.

Der Aufbau des Buches ist übersichtlich. Nach einer deutschen und englischen Zusammenfassung des Inhaltes folgen sechs selbständige, doch ineinandergreifende Kapitel, die jeweils eine deutsche und eine englische Zusammenfassung und ein Literaturverzeichnis abschließt.

Kapitel 1 bespricht die Entwicklung der Bewegungsphysiologie, die aufrechte Haltung, die Fortbewegung, die Armzielbewegungen, Gleichgewichtskontrolle und Reflexregulation. Hervorzuheben sind jene Beobachtungen, die sich auf die kortikale Regelung, die Entstehung des Automatismus, des Lernens der Bewegung, Ballwerfens, Kugelstoßen usw. beziehen. Das Thema des zweiten Kapitels ist die Physiologie und Pathophysiologie des, die wichtigste Station der Menschwerdung kennzeichnenden, aufrechten Stehens. Wichtig ist das dritte Kapitel, das die elektromyographischen Untersuchungen bei komplexen Bewegungsabläufen erörtert und wertvolle Richtlinien zu bewegungsphysiologischen Studien bietet.

Auf das Interesse von Pädiatern, Neurologen und Orthopäden kann das kurze vierte Kapitel über die Entwicklung des Zweibeinganges beim Kleinkind rechnen. Kapitel 5 behandelt die Störungen von Gang und Balance nach spinalen- und Hirnläsionen und ist vom diagnostischen und therapeutischen Standpunkt außerst wichtig. Die Beobachtungen an Kindern mit cerebral palsy können bei der konservativen oder aber chirurgischen Behandlung dieser Patienten gut verwendet werden.

Im letzten Kapitel wird die Anwendung der motorischen Forschungsergebnisse beim sportlichen Krafttraining besprochen. Es wird betont, daß neben der Muskelquerschnittsvergrößerung die neuronale Anpassung einen wichtigen Faktor darstellt.

Das Werk dürfte zur Weiterforschung anregen, sich aber auch zur praktischen Verwertung bei der Therapie von Bewegungsstörungen nützlich erweisen und so die Zielsetzung der Autoren erfüllen.

T. Vizkelety

Onkologie. Redigiert von B. Kornhuber XVI + 184 Seiten mit 12 Abbildungen und 9 Tabellen. Springer-Verlag, Berlin—Heidelberg—New York—Tokyo 1984. Preis DM 32,—

Das in der Reihe „Pädiatrie: Weiter- und Fortbildung" erschienene Buch befaßt sich mit den wesentlichsten Teilgebieten der pädiatrischen Onkologie: akute lymphoblastische und myeloische Leukämie, Medulloblastom und Ewing-Sarkom. Die Kapitel der Arbeit decken also nicht das ganze Spektrum von Tumoren. Das Ziel des Werkes weicht von den im ähnlichen Themenkreis bekannten Arbeiten ab, es trachtet lediglich den Nichtonkologen zeitgemäße Information über die Möglichkeiten der Diagnostik und Therapie zu bieten. Heute werden diese Kranken zwecks komplexer Behandlung in Zentren

mit entsprechender Erfahrung konzentriert. Mit der Schilderung der diagnostischen und therapeutischen Möglichkeiten und der heute erreichbaren Heilungsaussichten wünscht das Buch die Überweisung der Kinder mit Malignomen an diese Zentren zu fördern.

Die einzelnen Kapitel wurden von erfahrenen und anerkannten Persönlichkeiten des Fachgebietes verfaßt. Es werden stets die Häufigkeit, und wo es möglich ist, auch die Ätiologie, sodann die klinischen Symptome, das hämatologische bzw. histologische Bild, die diagnostischen Untersuchungen und die Stadieneinteilung erörtert. Ferner ist in den Kapiteln die Beschreibung der zur Zeit verbreitetesten Formen der Therapie mit den eventuell notwendigen supportiven therapeutischen Maßnahmen zu finden, und abschließend sind die prognostischen Aussichten angedeutet. Mit ausgewählten Literaturangaben trachtete der Redakteur die Interessenten zu intensiverer Information zu verhelfen; diese findet man am Ende der betreffenden Kapitel oder aber in den Text eingeschaltet. Von den Kapiteln ist die Ausführlichkeit der Besprechung der Leukämie (Henze, Creutzig) und des Rhabdomyosarkoms (Treuner und Niethammer) hervorzuheben. Abschließend ist ein kurzes Kapitel dem Ultraschall in der Tumordiagnostik gewidmet.

Das Buch soll dem Nichtonkologen, dem in Klinik und Praxis tätigen Kinderarzt in gedrängter doch klarer und gut übersehbarer Form das Wesentliche der neuen Kenntnisse der zeitgemäßen pädiatrischen Onkologie vermitteln.

D. Schuler

Serology of Tuberculosis and BCG Vaccination edited by W Fox. Advances in Tuberculosis Research Vol 21. VIII + 252 pages with 5 figures and 24 tables. Karger, Basel 1984. Price: DM 198,—

The results achieved in research on the immonology of tuberculosis and BCG vaccination are summarized in the three papers of this book.

The first paper, written by J. M. Grange on the humoral immune response in tuberculosis, on its nature, biological role and diagnostic usefulness, evaluates the serological methods carried out in parallel with the cultural diagnosis of tuberculosis. The diagnostic procedure of tuberculosis would be much easier if the bacteriological methods could be replaced by reliable immunological methods that are technically simple and less expensive.

Exact knowledge of protein, polysaccharide and lipid structure of the complex antigen of mycobacteria is indispensable for the use of seroimmunological methods of tuberculosis. Comparative research by serodiagnostic methods on antigen structure would add much to the solution of taxonomic questions as well.

The following serological methods are evaluated by the author: complement fixation, agglutination, haemagglutination, haemaggregation, latex agglutination, precipitation, gel diffusion, fluorescent antibody test, RIA and ELISA.

The initial methods in tuberculosis serology were CFT — introduced in 1901 —, agglutination and precipitation; since 1945 haemagglutination and antigen structure analysis; and at present, research on the complex interactions of humoral and cell-mediated immunity furnishes much new information. Introduction of monoclonal antibodies has opened a promising field.

The second paper (H. G. ten Dam: Research on BCG vaccination) reviews the contradictory results of controlled trials on the protective value of BCG vaccinations carried out since 1940. The discrepancy in this respect may have many sources: number of live bacteria per vaccination dose, mode of vaccination, viability of the vaccine, residual virulence and allergization potency of the BCG substrain used for cultures, preservation techniques, cross immunization between BCG and atypical mycobacteria of the environment.

The present controversy on the protective value of BCG vaccination has been

elicited by the evaluation of data of a 7.5 year follow-up trial in India initiated in 1968. The vaccination had no protective value as compared with placebo, irrespective of the dose or the kind of vaccine.

In the mind of WHO experts, this "inefficacy" of the vaccination may be attributed to any of the following factors: faultive grouping based on prevaccination tuberculin sensitivity, reduced virulence of the so-called Indian M. tuberculosis, extinction of BCG allergy within 2.5 years, high incidence of leprosy, high rate of exogenous reinfection, massive prevalence of atypical mycobacteria in the environment, shortened duration of protection by BCG, use of lyophilised BCG vaccines.

In contrast to the unfavourable results obtained in India, many field studies — conducted in Manchester, Hamburg, Malaysia, Singapore and Korea — have demonstrated a protection rate of 60—90% in children vaccinated with BCG.

The third paper (Lotte, A,. Wasz-Höckert Poisson, N., Dimitrescu, N., Verron, M., Couvet, E.: BCG complications: estimates of the risks among vaccinated subjects and statistical analysis of their main characteristics) offers a complete review of all complications observed since the introduction of BCG vaccination in 1921. The number of BCG vaccinations carried out within the framework of national and WHO/UNICEF campaigns both before and after the Second World War is estimated at 1.2—2.0 thousand millions. Decreasing tuberculosis morbidity, the necessity of evaluation of the benefit/risk ratio by adequate adaptation of the BCG vaccination calendar made an international comparison of risks of BCG vaccination imperative.

This is the first classification of BCG complications based on clinical, bacteriological, histological and biological data. compulsory vaccination systems, distortions in incidence figures caused by inaccurate registration are taken into consideration. The incidence of early local complications like suppurative lymphadenitis can be reduced by the use of lyophilised vaccine and improved vaccination technique. In the few fatal cases, a deficiency of cell-mediated immunity can always be detected.

L. Lugosi

New MI, Levine LS: *Congenital adrenal hyperplasia* Monographs on Endocrinology Vol 26. IX + 88 pages with 35 figures and 6 tables. Springer-Verlag, Berlin—Heidelberg—New York—Tokyo 1984. Price DM 72,—

New and Levine, the internationally well-known experts of adrenal endocrinology, summarized the recent knowledge in the field of congenital adrenal hyperplasia. In this short monograph the reader will be acquainted with normal as well as pathological steroidogenesis. The modern biochemical and enzymological methods allowed to discover the enzyme defects which result in various intersexual disorders. The basic defect must be well defined since aetiological diagnosis and adequate therapy depend on the precise biochemical diagnosis.

The 4th chapter deals with the latest discoveries. In the adrenal cortex the zona fasciculata and zona glomerulosa seem to be two functionally distinct glands; it is therefore easy to understand the difference of clinical symptoms associated with apparently the same enzyme defects.

The main task of the monograph is to clarify the biochemical and endocrinological background of congenital adrenal hyperplasia. Therefore the chapter dealing with treatment is too short and somewhat superficial. From the practical point of view, more concrete, useful and guiding data would be needed, since for patients with such diseases the crucial questions are the sex assignment and therapy.

The last two chapters deal with genetics and prenatal diagnosis of the various forms of congenital adrenal hyperplasia. This part will mean a great help for genetic counsellors, too.

The text is easy to read, the illustrations are excellent. The well-chosen literature contains the latest data, and the short subject index is enough for orientation. The book may be recommended to paediatricians, endocrinologists as well as geneticists who are attending patients with congenital adrenal hyperplasia.

P. Kiss

W. Storm: *Neugeborenensepsis und Intensivpflege* Sepsis-Diagnostik bei intensivpflegebedürftigen Neugeborenen. 102 Seiten mit 8 Abbildungen und 33 Tabellen. *Perimed* Fachbuch-Verlags-Gesellschaft, Erlangen 1984. Preis DM 38,—

In dem vorliegenden Buch werden die an der Neugeborenen-Intensivabteilung der Universitätskinderklinik Düsseldorf gewonnen reichen Erfahrungen des Autors zusammengefaßt.

Die Neonatologen haben schon lange vermutet und wissen heute bereits, daß die schwere Neugeborenensepsis, die allgemein der maschinellen Beatmung zugeschrieben wurde, nur in einem minderen Teil der Fälle die Folge der Intensivpflege oder von hygienischen Versäumnissen ist. In dieser Arbeit wird dies auch wissenschaftlich unterstützt. Die Diagnose der Sepsis kann durch die klinischen Symptome, die positive Blut (Liquor) Bakteriologie und beweiskräftige Laborbefunde gesichert werden.

Die Ursachen der zufolge der Intensivpflege, namentlich der intravaskulären Katheterisierung, maschinellen Beatmung, Infusionen, parenterale Ernährung und deren Komplikationen herbeigeführten Sepsis werden eingehend besprochen und betont, daß die im Laufe der Intensivbetreuung ermittelte positive Blutbakteriologie Kontamination, vorübergehende oder persistierende Bakteriämie bedeuten kann. Desweiteren folgt die kritische Erörterung sämtlicher zum Beweis der Neugeboreneninfektion bis jetzt dienenden Laborverfahren; nach den Erfahrungen des Autors soll die Diagnose lediglich die gleichzeitige Anwendung mehrerer Laborverfahren beruhigend sichern, da der diagnostische Wert einiger Methoden nicht eindeutig ist. Die Bedeutung der hämatologischen Blut- und Liquoruntersuchungen und die bakteriologische Untersuchung von Urin und Mageninhalt wird hervorgehoben und von den diagnostischen Tests die mikroskopische Untersuchung der peripheren Blutausstrichen zum Nachweis von intrazellulären Bakterien, ferner die Gegenstrom-Immunelektrophorese besprochen. Ohne entsprechende Klarstellung der Infektion kommt es oftmalig zur ungerechtfertigten Antibiotikumbehandlung mit all ihren Nebenwirkungen und Gefahren.

Das kurze, doch sehr aktuelle Buch soll Neonatologen, Geburtshelfer und Laborärzten empfohlen werden.

G. Korányi

D. R. Buckle, H. Smith: *Development of anti-asthma drugs.* XII + 403 pages, Butterworths, London 1984. Price £ 46.00

This book reviews anti-asthmatic drugs, offers a critical evaluation of their efficacy and outlines research of the near future and its expected achievements. Part 1 sums up the well-known facts and knowledge concerning pathomechanism, manifestations and epidemiology of bronchial asthma. The role of mediators and their significance in pharmacotherapy of asthma are discussed in the two subsequent sections. In recent years, much knowledge has been gathered on histamine, the first and best studied mediator: we have now a better insight into histamine release and its interactions with other mediator substances, into the causes of relative ineffectiveness of H_1 antihistamines in asthma. The chemical structure of the leucotrienes has been fully and their biological effects relatively exactly clarified during the last years. Research on their antagonists is still in the experimental phase, clinical

practice has not yet profited of these experiments. Much progress has been achieved with the platelet activating factor (PAF), its role in inflammation and in immediate type hypersensitivity reactions. No antagonists to this factor have yet been detected.

The third section deals with the drug therapy of asthma. The material is discussed according to the various mode of actions of the drugs: (i) competetive antagonism; (ii) functional antagonism; (iii) inhibition of mediator release; (iv) specific hyposensitization.

ad (i). This group of drugs comprises the H_1-type antihistamines, the SRS-A antagonists and the anticholinergic compounds. In the last subgroup, research of many years has resulted in very effective drugs like ipratropium and oxytropium; they are ineffective, of course, if there is no vagal component in the mechanism of bronchospasm.

ad (ii). Competitive antagonism is a characteristic feature of drugs acting on the adrenoreceptors, theophylline and prostaglandins. The effectiveness of beta-receptor influencing drugs is beyond doubt; in principle, antagonists to the alpha-receptors, present in the lung, may be of clinical importance, but this approach does not work in practice. The clinical efficacy of prostaglandins is rather limited by the fact that there are at least two types of receptor of opposite action against these compounds.

ad (iii). Among the drugs inhibiting mediator release, disodium cromoglycate plays an outstanding role. Its effect is fully discussed and also the drugs that can be administered in case of failure of disodium cromoglycate are dealt with. According to the authors, corticosteroids belong to this group; it is generally believed that in the year 2000 they will be still in use since this type of therapy is effective, well elaborated and not too expensive. They think that no real progress can be expected in the field of oral and parenteral steroids. With the inhaled preparations a more favourable mode of application and shorter plasma half time may be achieved. By inhibiting the metabolism of arachidonic acid a favourable effect can be attained in the cyclo-oxygenase and lipoxygenase pathways. During the last decade a large number of non-steroid antiphlogistic drugs have been developed: their clinical usefulness in joint diseases, antipyresis, the treatment of patent ductus arteriosus is well established, but the great breakthrough in asthma therapy is not yet in sight.

ad (iv). The chapter on specific hyposensitization is one of the most thoroughly composed parts of the book. The task of the contributing authors — Moran and Wheeler — was not easy since the efficacy of this form of treatment has not been fully established in spite of 75 years' clinical experience. All pros and cons are listed here. Confirmation of the favourable effect is of relative value, they think that the new purified preparations are a great but not decisive progress. In their opinion, research should be concentrated upon specific IgE suppression, suppression of isotypes and development of "allergiades" of favourable effect.

This book is primarily destined to experts interested in details. It is carefully composed, offers an excellent review of the present situation and gives a balanced outlook at future research and achievements to be hoped. The tables, chemical formulas and figures have an own numeration within each chapter, each section is followed by a detailed list of references. The book is warmly recommended to all paediatricians and physicians treating asthmatics.

E Cserháti

13th Annual Meeting of the European Working Group for Cystic Fibrosis

Jerusalem, Israel

November 3—8, 1985

For further information please contact:

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Israel

POTESEPTYL

tablets, syrup

POTESETTA

tablets

Potential sulfonamide preparation

Composition

	POTESEPTYL		POTESETTA
	in tablets	in 50 ml syrup	in tablets
Trimethoprim	0,08 g	0,4165 g	0,02 g
Sulfadimidine	0,40 g	2,0385 g	0,10 g

Effect

The drug contains two components with antibacterial effect which inhibit the synthesis of the bacterial folic acid in the following way. Sulfadimidine inhibits the paraamino – benzoic acid – dihydrofolic acid phase whereas trimethopim inhibits the dihydrofolic – tetrahydrofolic acid phase of the folic acid synthesis, respectively. The growing of a large number of both Gram negative and Gram positive bacteria is inhibited by this double blockade of ferments.
Owing to the synergy the bactericidal effect can be reached with smaller doses of the drugs and with more safety i.e. less chance for the development of resistant bacteria. A high concentration of the drug is formed in the bile and is excreted in the urine mainly in this active form.

Indications

Infections of the upper and lower respiratory tracts respectively: acute and chronic bronchitis, bronchiectasia, pneumonia, tonsillitis, pharyngitis.
Diseases of the sexual organs: gonococcusurethritis, prostatitis.
Infections of the kidney and urinary passage: aucte and chronic cystitis, pyelitis, pyelonephritis, urethritis.
Inflammatory diseases of the gallbladder and biliary duct: cholecystitis, cholangitis.
Infections of the gastrointestinal system: enteritis, abdominal typhus, paratypoid, dysentery.
Skin infections: pyoderma, furuncle, obscess, wound infection.

Contra-indications

Hepatic and renal failures, blood-discrasia, sensitivity to trimethoprim and sulfonamide and pregnancy. It should not be administered to prematures, newborn infants and infants up to the age of 6 weeks, to nursing mothers as well.

Dosage

In case of acute infection the compound has to be given at least for 4 days, and generally at least 2 more days in the symptomfree condition.

For adults

Initial dose: 2 times 2 POTESEPTYL tablets
Maintenance dose: 2 times 1 tablet
Maximal dose: 2 times 3 tablets (in the morning and in the evening after meals).

For childern

The usual daily dose is 6 mg of trimethoprim + 30 mg of sulfadimidine/kg of body weight, divided into two parts.

Accordingly, the following dosage is recommended for childern:

	POTESEPTYL		POTESETTA
	tablets twice daily	syrup twice daily	tablets twice daily
at the age of 1–3 years	1/4	2,5 – 5 ml	1–2
at the age of 3–6 years	1/2	5 – 7,5 ml	2–3
at the age of 6–12 years	1	7,5 – 10 ml	3–4

(In the morning and in the evening after meals)

1 dosing spoon (5 ml syrup) corresponds 40 mg of trimethoprim and 200 mg of sufadimidine.

Side effects

Indisposition, headache, exanthema from medicine, gastric complaints. Rarely temporary damage of haemopoietic system can be observed (leucopenia, decrease of the platelet count and the folic acid level). But after administration of folic acid these values return to normal quickly.

Precautions

In case of limited renal function – to avoid the danger of accumulation – only reduced doses should be given (it is advisable to determine the plasma concentration). During the therapy to assure a proper absorbtion sufficient quantites of water should be given to the patient. IIf exanthema occured the administration of the drug should be discontinued. Precaution is recommended in the case of folic acid deficiency anaemia, in the treatment of chronic alcoholics and patients suffering from RA, who are given immunsupressive drugs.

Drug-interactions

Since sulfonamides outplace some drug molecules bound to proteins in patients taking Syncumar – haemorrhage, in patients taking oral antidiabetics – hypoglykaemia can be caused by POTESEPTYL preparations. Sulfonamides inhibit also the metabolism of hydantoins in the liver so POTESEPTYL can cause toxic symptoms in patients who are treated with Phenytoinum tablets or injections. The therapeutical serum level of sulfonamides can be raised by salicylates and the phenylbutazon up to a toxic value.

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by

A. CZEIZEL and G. TUSNÁDY

In English. 1984. XV + 358 pages, 31 figures, 123 tables. 17×25 cm.
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Congenital abnormalities represent a major problem both from a medical and a social point of view. The nine most common isolated congenital abnormalities in Hungary, accounting for about 60% of all congenital abnormalities have been studied. The birth prevalence of these malformations was determined in the same population by using identical methods. Complex epidemiological, teratological and genetic examinations were also carried out. A fundamental similarity was found in the aetiology of the diseases studied. The twin and family studies unequivocally verified heredity while the epidemiological and teratological examinations confirmed the role of the environment factors. Special computer programmes proved the validity of the multifactorial threshold model, and the recurrence risk, so essential in genetic counselling, could be determined as well. The data relating to first-degree relatives provided a comprehensive picture of the isolated common congenital abnormalities in Hungary, serving at the same time as a basis for research in other countries.

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THE PATHOPHYSIOLOGY OF INFANTILE MALNUTRITION

PROTEIN-ENERGY MALNUTRITION AND FAILURE TO THRIVE

by

Prof. E. KERPEL-FRONIUS M. D.

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Semmelweis University Medical School, Budapest
Member of the Hungarian Academy of Sciences

In English · 1983 · 312 pages · 47 figures · 74 tables 17 × 25 cm
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ISBN 963 05 3222 0

This work is an attempt to compare the various types of undernutrition, to identify the features common to each type, and also to relate them to the specific and other complicating or ecological causative factors. It also seemed rewarding to draw a parallel between the mass disease seen in the Developing World and the now rare forms due to organic disease or psychological causes in the advanced countries. It was also attempted to fit in the contemporary literature early information published by the German and French classics of paediatrics, acquired at a time when infantile malnutrition was still frequent in Europe. In this context the experiences collected during the famine in the Second World War are described as well. It is endeavoured by the auther to reconcile, so far as possible, contradictory opinions concerning the pathophysiological features of these conditions and to trace the routes of the different opinions.

In the 24 chapters of the book, illustrated by 47 figures and 74 tables, among others the protein, carbohydrate, fat and water metabolism, the endocrine problems, the changes in circulation, renal function, thermoregulation, as well as the influences of infections and of diarrhoea are discussed in detail. Important chapters analyze the possible immediate causes of death, and the long-term effects on development and intellectual functions.

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Neonatal Ventricular Haemorrhage: Treatment by Puncture

G Simon

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Transfontanellar ventricular puncture and intermittent lumbar punctures were carried out in 10 patients affected by intraventricular haemorrhage during the period between 1980 and 1983. In only three of them was later a shunt necessary for treatment of hydrocephalus. Six children developed normally, in two severe mental retardation ensued, and two died later, one of heart failure after cardiac surgery and one of ventriculitis following shunt implantation. Puncture therapy can be recommended for intraventricular haemorrhage of the neonate.

The most frequent and most severe form of intracranial haemorrhage in the neonatal period is periventricular and/or intraventricular haemorrhage (IVH). The disorder plays an important role in perinatal morbidity and mortality [17]. In recent years several reviews on the pathology and aetiology of IVH have been published [2, 6, 7, 8, 21, 23] and also an excellent summary of the clinicopathological findings [17]. The affected babies are mostly premature but the disorder may also occur in mature newborns [5, 9, 16]. Follow-up of the survivors [13] has shown that a favourable outcome can be expected in cases affected by IVH of stages I or II according to the classification of Papile et al. [18]. The advent of sonography and computer tomography allowed to diagnose the condition in vivo and a more exact follow-up of the haemorrhage itself [1, 3, 14, 18].

Few reports deal with the therapy [15, 19, 24]. There is almost general agreement that there is no effective treatment, the overwhelming majority of patients dies, and the survivors are severely damaged.

Continuous drainage of IVH was based on the idea that blood in the CSF plugs the resorptive surface, thus resulting in intracranial hypertension after the disappearance of blood from the CSF. This in turn leads to further ventricular dilatation and brain damage. Removal of blood by drainage prevents obstruction of the resorptive surfaces and its consequences [19]. This has been supported by animal experiments [20]. Mantovani et al. [15] found that in cases of IVH daily lumbar puncture is not a sufficient method for prevention of hydrocephalus.

Material and Method

Between January 1, 1980, and December 31, 1983, transfontanellar ventricle puncture was performed in 18 instances in ten

TABLE I

Serial number of patient	Gestational age, weeks, Gender	Birth weight, g	Course of pregnancy and delivery	Apgar score	Postnatal events	Cerebrospinal fluid
1	41 M	3600	25 yrs, parity: 2, oedema, precipitated delivery	2/3/5	Resuscitation, spontaneous respiration after 10 minutes, 9 hours later generalized fits, sustained inconsciousness	Lumbar and ventricular puncture at age of 12 hours: massive blood. Two ventricular punctures, 3 lumbar punctures
2	32 M	1550	28 yrs, parity: 2, breech delivery (Bracht extraction)	3/4	Resuscitation, spontaneous respiration after 5 minutes. Repetitive apnoeic spells, generalised muscle hypotension, fontanellar bulging	Lumbar and ventricular puncture at 36 hours. Blood containing fluid. Total protein: 2.2 g/l. One ventricular puncture, 3 lumbar punctures
3	41 F	2380	17 years, primipara, during pregnancy Henoch-Schönlein purpura, meconium containing amnial fluid	8/9	Clonic fits on extremities at 36 hrs. At 48 hours: bulging fontanelle, right palpebral ptosis, repetitive apnoeic spells	Ventricular punctures at 48 h, dark-yellow fluid, total protein: 1.7 g/l. One ventricular puncture, 3 lumbar punctures
4	33 F	1660	37 yrs, parity: 3, EPH gestosis, fragmented, defective placenta	6/7	36 h: spactic muscles, opisthotonus, ocular symptoms	Lumbar and ventricular puncture at 48 hrs, blood containing CSF, total protein: 1.94 g/l. Two ventricular and 2 lumbar punctures
5	41 M	3950	25 yrs, primipara, 2/3 earlier imminent abortion	2/3	Admitted from another ward at 4 h. Spastic muscles, opisthotonus, ocular symptoms, small fontanelle	Lumbar and ventricular puncture at 12 hours, blood in CSF. Total protein: 1.21 g/l. 3 ventricular and 3 lumbar punctures
6	41 M	4130	33 yrs, parity: 2. Protracted expulsion phase	4/7/8	24 h: irritability, spastic muscles, generalized fits, ocular symptoms, small fontanelle	Lumbar and ventricular puncture at 48 hrs, blood containing CSF. Total protein; 5.1 g/l. Tvo ventricular, seven lumbar punctures

7	40 M	3620	33 yrs, parity: 3, cephalic presentation, uneventful delivery	9/10	After 48 h repetitive apnoeic spells, spastic muscles	Lumbar and/ventricular puncture at 72 h, blood containing CSF. Total protein: 2.94 g/l. Two ventricular and 8 lumbar punctures
8	36 M	2740	22 yrs, primipara, hypertension, EPH gestosis, protracted extrusion phase	6/9	22 h: apnoeic spells, muscular hypotension	Lumbar and ventricular puncture at 36. Total protein 7.42 g/l. Two ventricular and 5 lumbar punctures
9	36 M	2750	26 yrs, parity: 3, cephalic presentation, uneventful delivery	9/10	24 h: irritability, apnoeic spells, partial exchange transfusion for hyperviscosity, 72 h: bulging fontanelle	Lumbar and ventricular puncture at 72 h, blood in CSF, total protein: 2,94 g/l, two ventricular and 5 lumbar punctures
10	41 M	3190	27 yrs, primipara, protracted expulsion phase, meconium stained amnial fluid	6/9	8 h: pallour, bradycardia, 24 h: ocular symptoms, spastic musculature, generalized fits	48 h: lumbar and ventricular puncture. Total protein: 2.02 g/l. One ventricular and 4 lumbar punctures

patients affected by IVH. Table I sums up the most important data of the perinatal period. The diagnosis was based on clinical symptoms which were not very characteristic and had much in common with those of hypoxaemic and ischaemic cerebral damage, on sonography and CSF cytology, the latter to exclude artificial bleeding. In addition, severity of symptoms (increasing intracranial pressure, convulsions, unconsciousness) was considered in indicating ventricular puncture.

The puncture was performed after diazepam pretreatment under sterile conditions, using a lumbar cannula. First the subdural space was punctured, then the needle was directed towards the frontal horn of the lateral ventricle. In none of the ten cases reported here was there a subdural haemorrhage, in nine cases the ventricular fluid contained macroscopic blood, in one patient it had a deep yellow colour. In order to avoid sudden volume reduction in the ventricles, the ventricular space was rediluted by injecting body-warm physiological saline; this also enhanced removal of cellular elements from the CSF. In patients in whom clearance of the fluid could not be achieved, puncture was performed on the opposite side 24 hours later. This treatment was supplemented by lumbar punctures carried out every day or every second day. The following drugs were used: synthetic vitamin K_1, vitamin C, rutin, calcium gluconate, oestriol disodium succinate, furosemide and phenobarbital and, for preventing infections, antiobiotics were applied. The patients received parenteral nutrition until consciousness had been regained. In nine patients leaving the department, neurohabilitation after Katona's method [10, 11, 12] was introduced.

Results

There are several methods for determining the time of onset of haemorrhage [3, 4, 22]. In our cases the diagnosis was established in two patients within 24 hours of age, in six between 24—48 hours, and in two in 72 hours. Protracted clearing up of the CSF was seen in four patients (Nos 6, 7, 8 and 9); in three of these (Nos 6, 7 and 8) implantation of a ventricular shunt was necessary. In the six remaining patients the CSF became clear gradually, there was no sign of rebleeding. The outcome of our cases in May, 1984, is demonstrated in Table II.

Discussion

A large proportion of patients affected by neonatal intracranial, periventricular or intraventricular haemorrhage dies and many of the survivors exhibit brain damage. Early diagnosis can be achieved by sonography. Severe clinical symptoms, signs of increased intracranial pressure and blood in the lumbar CSF indicate a transfontanellar puncture of the lateral ventricle. Removal of the blood, prevention of obstruction of the resorptive surface and of sustained intracranial hypertension reduces the mortality and improves the quality of life of the survivors.

Since transfer of the newborn affected by acute haemorrhage is not recommended, the treatment must be carried out in the local pathological newborn ward. A few days after onset of intraventricular haemorrhage, when transfer is already possible, initiation of treatment is too late and the results are disappointing.

TABLE II

Serial number of patient	Age at checking, months	Hydro-cephalus	Shunt	Motor handicap	Epilepsy	Other handicaps	Mental development	Note
1	42	—	—	spastic tetraplegia	BNS 4 mo	—	Severely retarded	Uneducable
2	36	—	—	—	—	hypermotility, disordered sleep	Coherent speech	
3	36	—	—	—	—	convergent squint	adequate for age	Wears glasses
4	36	—	—	—	—	—	adequate for age	
5	10	—	—	—	—			Dropped out, emigrated
6	12	+	+	retarded motor development	—	—	retarded	Checked in National Institute of Neurosurgery
7	12	+	+	retarded motor development		secondary generalised	slight retardation	12 months: CT, shunt, 3 mo later death of ventriculitis
8	12	+	+	—	—	—	adequate for age	
9	6	—	—	motor and generalised muscular hypotension	—	transposition of great arteries	slightly retarded	6 mo: death after cardiac surgery
10	10	—	—	—	—	convergent squint	adequate for age	

We did not encounter convulsions, apnoea, increase of bleeding tendency or infection during puncture treatment.

Because of the small number of cases and the lack of a control group, valid conclusions cannot be drawn from our experience, but it appears encouraging against the pessimistic approach to therapy of intraventricular haemorrhage: 6 out of 10 patients are intact survivors.

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Examination of the Ouabain-Sensitive Na-K Pump in Essential Hypertensive Children of Normal Body Weight

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The ouabain-sensitive Na-K pump of erythrocytes was examined in 17 normotensive and 15 essential hypertensive children aged 6—16 years. Children who proved to be fat according to skinfold measurements were excluded from the examinations. The activity of the ouabain-sensitive Na-K pump was assessed by measuring the ratio of Na-ion efflux and K-ion influx through the erythrocyte membrane previously treated with para-chloro-mercury benzol sulphonate. In essential hypertensive children the ratio of the Na/K fluxes was found to be characteristically 5.8 ± 2.0, showing a mathematically significantly more active ($p < 0.05$) Na-K pump function than in the control group 9.6 ± 5.8. According to the results, however, the method is suitable only for the separation of groups and not of individuals.

In the developed countries some 50% of the mortality is caused by diseases of the circulatory system. During the last decade this high mortality ratio started to decrease in several countries, so in Finland [11], Belgium [12] and the United Kingdom [9], among others. According to WHO [23] the mortality of cardiovascular diseases has considerably been reduced in a large part of the world, while in other countries e.g. in Hungary, it still increases moderately. In 1960 out of 10 000 Hungarian inhabitants 45 had died from cardiovascular disease while in 1982 this number was 73.

In countries where the mortality has decreased, the health organizations carried out an effective prevention programme, trying to recognize the earliest possible the risk factors of the cardiovascular system and to eliminate them. One of the best known risk factors present in 10—20% of the grown-up population, is essential hypertension.

According to Rossi and König [20], the frequency of hypertension in childhood is only 1—3%. According to Bühlmeyer [2] hypertension in childhood is of renal origin in 79% and of essential character only in 5%; other workers have observed essential hypertension in children more frequently. All data state, however, that essential hypertension begins in childhood, and that its rate increases with age.

According to present knowledge, essential hypertension is the consequence of multifactorial causes [4]. It seems to manifest itself under the effect of environmental factors in

genetically determined persons. If this hypothesis would agree with the facts, early recognition of the endangered persons were possible with the help of genetical markers, and thus the onset of the disease could be prevented.

In the last 15 years, several selective and non-selective population studies have dealt with the cardiovascular risk factors. Specific investigations were carried out primarily in children whose parents had had cardiac infarction in their young days. In their case it is namely likely that under similar aetiologic and familial circumstances a metabolic disturbance of the lipoproteins would occur more frequently than in the healthy population [10]. At the same time, other authors examined the erythrocyte membrane. Its ion content and transport mechanism were compared in a normotonic population and in a population with essential hypertension and their direct relatives.

Some of the main steps in research were as follows. In 1960 Losse et al observed a different Na content [14] and in 1977 Poston et al [19] a different Na permeability and Ca-binding power of the red blood cell membrane and an increased Na-K ATP-activity in hypertonics compared to the control group. In 1978 new data were reported on the lithium ion transport of erythrocytes [16] and on their Na-K pump [21]. Then Garay and Meyer [6] described a new test which is able to detect the changed activity of Na^+ and K^+ flux in the erythrocytes of patients with essential hypertension. This was soon followed by the observation of Meyer et al [15] of the activity decrease of Na-K cotransport in ouabain treated erythrocytes in patients with essential hypertension and in their direct descendants. These results have been confirmed by Davidson et al [4].

Na-Li exchange can similarly be examined in the membranes of ouabain inhibited erythrocytes; it was found to be increased in the erythrocytes of patients with hypertension [3, 5, 22].

From among the above-mentioned observations the most important ones from the point of view of essential hypertension are shown in Table I. The present work deals with the ouabain sensitive Na-K pump (Fig. 1) in normotensive and essentially hypertensive children.

TABLE I

Transport processes in the erythrocytes of essentially hypertensive (EH) and normotensive patients

Transport process	Ions	Inhibitor	Transport protein	Alteration in E.H.
Na-K pump	**Na(Li), K(Li)**	**ouabain**	**Na-K ATP-ase**	increase
Na-Li countertransport	**Na, Li**	**phloretin**	**undefined**	increase
Na-K cotransport	**Na, K**	**furosemide**	**nndefined**	decrease

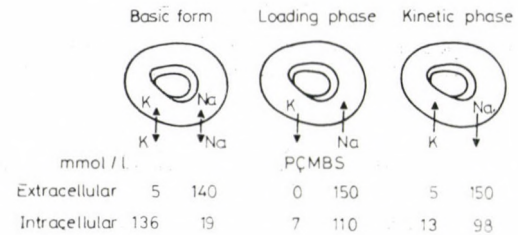

FIG. 1. Simplified scheme of method used for examination of the ouabain sensitive Na-K pump

PATIENTS AND METHODS

Data of 17 healthy normotensive children whose familial history did not reveal hypertensive relatives were compared with those of 15 earlier examined children who were treated for hypertension. The diagnosis of essential hypertension was qualified on the basis of routine examinations [20]. The age of the children ranged from 6 to 16 years. Their skinfolds including the calf skinfold were measured with Holtain caliper according to the International Biological Program (IBP) on the left side of their body. Children who proved to be fat on basis of the Parizková and Roth [17] formula were excluded from the examinations.

Blood pressure of the children was measured on the right upper arm in sitting position, after 5 minutes of relaxation, with a mercury tonometer using a cuff suited to the width of the upper arm. To the patients subjected to examination no drugs have been administered for one week prior to blood analysis which after 12 hours of starvation was done at 8 o'clock a.m.

The ouabain sensitive Na-K pump of the erythrocytes was examined by Garay and Meyer's method [6] with some modifications. Freshly collected heparinized venous blood was centrifuged at 1750 g, at a temperature of 4 °C for 5 min. The red blood cells obtained were washed twice in 20 vol per cent concentrated solution containing 150 mmol/L NaCl; 2.5 mmol/L $MgCl_2$; pH = 7.4±0.02, and then they were placed into a Na-loading solution of the following composition; 150 mmol/L NaCl; 2.5 mmol/L $MgCl_2$; 2.5 mmol/L Na_2HPO_4; 0.1 mmol/L PCMBS (parachloromercury benzolsulphonate); 6 mmol/L glucose; pH 7.4±0.02. The red blood cells were incubated in this solution for 20 hours and 4 hours later the solution was exchanged for a fresh one, centrifuged and the supernatant was weighed and incubated in a solution containing 150 mmol/L NaCl; 2.5 mmol/L $MgCl_2$; 2.5 mmol/L Na_2HPO_4; 6 mmol/L glucose and 4 mmol/L cysteine at a temperature of 37 °C for 1 hour, in order to wash out the PCMBS.

One ml of the suspension obtained by centrifugation at 1750 g at 4 °C for 5 min was mixed in Ringer's solution composed of 145 mmol/L NaCl; 5 mmol/L KCl; 2.5 mmol/L $MgCl_2$; 2.5 mmol/L Na_2HPO_4; 10 mmol/L glucose; pH 7.4±0.02. Two ml of this were then incubated for 0, 1, 2, 3, 4 and 5 hours at 37 °C, centrifuged as described above, then washed twice with 150 mmol/L choline chloride. To each incubation time two parallel samples belonged.

The samples obtained were haemolysed in 4 ml distilled water and their Na^+ and K^+ content was measured by flame photometry. In this way the original volume could be calculated. The volume loss occurring between the consecutive steps was checked by haemoglobin and haematocrit determination and then corrected.

The ratio of Na-K fluxes was calculated with the least squares method.

TABLE II

Na^+ and K^+ ion-concentrations of erythrocytes of normotensive and hypertensive children after PCMBS treatment. Ion-transport in mmol/l in erythrocytes, measured hourly

	Na_{effl}/K_{infl}	Na_{start} mmol/l	Na_{effl} mmol/l/h	K_{start} mmol/l	K_{infl} mmol/l/h
Control	9.6	104	2.6	6.7	0.42
n = 17	±5.8	±23	±1.1	±5.3	±0.34
Hypertensive	5.8	111	3.4	9.6	0.63
n = 15	±2.0	±18	±1.5	±7.0	±0.29
p <	0.05	0.35	0.15	0.2	0.1

Abbreviations:

Na_{start}/K_{start} = ion concentrations after PCMBS treatment
Na_{effl}/K_{infl} = ion concentrations
Na_{effl}/K_{infl} = quotient of ions and out of the cell; ratio

RESULTS

In conformity with data in the literature, the intracellular Na^+ concentration of erythrocytes treated with PCMBS of hypertensive patients was found to be high, 111±18 mmol/L, while the value in the control group was 104±23 mmol/L. A similar non-significant increase was observed in the original intracellular K^+ concentration, 9.6±7.0 mmol/L versus 6.7±5.3 mmol/L in the controls. Examining the Na-K pump of erythrocytes in hypertensive patients, there was an increased intracellular accumulation of K^+ (K_{infl}) and also an increased Na^+ efflux (Na_{effl}) as compared to the controls. To judge the operation of the pump, the ratio

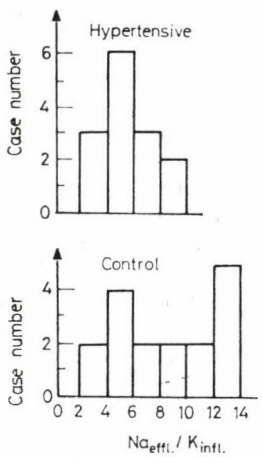

FIG. 2. Number of essentially hypertensive and normotensive children plotted against Na_{effl}/K_{infl}

Na_{effl}/K_{infl} was examined. In the control group it amounted to 9.6 ± 5.8, while in the essentially hypertensives to 5.8 ± 2.0; the difference was significant ($p < 0.05$) (Table II). Plotting the case numbers against Na_{effl}/K_{infl} (Fig. 2), the majority of Na_{effl}/K_{infl} values of hypertensive patients lay under 6, while those of the controls were usually higher. It was also remarkable that while the values of the hypertensives were evenly distributed along an incidence maximum, those of normotensives were either above 12, or near to the values of hypertensives.

Discussion

The purpose of this work was to find a method which would allow to select from among the total population those children who at the time of the examination were still normotensive, but were inclined to develop essential hypertension. Since for technical reasons the effectiveness of the various methods cannot be measured directly, therefore an inverse situation was created wherein children diagnosed as essentially hypertensive were separated from the normotensives.

The results obtained permit to outline a hypothesis. In the erythrocytes of patients, the K-Na cotransport decreases and consequently the Na^+ content of cells is elevated, causing a volume increase in the erythrocytes. The ouabain sensitive Na-K pump is not always able to compensate this pathological situation. According to Blaustein's hypothesis [1] the mechanism takes place not only in erythrocytes, but also in all kinds of cells, thus also in the smooth muscle cells. The increased Na^+ content of the smooth muscle cells may inhibit the Na-Ca exchange. The increased Ca^{2+} concentration would then lead to a constriction of smooth muscle cells, to vasoconstriction and hypertension.

Some other authors assumed that essential hypertension was caused by the increased activity of a Na-transport inhibitor circulating in the blood [13], but other researchers refused this concept [22].

Postnov et al [19] also assumed the existence of a hypothetic substance. They observed a reduced Na^+ efflux when incubating the leukocytes of normotensive subjects with the plasma of hypertensive patients.

According to various hypotheses, hypertension is presumably a multifactorial disease. However, the ion content, as well as the ion exchange through the erythrocyte cell membranes of essentially hypertensives and of their relatives differ from that of normotensives. This observation would allow the following considerations. The symptom-free risk population could be recognized in due time and, by changing the environmental factors, the prevention of hypertension could be possible. The distinction between primary and secondary hypertension could be done with a single examination [7] and so the environmental and genetic causes of essential hypertension could be

differentiated. These possibilities could, however, be only realized if the techniques are further improved. The results obtained by the currently used methods including those obtained with the ouabain sensitive Na-K pump, are suitable only for the separation of groups but because of the wide scatterings they are inadequate for the diagnosis of individual cases. Besides, due to diagnostic difficulties some secondary hypertensions are misdiagnosed for essential hypertension, and the control group may contain such normotensives who on the basis of their genetic characteristics are inclined to hypertension. To this last group may have belonged the four normotensive children included in our examination, whose Na_{effl}/K_{infl} values were similar to those of hypertensive patients.

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Treatment of Neonatal Hyperbilirubinaemia with Flumecinolone, a New Enzyme Inducing Drug

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The effect of flumecinolone, a new drug with enzyme inductor properties, on non-haemolytic hyperbilirubinaemia of term and premature newborns has been investigated. Prophylactic treatment with the drug prevented the development of severe hyperbilirubinaemia. Alone or in combination with phototherapy, flumecinolone inhibited the steep rise of serum bilirubin in premature infants. A similar effect has been shown in term babies with haematomas. The new drug is void of all side-effects of phenobarbital.

A significant proportion of neonatal hyperbilirubinaemias is caused by decreased glucuronyl transferase activity in the liver. The activity of this enzyme can be induced by phenobarbital. This knowledge was the basis for the introduction of phenobarbital treatment of icterus gravis neonatorum [7]. This drug is not any more widely used for this purpose because of its side-effects [1]: it takes 3—4 days to attain the maximum effect, the drug has to be applied intramuscularly, accumulation of the drug may lead to depressed respiration and it is less effective than phototherapy.

Flumecinolone, 3-trifluoromethyl-α-benzhydrol (Zyxorin®, Gedeon Richter Ltd. Budapest, Hungary) acts in the liver as an enzyme inductor but, in contrast to phenobarbital, it does not depress respiration, does not inhibit imprinting, nor leads to paradoxical irritability or hyperactivity, and can be applied by mouth [9]. After completion of pharmacological studies in adults the drug has now been tested in paediatric patients as well.

We have examined the effect of flumecinolone on the course of the bilirubin level of premature babies, to test whether the drug was suitable for the treatment of non-haemolytic hyperbilirubinaemia of premature infants. In addition, the prophylactic effect of the drug on hyperbilirubinaemia has also been tested in term babies having haematomas.

Material and Method

56 newborns with moderately low birthweight, all born after completion of the 35th week of gestation, were treated with flumecinolone, 30 mg/kg bodyweight in a single dose daily for 3 days. Their mean weight was 2285 g, mean gestational age

was 36.5 weeks. 52 babies had a five-minute Apgar score higher than 7, 4 had a value equal to or lower than 7. There was no case of Rh-incompatibility, ABO incompatibility was present in one case. Flumecinolone therapy was started whenever the level of indirect serum bilirubin increased above the value of 180 μmol/l.

Flumecinolone alone was applied in 19 cases, combined with phototherapy in other 19 cases. This latter was started if the serum bilirubin level remained at the margin of indication for exchange transfusion by 24 hours after introduction of flumecinolone treatment. 18 premature infants were treated only with blue light lamps (Medicor KLA-21). Serum bilirubin was determined by the method of Jendrassik and Gróf. During the first ten days of life blood and reticulocyte counts, prothrombin time, serum sodium and potassium, serum GOT, GPT and γ-GT, total protein and albumin, immune electrophoresis, serum creatinine, blood urea nitrogen, blood and urinary glucose were regularly checked; the same laboratory tests were carried out some time during the subsequent weeks. 15 term babies (mean birthweight 3320 g, mean gestational age 40.0 weeks) with extensive haematomata were treated with a single daily dose of 30 mg/kg flumecinolone for 4 days from the first day of life.

Results

The course of serum bilirubin of the 19 preterm babies treated with flumecinolone alone is represented by Line 1 in Figure 1. Line 2 shows the changes seen in the 19 premature babies treated with flumecinolone and additional phototherapy, while Line 3 stands for the mean values of the 18 prematures treated with phototherapy alone. Table I shows the numerical mean values and their standard deviations in the three treatment groups. From Figure 1 and Table I it can be seen that flumecinolone treatment, alone or in combination with phototherapy, keeps the bilirubin level below that observed in infants receiving only phototherapy. At first sight it seems curious

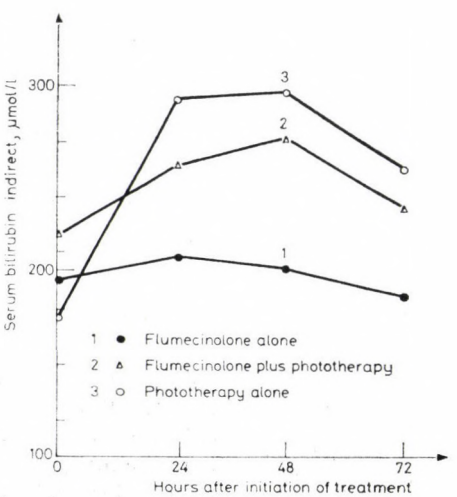

Fig. 1. The course of hyperbilirubinaemia in 56 preterm babies

TABLE I

Serum bilirubin levels in three groups of jaundiced preterm newborns, means and standard deviations

Treatment groups	n	Serum bilirubin, indirect, μmol/l							
		Before therapy		24 hours after initiation of therapy		48 hours after initiation of therapy		72 hours after initiation of therapy	
		mean	SD	mean	SD	mean	SD	mean	SD
Group 1									
Flumecinolone	19	195	51	205	85	205	83	185*	70
Group 2									
Flumecinolone plus phototherapy	19	220	65	255	39	270	51	235*	60
Group 3									
Phototherapy	18	175	65	290	51	295	62	255**	72

* no significant difference from initial value
** significant difference from initial value, $p < 0.01$ (paired t-test)

that Line 2 runs a higher course than Line 1; the mode of selection, however, fully explains this difference: since additional phototherapy was only introduced in case of a high bilirubin level observed 24 hours after initiation of drug treatment, i.e. in spite of flumecinolone treatment, the initial values were already higher in the babies needing subsequent additional phototherapy. The data show that flumecinolone, alone or combined with phototherapy, is capable of preventing a further rise in indirect serum bilirubinaemia without blood-group incompatibility. After 72 hours the mean serum bilirubin level of infants treated with flumecinolone alone was lower than their own initial mean value. In the group treated with flumecinolone plus phototherapy the 72-hour value did not differ from the initial value while in babies treated with phototherapy alone the mean value observed 72 hours after introduction of therapy was significantly higher than the initial mean of the same group.

In 15 term newborns affected by haematoma (face, limbs or cephalhaematoma) flumecinolone prophylaxis was started at the age between 12 and 24 hours. The results are demonstrated in Figure 2 and Table II. Figure 2 shows the course of serum bilirubin of normal and pathological newborns during the first week of life. The moderating effect of flumecinolone on the rise of serum bilirubin can clearly be seen. From Table II it can be noticed that the difference was significant from the third day of life. Exchange transfusion had to be carried out in one patient receiving flumecinolone prophylaxis and three patients left untreated.

Four cases deserve further attention.

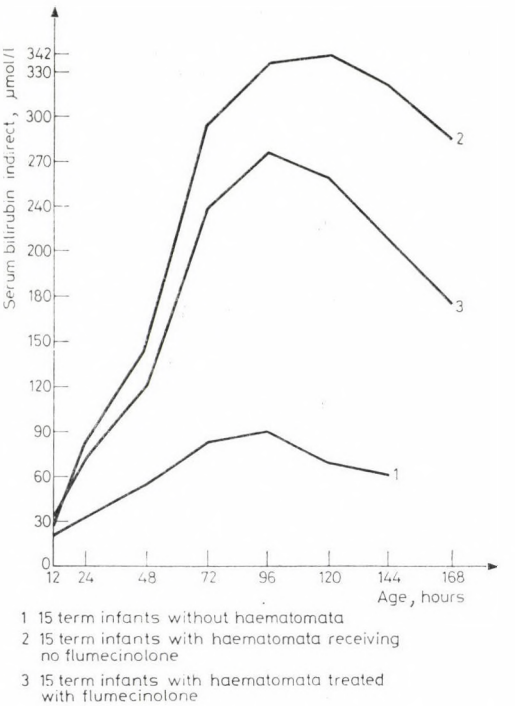

FIG. 2. The effect of flumecinolone on serum bilirubin of term newborns with and without heamatoma

In a newborn with a birthweight of 2700 g and a gestational age of 40 weeks, affected by AO incompatibility, flumecinolone combined with phototherapy did not prevent the rise of serum bilirubin to a level necessitating exchange transfusion.

In a newborn weighing 3100 g, and of 39 weeks gestational age, phototherapy had to be supplemented with D-penicillamine in order to avoid exchange transfusion. After blood-exchange a rebound ensued, the patient's serum bilirubin persisted a-

TABLE II

Effect of flumecinolone on serum bilirubin in term newborns with haematomas

Age in hours	n	Serum bilirubin, indirect, µmol/l means and standard deviations							
		12	24	48	72	96	120	144	168
No flumecinolone	15	mean 26	160	160	294	336	338	333	284
		SD 8	35	50	61	61	65	54	54
Flumecinolone	15	mean 32	74	140	240	278	260	216	174
		SD 10	33	48	60	63	66	55	52
Significance of difference	p<	—	—	—	0.05	0.02	0.01	0.001	0.001

round 350 μmol/l; this prompted us to initiate flumecinolone therapy on the 13th day of life. By 72 hours after introduction of the drug the serum bilirubin level had dropped to 140 μmol/l.

In two term babies affected by prolonged neonatal jaundice, flumecinolone treatment induced a rapid, marked fall in serum bilirubin.

No side-effects have been encountered. The parameters listed above (blood picture, prothrombin time, blood gases and electrolytes, serum total protein, liver and kidney function tests) remained normal during the first 10 days of life and showed no pathological changes at later checking, either.

Discussion

Introduction of phototherapy into treatment of hyperbilirubinaemia of term and preterm newborns has caused a marked fall in the number of exchange transfusions [2]. Lakatos et al have proposed the use of D-penicillamine for the same purpose [4]. Haemocarbo-perfusion has also proved useful in abolishing extreme hyperbilirubinaemia [6]. Phenobarbital alone or combined with diethylnicotinamide has been shown effective in preventing severe hyperbilirubinaemia [8] and clofibrate has also been used for treating jaundice of term newborns [5]. Flumecinolone has been demonstrated to induce bilirubin conjugation in the liver [10].

In this study preterm newborns needing no intensive care have been investigated. The mean serum bilirubin level 72 hours after initiation of treatment was significantly lower in the group treated with flumecinolone than in newborns treated with phototherapy; the same holds for the combined treatment using flumecinolone and phototherapy. We therefore recommend flumecinolone therapy in addition to phototherapy or D-penicillamine for treating hyperbilirubinaemia of preterm babies born after the 36th week of gestation because flumecinolone has been observed to moderate the hyperbilirubinaemia and to have no toxic or side-effects.

In addition, haematoma is an important factor in eliciting neonatal jaundice, ranking after blood-group incompatibilities, asphyxia and infection [3]. Flumecinolone, a compound void of the side-effects of phenobarbital, is the drug of choice in treating newborns affected by haematoma.

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Simultaneous Occurrence of Diabetes Mellitus and Coeliac Disease

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Subtotal villous atrophy in the proximal jejunum was observed in six patients affected by juvenile diabetes. Introduction of gluten free diet invariably led to clinical improvement, in the four patients in whom also rebiopsy was performed the jejunal mucosa exhibited improvement. In all cases gluten sensitive enteropathy was diagnosed after the onset of diabetes. Marked stunting in growth, strikingly labile carbohydrate tolerance, pronounced proneness to hypoglycaemia or development of Mauriac's syndrome were the symptoms pointing to coeliac disease. Protracted diarrhoea was seen only in two patients, pronounced deceleration in weight development occurred in none of the six children. In four patients out of six the presence of both HLA B8 and DR3 antigens was demonstrated, in a fifth patient only DR3 was present; this suggests a common genetic background of the simultaneous occurrence of the two disorders. Untreated coeliac disease aggravates preexisting diabetes. The importance of early recognition of latent coeliac disease is stressed.

A close relationship between coeliac disease and diabetes is supported by the fact that certain HLA antigens show a much higher frequency in both disorders than in the general population. The simultaneous occurrence of the antigens B8 and DR3 is characteristic of both disorders while a high incidence of B15 and DR4 has been only observed in diabetes and these latter antigens exhibit no increased frequency in coeliac disease [9]. In a previous study we demonstrated a 32.8% occurrence of B8 in juvenile diabetes [1], while in coeliac disease B8 was demonstrated in 66.7, and DR3 in 77.8% of the cases [5].

This study has been performed to analyse the clinical features and establish the incidence of HLA antigens in six patients affected by both diabetes mellitus and coeliac disease.

Material and Methods

Of the patients two were boys and four were girls. Their age varied between 0.5 and 7 years at the onset of diabetes the diagnosis of which was based on the characteristic history, clinical and laboratory findings. At the time of the first jejunal biopsy their age ranged between 2.5 and 11.5 years. Coeliac disease was diagnosed on the basis of subtotal villous atrophy of the jejunal mucosa and clinical and mucosal improvement after introduction of a gluten-free diet. No rebiopsy has yet been performed in patients 5 and 6 because of shortness of time having elapsed since the first biopsy. In the morphometric clas-

TABLE I

Clinical data and HLA antigens of patients

Serial number of patient	Sex	Age in years at onset of diabetes	Age in years at time of 1st jejunal biopsy	Duration of treatment with gluten-free diet (years)	Height deficit in SD		Height corrected weight in SD deviating from the mean	
					before introduction of gluten-free diet	after	before introduction of gluten-free diet	after
1	girl	2.25	9.5	1.0	−1.9	−1.5	+0.1	+2.0
2	girl	1.5	2.5	7.75	−2.4	−4.0	−0.6	+3.5
3	boy	0.75	9.75	2.25	−2.75	−4.0	−0.6	+2.7
4	girl	7.0	11.5	1.0	−1.8	−1.6	+0.4	+0.9
5	boy	0.5	3.75	0.5	−6.0	−5.6	+0.6	+1.8
6	girl	5.5	5.75	0.5	−1.8	−2.3	−1.0	+1.8

sification of the jejunal mucosa the principles of Kuitunen et al [7] were applied. The antigens HLA A, B and C were determined by the standard NIH method, the DR antigens by the lymphocyte cytotoxicity micromethod of prolonged incubation time.

RESULTS

The findings are summed up in Table I. The onset of diabetes preceded the first manifestation of coeliac disease in all six patients. At that time the deficit in height of these patients ranged between −1.8 and −6.0 SD for age while their height corrected weight was normal. There was no improvement in age related height during the gluten-free diet; in fact, in two patients there was a further aggravation of the deficit. Weight gain ensued in all patients during the diet. The first symptom pointing to coeliac disease were diarrhoea and hypoglycaemia in one patient, only diarrhoea in one patient, appearance of Mauriac's syndrome in three patients and herpetiform dermatitis in one patient. In patient 3 diabetes and coeliac disease were accompanied by autoimmune haemolytic anaemia and chronic persistent hepatitis.

B8 was found in four and DR3 in the same four and in one additional patient. None of these five patients possessed B7. The presence of antigens B7 and DR2 is not characteristic of either diabetes or coeliac disease [1, 5]; these two antigens, quite conspicuously, were present in the patient affected by additional autoimmune haemolytic anaemia and chronic persistent hepatitis, but this patient had no B8 or DR3.

DISCUSSION

In the majority of our cases coeliac disease was suspected because of the appearance of non-enteral symptoms, impaired growth, labile carbohydrate metabolism and unexpected hypo-

having both diabetes and coeliac disease

Symptoms raising the possibility of malabsorption	Associated disorders	HLA antigens
Diarrhoea, Hypoglycaemia		A3, 29, B12, Bw35, Cw4, DR3, 5
Mauriac syndrome		A1, 3, B8, 12, DR, 4
Diarrhoea Mauriac syndrome	Autoimmune haemolytic anaemia, chronic persistent hepatitis	A3, 31, B7, Bw35, DR2, 5
None	Dermatitis herpetiformis	A1, 2, B8, 39, DR2, 3
Mauriac syndrome		A1, 2, B8, DR2, 3
Mauriac syndrome		A2, Aw24, B8, Bw50, DR3

glycaemia. In 13 patients out of 14 reported by Walsh et al [11] coeliac disease developed 5—15 years after the onset of diabetes.

Both coeliac disease and diabetes may be associated with autoimmune disorders, e.g. thyroiditis, rheumatoid arthritis, chronic persistent hepatitis, bronchial asthma or vitiligo [9, 10]. In one of our patients gluten sensitive enteropathy was accompanied by dermatitis herpetiformis. In all four similar cases of Holt and Blockweil [6] herpetiform dermatitis appeared after the onset of diabetes. In another patient of our material, diabetes was accompanied by chronic persistent hepatitis and autoimmune haemolytic anaemia.

The question emerges of the relationship between diabetes and coeliac disease. The significantly higher incidence of the same HLA antigens points to a close genetic relationship and may explain the association of the two disorders. Lecornu et al [8] observed decreased somatomedin levels in gluten sensitive enteropathy. This may partly explain the retarded growth of our patients. It is still obscure why the onset of diabetes precedes by so much time the recognition of coeliac disease. It may be anticipated that the onset of coeliac disease precedes the appearance of diabetes but it is not recognized because of the paucity of symptoms. Visacorpi [10] has made the interesting observation that diabetes develops in 4% of all patients affected by coeliac disease but in 10% of those in whom gluten sensitive enteropathy is diagnosed after completion of the second year of life. Among 24 diabetic patients simultaneously affected by coeliac disease the latter was diagnosed before the end of the second year of life in only two patients [2]. This is a curious finding since isolated coeliac disease is diagnosed during the first two years in the overwhelming majority of cases. From this it

may be anticipated that unrecognised, and therefore untreated, coeliac disease may predispose to a pathological carbohydrate metabolism. It is known that the gastric inhibitory peptide (GIP) released from the jejunum influences insulin secretion. Besterman et al [2] have shown in untreated coeliac patients that a test meal resulted in a significantly lower and flatter curve of plasma GIP resp. insulin than in healthy controls. Similar findings were obtained by Desjeux et al [4] in patients affected by villous atrophy of the jejunal mucosa, and Nutramigen administered directly into the duodenal lumen elicited a smaller increase in insulin levels than in control subjects with normal jejunal mucosa. Carson et al [3] found that gluten loading of coeliac patients provokes an increase in plasma glucagon. All these findings indicate that in coeliac disease, if untreated, the diabetic condition may be aggravated.

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Influence of Anticonvulsant Drugs on Thyroid Hormones in Epileptic Children

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Thyroid function tests were studied in epileptic children undergoing long-term anticonvulsive therapy with phenytoin, primidone or mephenytoin. Serum T_4 was decreased in all three treated groups, serum T_3 was diminished only in those treated with phenytoin or primidone. FT_4 was also significantly decreased while serum TSH and TBG were not affected in the treated patients. The effect of anticonvulsant drugs on thyroid hormone catabolism and peripheral conversion of T_4 seems to be important in these alterations.

In recent years several investigations have repeatedly documented that some anticonvulsants caused different alterations of thyroid hormone metabolism in adults [1, 5, 8, 9, 13, 14]. In this study we investigated the effects on the thyroid system of three anticonvulsive drugs commonly used in epileptic children.

Patients and Methods

Four groups of children aged 3—12 years were investigated. Three of them consisted of patients with epilepsy. They received phenytoin, primidone or mephenytoin therapy; the fourth group was the control one. The composition of these groups is demonstrated in Table I.

Abbreviations

T_4	thyroxine
T_3	triiodothyronine
FT_4	free thyroxine
TSH	thyreotropic hormone
TBG	thyroxine binding globulin
rT_3	reverse triiodothyronine

Phenytoin was administered to 15 children over a period of 6 months to 11 (mean, 4 3/12) years. Eleven patients received primidone for 7 months to 7 6/12 (mean, 3 6/12) years, and seven patients were given mephenytoin for 6 months to 3 1/12 (mean, 2 1/12) years. The patients were selected by excluding those with goitre or a history of thyroid disease. The diagnosis of euthyroidism was established

Table I

Groups investigated in the study

Group	n	girls/boys
Phenytoin	15	8/7
Primidone	11	6/5
Mephenytoin	7	3/4
Control	11	6/5

by careful clinical investigation, and with no knowledge of the results of thyroid function tests. The patients receiving anticonvulsant medication were taking no other drug. The control group comprised children without epilepsy and thyroid disease.

Serum was separated immediately from blood samples drawn at 08 hours and stored at −20 °C until analysis. Serum total T_4 and T_3 were determined by RIA (kits of the Isotope Institute of Hungarian Academy of Sciences). Serum free T_4, TSH and TBG were measured with commercial RIA kits (Amerlex free T_4 RIA, Radiochemical Centre, Amersham; RIA-mat-TSH, Byk Mallinckrodt; TBG RIA, CEA Sorin). All assays were performed in duplicate, statistical analysis was done with the standard *t* test.

Results

Figure 1 shows thyroid hormone concentrations in the different groups; individual values are indicated. In the treated groups, serum total T_4 level was lower than in the control group. Serum T_3 concentration was also lower in the groups receiving phenytoin and primidone. In these two groups, the decrease in serum FT_4 concentration was significant. In children treated with mephenytoin, serum TSH and TBG were unaffected by anticonvulsants (Table II).

Discussion

Phenytoin was the first anticonvulsant the decreasing effect of which on serum thyroid hormone concentrations was demonstrated with the protein bound iodine (PBI) method [11]. This observation was amply verified in later studies using the more specific competitive protein binding (CPB) and radioimmunoassay (RIA) methods for serum T_4, and the same effect of other anticonvulsant drugs [6, 9, 10, 14], together with the decrease in serum total T_3 concentration was also demonstrated.

The exact mechanism of the alteration of serum thyroid hormone con-

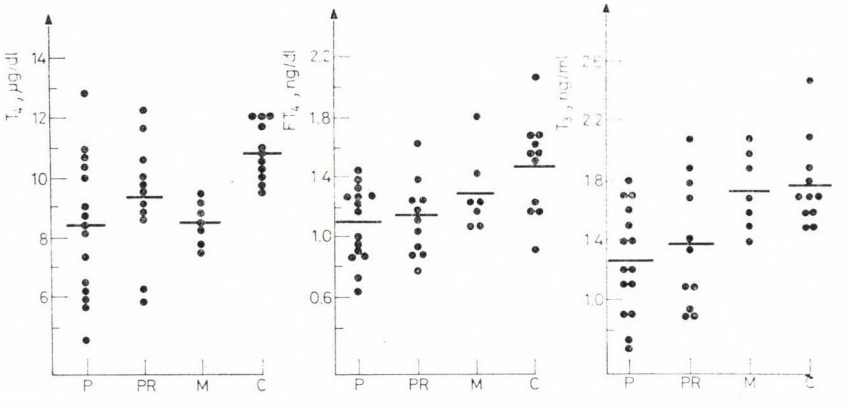

FIG. 1. Individual values of serum T_4, FT_4 and T_3 in different groups. P = Phenytoin; PR = Primidone; M = Mephenytoin; C = Control

TABLE II

Serum T_4, FT_4, T_3, TSH and TBG in children on long term treatment with various anticonvulsant drugs

Groups	(n)	T_4 μg/dl	FT_4 ng/dl	T_3 ng/ml	TSH μE/ml	TBG ng/ml
Phenytoin	(15)	8.42** ±2.31	1.10** ±0.24	1.25** ±0.42	2.53+ ±1.05	23.9+ ±5.28
Primidone	(11)	9.37* ±1.97	1.14** ±0.27	1.38* ±0.43	2.62+ ±1.23	25.2+ ±5.56
Mephenytoin	(7)	8.58*** ±0.50	1.31+ ±0.35	1.75+ ±0.32	2.81+ ±1.08	24.3+ ±2.97
Control	(11)	10.98 ±0.97	1.50 ±0.31	1.78 ±0.29	2.36 ±1.12	24.5 ±4.27

+ $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

centrations in patients treated with anticonvulsants is not known. It was suggested that these drugs would decrease the level of thyroid hormone binding proteins [14, 15], but in our groups we could not find any difference in the TBG level. It was also assumed that the anticonvulsants decreased the thyroid hormone concentration by displacing them from their protein binding. Although the mechanism, an increase in the free/bound thyroid hormone ratio was observed in vitro [12], we could not find it in our patients.

An increased catabolism of thyroid hormones might also be the reason for the diminished serum thyroid hormone concentration. Acceleration of the T_4 clearance by phenytoin was observed [8] and converting enzyme activity in the rat liver was also stimulated [7]. T_4 catabolism is increased by phenytoin via stimulation of the hepatic microsomal system [8]. The increased conversion of T_4 to T_3 [3] may explain why serum T_3 and T_4 decrease in a different manner. For example, in our patients treated with mephenytoin the decrease in T_4 was significant, while the decrease in T_3 was not significant. It seems that different anticonvulsants increase differently the activity of enzyme-caused degradation of thyroid hormones or catalyze the conversion of T_4 to T_3.

Monodeiodination of T_4 in peripheral tissues produces not only T_3 but also a metabolically almost inactive reverse T_3 (rT_3) [2, 4]. The thyroid hormone status can thus be influenced by different effects of long-term anticonvulsant therapy on this dual pathway of T_4 metabolism. Still, significant differences in serum rT_3 concentration have never been found in patients treated with anticonvulsants [10].

It is worth mentioning that a decrease in thyroid hormone levels of patients receiving long-term anticonvulsant therapy was significant only statistically: all the patients were

euthyroid with a normal concentration of serum TSH. The test is therefore particularly valuable in patients receiving anticonvulsants when hypothyroidism is suspected.

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Prenatal Diagnosis of Ascites Caused by Cytomegalovirus Hepatitis

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The case of a 23-weeks-old fetus is described in whom prenatal ultrasonography revealed ascites accompanied by an increased alpha-fetoprotein concentration in the amnial fluid. Detailed embryopathological work-up carried out after induced abortion demonstrated generalized cytomegalovirus disease and furnished histological proof of transplacental propagation.

Epidemiological studies have shown that the ubiquitous cytomegalovirus, a member of the herpesvirus family, is the most frequent causative agent of congenital viral infections in man [14, 15]; about 1% of all neonates are affected. Its incidence varies between 0.5—2.0%, it is more widespread in populations living under primitive conditions [7, 14, 15, 17, 18]. Congenital infection is due to transplacental transmission, the mother is usually a latent carrier [6, 10, 14]. The virus can be transferred by the infected genital secretions during labour or by breastmilk during the postnatal period. In infants and children staying in hospital iatrogenic infection may occur by blood transfusion or organ transplantation [3, 4, 5, 6, 8, 11, 12, 14, 16, 19].

In this paper we report on inclusion body disease due to generalized cytomegalovirus infection developing in utero in a 23-weeks-old fetus in whom subsequent histopathology proved transplacental propagation.

Report of a Case

C. K. E., a 23 years old woman had visited our genetic counselling clinic during the 21st week of her first pregnancy because her father had had hidden spina bifida, rubella had occurred in her environment during the early stage of this pregnancy and because she had contracted a febrile upper airways infection two weeks earlier. She had a negative family history and had never received blood transfusions. Her serum AFP level was found to be 100 ng/ml, a normal value for 21 weeks according to our norm. Ultrasonography, performed by a Picker LS 2000 device revealed oligohydramnios, thickened placenta and extensive ascites of the fetus (Figs 1 and 2). Eighteen ml amnial fluid was then obtained by transabdominal amniocentesis; its AFP content was much elevated, 34 400 ng/ml (> 2.5 M), cytological examination demonstrated regular epithelial cells and fetal erythrocytes. There was a rubella antibody titre of 1 : 64 and a CMV antibody titre of 1 : 16 in the maternal serum. The couple applied for abortion in view of the ultrasonographic finding and the high AFP content of the amnial fluid. Abortion was induced by extraovular 0.1% Rivanol combined with oxytocin infusion. There were no

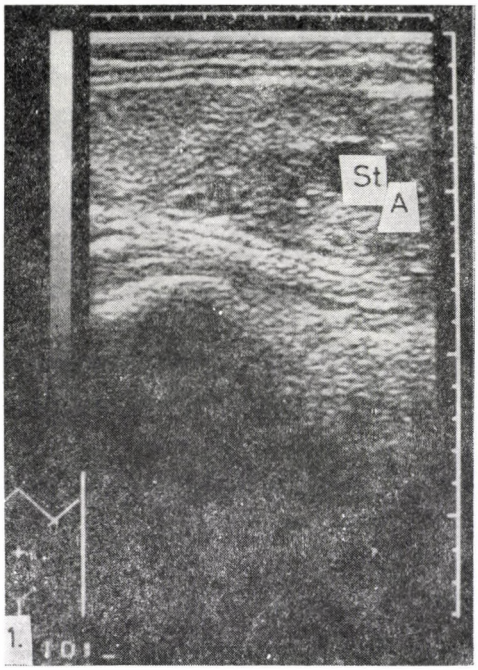


FIG. 1. Ultrasonography. Longitudinal section of fetus revealed ascites distending the abdomen (A), distension of the stomach (st) and conglomeration of the intestines

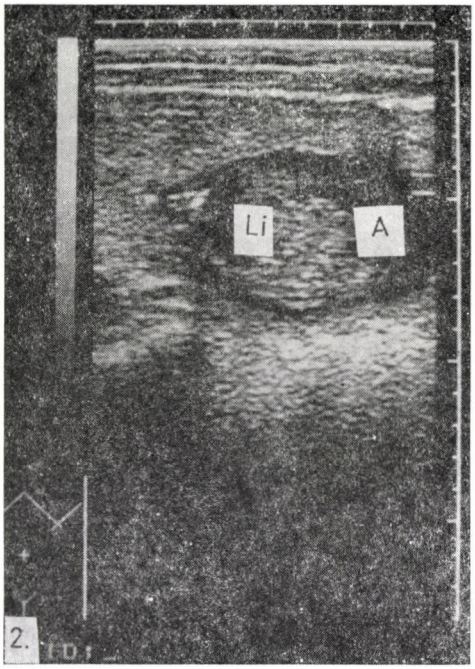


FIG. 2. Ultrasonography. The transversal section at the level of the abdomen demonstrates the presence of ascites (A) between the liver (Li) and the abdominal wall

complications. Detailed pathological examination of the fetus and the placenta was carried out.

There were petechiae on the skin all over the body, the subcutaneous tissue was oedematous and of gelatinous appearance. The abdomen was distended, the abdomen protruded 5 mm above the thoracic level (Fig. 3). A normal intrathoracal situs was seen, diffuse punctuated haemorrhagic foci were found on all serous membranes. The intrathoracic organs showed no abnormality. The abdominal cavity contained 40 ml light-yellow fluid. The abdominal

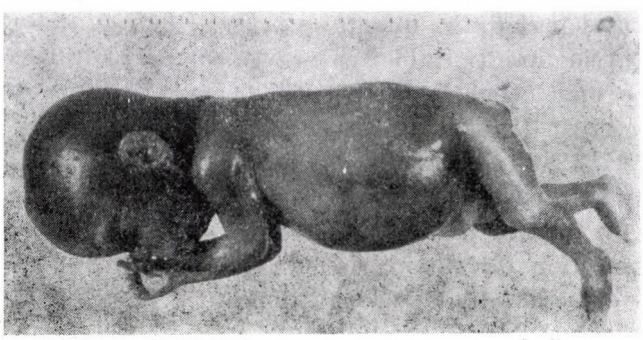
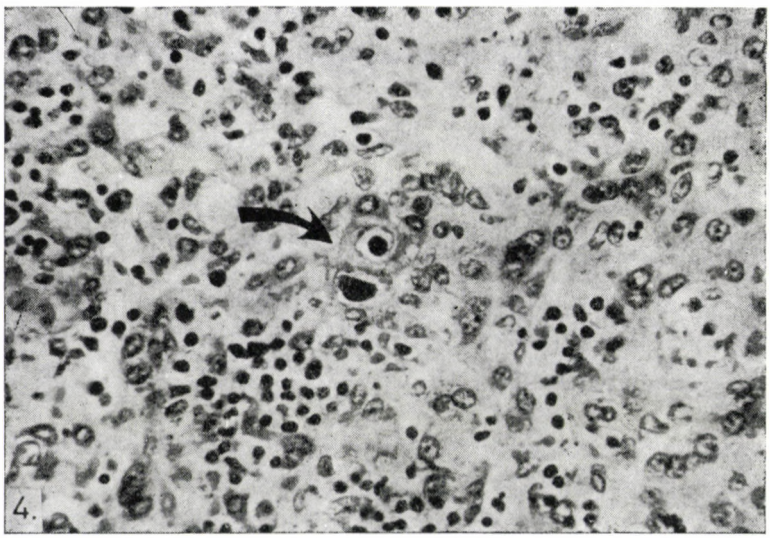


FIG. 3. The aborted fetus shows a striking prominence of the distended abdomen

situs was normal. The hepatic margin exceeded the costal margin by 10 mm, the surface of the liver exhibited diffuse, fine, uniform granulation. No macroscopic changes could be detected in the remaining abdominal and pelvic organs. The shape of the brain was normal, its surface was smooth and exhibited no gyration yet, the cerebral ventricles were of normal width.

Histology showed that the lobular structure of the liver was retained only in



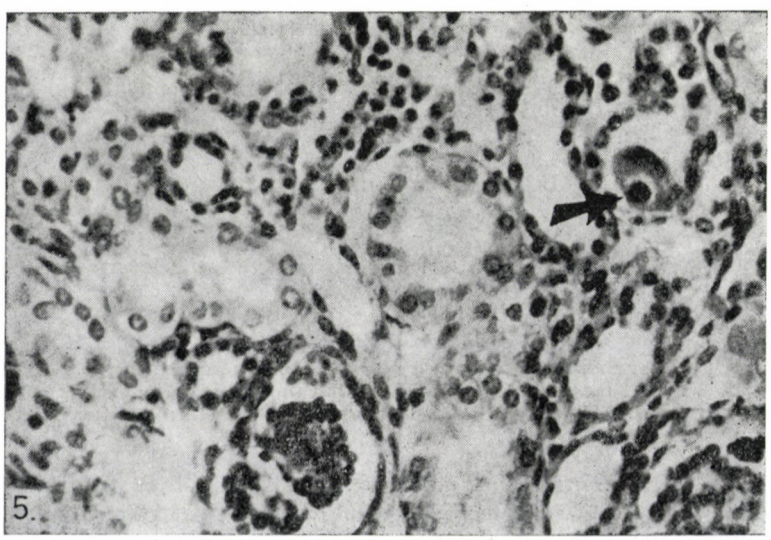


FIG. 4. Irregular pattern of liver cell trabeculae. Nuclear inclusion bodies in the liver cell indicated by arrow, extramedullary haemopoiesis in the sinuses. Haematoxylin-eosin staining, 400-fold magnification

FIG. 5. Inclusion body in an epithelial cell of a renal tubule (arrow). Haematoxylin-eosin staining, 400-fold magnification

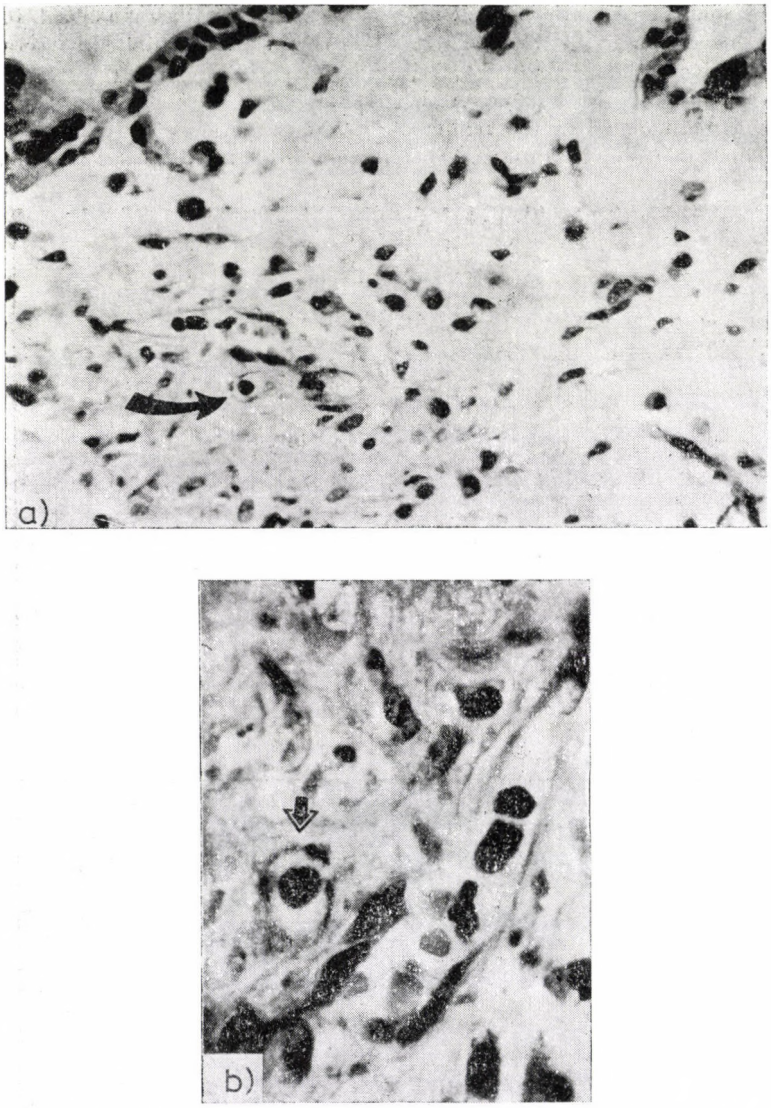


FIG. 6. a) Intranuclear inclusion body in a placental stroma cell (arrow). Haematoxylin-eosin staining, 400-fold magnification. b) The same inclusion body by larger magnification (arrow). Haematoxylin-eosin staining, 1000-fold magnification

small areas, a large proportion of the parenchyma consisted of disintegrated liver cells. Bile duct proliferation, proliferation of collagen fibres, lymphocytic infiltration and connective tissue formation, also propagating between the liver cell trabeculae, were encountered. In some areas ballooning liver cells, focal necrosis and intracytoplasmic retention of bile pigment were observed. Characteristic intranuclear cytomegalovirus inclusion bodies were seen both in epithelial cells of the bile ducts and in the hepatocytes (Fig. 4). Similar intranuclear inclusion bodies were

found in the kidneys, in the mesangium of the glomeruli and in the tubular epithelium (Fig. 5), in the alveolar epithelial cells of the lungs, in the glandular and ductal epithelium of the pancreas and in the stroma cells of the placenta (Fig. 6).

Discussion

Neonatal cytomegalovirus disease can take two forms: the newborn may suffer a congenital infection due to intrauterine transmission or may contract the infection during the perinatal period [6]. Intrauterine transmission usually takes place during late pregnancy and its source may be either a recently acquired maternal infection or a reactivation of previously contracted, latent maternal infection [9, 14]. The latter form indicates that maternal antibodies do not sufficiently protect the fetus [13]. Infection with the virus usually leads to chronic disease of the fetus, newborn or infant, with alternating exacerbations and periods of latency accompanied by sustained shedding of the virus. In certain cases acute symptoms may develop but the chronic form with late onset is more frequent [15]. The incubation time of the infection acquired during the perinatal period is 4—12 weeks, 8 weeks on the average. Typical symptoms develop in 5% of all congenital infections, another 5% exhibit atypical clinical symptoms and in 90% of all infected cases there is no manifestation during the neonatal period [2, 7]. Clinically typical cases are characterized by multiple organ affection, the reticuloendothelial system and the central nervous system being most involved. Petechiae, hepatomegaly, splenomegaly and jaundice are the most striking features. Since cytomegalovirus infection is the most frequent cause of congenital hepatitis, liver biopsy and its careful histological examination has been recommended in cases affected by hepatosplenomegaly and septic jaundice [1]. Teratogenicity of the virus is a matter of controversy.

Prognosis of infants affected by congenital cytomegalovirus infection is good in the majority of cases but it has been proved that loss of hearing, chorioretinitis, neurological and dental sequelae may develop, manifesting usually during the second year of life [15]. In our case the most severe abnormalities were found in the liver, the pathological finding corresponded to that of red hepatic atrophy. The consequences of the liver changes — hypoproteinaemia due to extensive destruction of hepatic parenchyma and ascites — allowed to arrive at the diagnosis by help of ultrasonography. The detailed histological examination has proved the presence of generalized cytomegalovirus disease and its transplacental propagation.

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Relationship of Maternal and Newborn (Cord) Serum Ferritin Concentrations Measured by Immunoradiometry

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Serum ferritin concentration was determined by immunoradiometry in venous blood samples of 45 pregnant women at term, in their babies' cord blood samples, and blood specimens obtained from 43 infants aged 3—12 months. The concentration of ferritin was higher in cord serum than in respective maternal samples and infant specimens. Low values were found in more than half of the maternal venous samples. Iron stores of newborns delivered by mothers with low serum ferritin concentration were lower than in newborns of mothers having normal ferritin levels. Serum ferritin measurement is a sensitive method to determine iron deficiency in pregnancy.

Storage iron is made up of two components: ferritin and haemosiderin [16]. Several studies have demonstrated a relationship between the serum ferritin concentration and the size of body iron stores in healthy adults, children and infants [3, 5, 8, 14, 15]. Parallel measurement of serum ferritin concentration in newborn and maternal blood samples may offer important information how maternal iron status influences fetal iron stores. To investigate this question we have attempted to determine the serum ferritin concentration in venous blood samples of pregnant women before delivery and their newborns' cord blood specimens.

Material and Methods

In the first series, serum ferritin was measured by immunoradiometry in cord blood samples of 14 newborns, and in venous blood samples of 43 infants. The newborns' gestational age was 38—41 weeks; the infants, aged between 3 and 12 months, were without hematological and infectious diseases and were grouped according to their age.

In the second part of the study cord and maternal blood samples were obtained from 45 patients having normal deliveries of live infants following uncomplicated pregnancies. All were between 38 and 41 completed weeks of gestation. No patient had had bleedings during pregnancy. A 3×250 mg supplement of elemental iron was provided daily from early pregnancy.

Serum ferritin was determined by immunoradiometry, utilising FER-ION kits (RAMCO Laboratories Inc., Houston, USA). Results below 10 ng/ml were considered low, as in our previous work [4]. In addition, serum iron (Fe), serum total iron-binding capacity (TIBC), and transferrin saturation (SI per cent = Fe/TIBC $\times$ 100) were also determined in 11 maternal blood samples having low ferritin concentration.

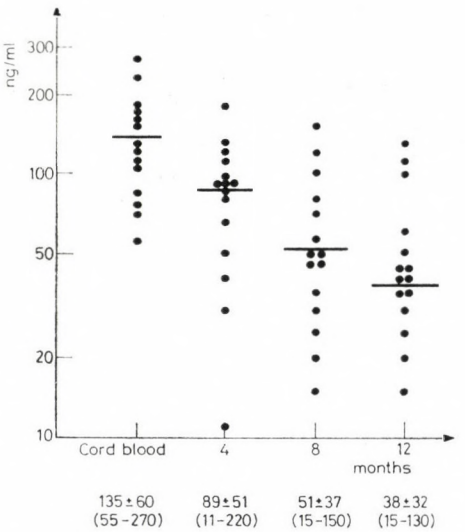

FIG. 1. Serum ferritin concentration during the first year of life

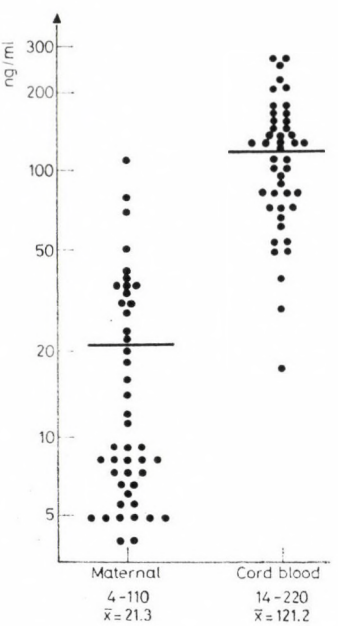

FIG. 2. Serum ferritin concentration in maternal and cord blood samples

Results

Figure 1 shows results obtained in the first series; individual values are indicated. Serum ferritin concentration was highest in cord blood samples, and it decreased during the first year of life.

In Figure 2 the serum ferritin in maternal and cord blood samples is demonstrated. Before delivery more than half of the mothers had a ferritin level lower than 10 ng/ml. Ferritin concentrations in cord blood samples were higher, above 10 ng/ml.

Serum ferritin in cord blood of newborns whose mothers had low and normal serum ferritin levels, can be seen in Table I. Newborns of mothers with normal ferritin level had a significantly higher serum ferritin concentration than babies of mothers with low iron stores.

Table I

Serum ferritin concentration in cord samples grouped according to maternal iron stores

Maternal iron stores	(n)	Maternal	Cord
		serum ferritin concentration (ng/ml)	
Low	(24)	6.5± 1.6	98.5±50.6
Normal	(21)	38.3±23.1	147.2±66.0
t-test			p<0.01

The parameters of iron metabolism in 11 mothers having low serum ferritin level are summarized in Table II, and their individual serum ferritin and Fe concentrations are demonstrated in Figure 3. The average

Table II

Parameters of iron metabolism in pregnant women having low ferritin concentrations (n = 11)

Parameters		X ± S.D.	range
Serum ferritin	(ng/ml)	7.5± 1.6	4.0—9.0
Serum Fe	(μmol/l)	13.8± 3.2	8.5—18
TIBC	(μmol/l)	99.3±23.1	59—132
SI	(%)	13.9± 5.4	8.8—23

TIBC = total iron binding capacity; SI = transferrin saturation

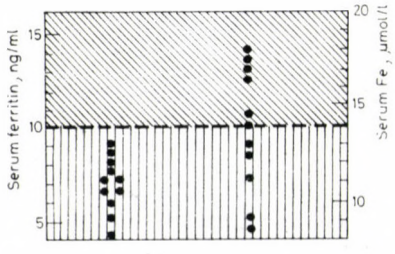

Fig. 3. Serum ferritin and serum Fe concentration of pregnant women having low iron stores (n = 11); individual values; ▨ = normal concentration, ▥ = low level

serum Fe concentration was at the lower border of the normal range. Some individual Fe levels were in the normal range despite the low serum ferritin concentrations.

Discussion

Introduction of the immunoradiometric method has made serum ferritin measurement accessible to practice [1, 9]. Using this method Addison et al [1] have described that ferritin is present in the serum of healthy persons. Further studies have elucidated that the serum ferritin level was closely related to iron stores or the iron content of tissues [3, 5, 8 15]. In childhood serum ferritin is a good discriminant between iron deficiency anaemia and infectious anaemia [4].

There are contradictory reports on the influence of maternal iron stores on the fetal ones [6, 7, 11]. It has been generally accepted that iron supply is increased during pregnancy and a considerable part of pregnant women shows iron deficiency before delivery [2]. On the other hand, it is also well-known that the fetus accumulates his iron stores in the last period of pregnancy [13]. Thus the maternal deficiency may be very important for the newborns' iron stores, and for iron deficiency anaemia in infancy.

The biochemical indices of iron metabolism are elevated in cord serum [6, 13]. Its ferritin concentration is higher than the maternal level. The range of serum ferritin concentration in cord and maternal blood samples were in our study in accordance with data in the literature [6, 11, 14]. It is in agreement also with other studies that the serum ferritin level is decreased during the first year of life [12].

We have pointed out that, despite of prophylactic iron therapy, the maternal ferritin is low at term in more than half of the cases. In our study the incidence of low serum ferritin concentration at term was higher than the findings of Kelly et al [6] and similar to those of Pácsa et al [10].

There was no direct correlation between individual maternal and cord ferritin concentrations which is in agreement with other findings [6, 11]. However, when the newborns were grouped according to whether their mothers had a low or normal ferritin level, there was a significant difference between the respective cord ferritin concentrations. Similar results were reported by Kelly et al [6] and it is therefore suggested that the fetal iron stores are reduced when the maternal stores are low.

Our results showed that the serum ferritin concentration is a more sensitive marker of the maternal iron stores than is the serum Fe level, since low serum ferritin levels can be registered despite of normal serum Fe concentrations. This means that the haemostatus of pregnant women may appear normal on the basis of their serum Fe concentration, when the low serum ferritin predicts an iron deficiency.

Serum ferritin measurement, in this manner, is a useful method to determine the iron deficiency in pregnancy. A low maternal serum ferritin level means a risk factor for the development of infantile iron deficiency anaemia.

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Sneezy Twins

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A curious monozygous pair of twins producing sudden and vehement nose blowing and/or sneezing during/after eating and drinking, when their stomach had achieved a certain stage of fullness, has been observed. The sneezing reflex could be registered in 4 male members of the family. Since there were neither neurological disorders nor significant alterations in their electroencephalographic activity, the phenomenon may be regarded as a special type of hereditary vegetative sensitivity. The trait seems to follow either an autosomal dominant or perhaps a Y-linked mode of inheritance.

Sneezing and coughing function as reflexes integrated by the medulla oblongata. They are set into action by different triggers acting on the respiratory epithelium and serve to eliminate noxious agents from the respiratory tract. Twenty years ago Everett [2] reported on sneezing reaction initiated by bright light and called it photic sneeze reflex; it could be elicited in 23% of the medical students of Johns Hopkins University. Recently, Peroutka and Peroutka [8] have given account of a family in which three males and one female reacted by sneezing if exposed to strong light. Beckman and Nordensen [1] obtained results similar to those of Everett in that the sneezer trait was observed in 24% of blood donors of Umeå, Sweden. On the basis of their family study they suggested that the trait might be inherited in an autosomal dominant fashion.

One of the present authors (G. F.), accomplishing a comprehensive twin study, found a twin pair among the participants whose sneezing reflex could be provoked in a different way.

Report of Cases

The members of a male twin pair introduced themselves as the "Sneezy twins". At that time they were 50 years old.

The phenomenon was observed in 4 male subjects belonging to three generations of their family (Fig. 1). The familial feature had been observed in the twins' father since his youth. The twins R. B. and G. B. are constantly watched by their colleagues with whom they have lunch. When the colleagues enter the common lunch room where the twins have already reached a certain stage of saturation, their repeated sneezing

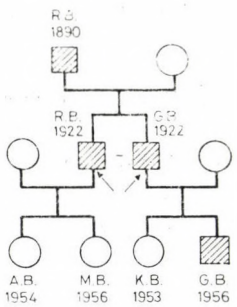

FIG. 1

is taken as an indicator of a good and abundant meal. The sneezing reflex of the twins is provoked by a full stomach.

The sneezing attack also appeared at puberty in a further male member of the third generation. None of the females of three generations were affected.

The diagnosis of zygosity of the twins was based mainly on various blood group and protein system determinations (AB0, Mn, Rh, Hp and Gm) [9], and some other anthropogenetic traits [3, 4, 5, 6, 7]. Based on these investigations, they were considered monozygous.

The twins were similar in physical appearance, although one of them (R. B.) weighed ten kg less than the other. This could be attributed to his allergy to milk, milk products, strawberries, raspberries and to different environmental factors. Both were right-handed and showed an R type of hand clasping and an L type of arm folding. Both were strong cigarette smokers. Both were tasters for phenylthiocarbamide and smellers for acetone and methylethylketone. Both of them performed a positive curling of the tongue. Their index numbers, after having drunk a standardized beetroot juice, were almost identical (0.090 and 0.114).

Neurological findings. G. B. had no neurological complaints. R. B. often had a headache. Brain nerve functions were normal in both. Deep reflexes were slightly intensified, especially in R. B. They both had mild hyperhidrosis of the palms. Movements, sense functions and coordination were intact. A tremometric examination, using a piezoelectric accelerometer, revealed no resting tremor but some degree of postural hand tremor could be registered in both. Visuomotor reaction time was corresponding to age.

In order to obtain a controlled repetition of their strange characteristic, the twins were examined at the University Neurological Department. Electroencephalography was performed at two occasions. During spontaneous registration, compression of the carotid sinus on both sides, bilateral bulbus compression and the Valsalva test were performed, then they were invited to smell amyl nitrate. Thereafter they were asked to

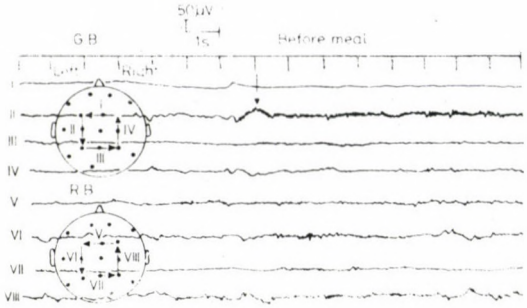

FIG. 2

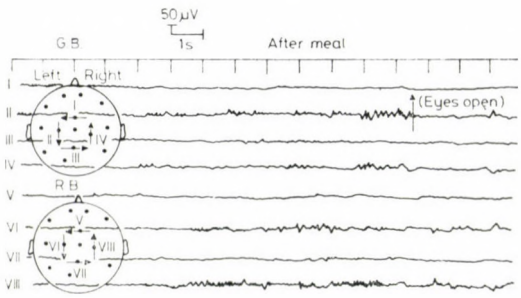

FIG. 3

consume Amolet biscuits and water; this combination leads to strong gastric and intestinal distension. These types of loading of mainly vegetative character did not activate sneezing in either of the twins. Simultaneously with exteroceptive stimulation of the vagus and trigeminus nerves a desynchronization in the EEG could be observed, related to an increase in the number of α-waves in background activity. Since the aphysiological distension of the stomach did not provoke sneezing, the twins were given an abundant lunch and electroencephalographic records were continuously taken before, during and after the meal (Figs 2 and 3). In the moment when a sensation of gastric fullness was achieved, a sudden and vehement nasal secretion and nose-blowing lasting for some minutes appeared in both persons simultaneously. The EEG, however, remained normal. No appreciable alterations in cortical electric activity were evoked by the sneezing reflex.

As described above, the phenomenon was observed in 4 male members of three generations of the family, including a monozygous pair of twins. The family tree suggests a dominant inheritance either autosomal or Y-linked. The latter would be an extreme rarity but its possibility cannot be discarded.

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Adenine Therapy in Lesch-Nyhan Syndrome

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In a 7-year-old patient with Lesch-Nyhan syndrome (LNS) the ^{15}N excess frequency was determined in the excreted uric acid after oral application of 27 mg ^{15}N glycine/kg body weight, using emission spectrometry. Incorporation of glycine into uric acid was considerably increased in untreated LNS in comparison with the control. This was due to the extremely increased endogenous de novo synthesis of purine. Allopurinol therapy caused only a gradual decrease of uric acid excretion. The pattern of purine excretion changed in favour of the better soluble oxipurines hypoxanthine and xanthine, by competitive inhibition of xanthine oxidase. In LNS, however, allopurinol had no uricostatic effect.

Therapy with adenine is an alternative to influence the de novo synthesis. After adenine application a decrease of the cumulative ^{15}N uric acid excretion occurs and the percentual proportion of ^{15}N uric acid in total ^{15}N excretion decreases. These changes are due to an inhibition of de novo purine biosynthesis. Adenine, however, must be applied in combination with allopurinol in order to avoid the formation of nephrotoxic 2,8-dioxiadenine by xanthine oxidase. Adenine therapy led to an improvement of the clinical course. No side-effects were observed.

Lesch-Nyhan syndrome (LNS) is a disease of purine metabolism with recessive inheritance. The de novo synthesis of purine is increased due to a defect or gross deficiency of the activity of hypoxanthine-guanine phosphoribosyltransferase (HG-PRT), and the daily turnover of uric acid is considerably increased. The excessive juvenile hyperuricaemia and hyperuricuria may lead to the formation of urate nephrolithiasis and nephropathy, gouty arthritis and tophi.

This is regularly associated with progressive neurological signs and symptoms such as aggressive automutilation, choreoathetosis, muscle spasticity and mental retardation. Whereas the complications of hyperuricaemia can be prevented by allopurinol, the drug has no effect on the development of CNS symptoms [18, 22]. Since children with LNS do not show signs of cerebral lesions at birth, there should be some means of prevention but numerous attempts [1, 3, 6, 10, 18] have failed to affect the progression of CNS lesions.

Therapy with adenine is a possibility to increase the nucleotide supply to the CNS. An inhibition of the de novo purine synthesis is anticipated due to the rise of purine nucleotide concentration through a feed-back mechanism. The present paper will describe the effect of adenine therapy

on purine metabolism by means of ^{15}N-tracerkinetic measurements and on the course of the disease of a 7-year-old patient with LNS.

Report of a Case

T. M., a girl, was born on 28. 9. 1976 and admitted at the age of 7 months. At that time the patient showed clear statomotor retardation, no traction response, no rotation from supine into prone position, no grasping, persisting infantile reflexes, jittery arms, athetotic movements.

The blood urea level was 490 μmol/l. Activity of HG-PRT in erythrocytes was reduced to 4% and in skin fibroblasts to 5% of normal (Table I). Thus, the diagnosis of LNS was confirmed. After administration of allopurinol (10 mg/kg body weight) and adjusting the blood urea level to 200—300 μmol/l the child was discharged. She was admitted again at the age of 5 years when she was moderately atrophic (11.2 kg) and had a short stature (3 sigma too small). Her oral mucosa, lips and both forefingers showed scars after bites.

Neurological findings. Vigilant visual contact to persons, speech consists only of some syllables; frequent changes between periods of motor rest and abrupt abnormal patterns of movement, absent traction response, stepping reflex not possible due to crossing of legs, spasm of adductors of fingers and hands, opisthotonus, response to pain and tactile contacts.

Paraclinical findings. Serum urea and creatinine, electrolytes, acid-base status and endogenous creatinine clearance were normal. No evidence of megalocytic anaemia, urea concentration on allopurinol therapy was between 200 and 300 μmol/l. No evidence of calculi in the urinary system on contrast urography and renal sonography. Isotope nephrogram normal on both sides, no evidence of gouty arthritis or tophi. EEG: mild to moderate diffuse disturbances, low voltage, no focal signs or evidence of convulsions.

Combined therapy with adenine and allopurinol has so far been continued for 18 months. During this time, a clear improvement has been observed. The child was more interested and open to contacts from the environment, her understanding of words had improved and the trend for autoaggression has clearly diminished. Only once were mild bites seen on the lips.

Methods

^{15}N tracerkinetic examination. Glycine is an essential element of de novo purine biosynthesis. It was used as ^{15}N glycine (96 at.-% ^{15}N); the oral dose was 27 mg/kg body mass. Then the incorporation rate into uric acid was determined. ^{15}N glycine was administered at 8 a.m. with fluid. Urine was collected over the following 3 days, the daily urine volume was measured, the excretion of uric acid was determined and aliquot samples were deep-frozen (−21°C) for ^{15}N-tracerkinetic examinations. A child with normal metabolism and with the same body mass served as a control.

a) LNS without therapy
b) LNS on adenine therapy
c) LNS on allopurinol therapy
d) LNS on combination therapy with adenine and allopurinol
e) control without therapy
f) control on adenine therapy.

The daily dose of allopurinol was 10 mg/kg body weight, that of adenine, 100 mg/kg body weight. The patient was given a normal diet with restriction of foods rich in purine.

HG-PRT was determined in the haemolysate and in fibroblast homogenates using the radiochemical method of Wehnert et al [20]. Serum and urine uric acid levels were determined enzymatically with uricase (katalase according to Kageyama [8]) (Fermagnost®-uric acid test). The concentrations of hypoxanthine and xanthine were determined in a parallel sample as uric acid equivalent by addition of 0.01 U/ml xanthine oxidase (Boehringer, Mannheim).

Isolation of uric acid. Determination of the ^{15}N labelling of uric acid requires its preparative separation and purification. The separation of uric acid from the collected urine followed a separation scheme designed for ^{15}N isotope analysis in NPN compounds [5]. Uric acid was separated from the other NPN constituents by means of adsorption chromatography to polystyrene sulphonic acid (column: 10×170 mm Dowex 50 WX8; eluent: water). After passage of the column, uric acid was crystallized in a 10 ml fraction and purified by transcrystallization in water.

^{15}N isotope analysis. ^{15}N isotope analysis requires transformation of the nitrogen compound studied into ammonia. Urea N and the N-compounds of urine were transformed into ammonium chloride by the Kjeldahl procedure and alkaline distillation. Determination of the relative ^{15}N frequency was performed by emission spectrometry with the ^{15}N analyser NOI-6 [12]. Isotope analysis required 55 μg ammonium chloride substance. The labelling values determined for uric acid and total N in the urine were mean values of a threefold determination with a relative standard error of 2.5%.

Results and Discussion

In the patient with LNS, HG-PRT activity decreased to 4% in erythrocytes and 5% in skin fibroblasts (Table I). The lack of HG-PRT activity prevented the re-use of the purine bases hypoxanthine and guanine and their synthesis into the respective nucleotides. The decrease of intra-

Table I

HG-PRT activity in erythrocytes and skin fibroblasts

	GMP nmol/l/h and μl erythrocytes	GMP nmol/l/h and g protein
Controls	$\bar{x}=105\pm11$ n=40	$\bar{x}=816\pm107$ n=4
Father	110	absent
Mother	101	273
Patient T. J.	4	40

Table II

Uric acid blood level in a patient with LNS without therapy and on treatment with adenine, allopurinol and their combination

Therapy	Uric acid blood level μmol/l
No therapy	617
Adenine 4×0.5 g daily	1073
Allopurinol 3×50 mg daily	256 ± 71 n=10
Adenine, 4×0.5 g and allopurinol, 3×50 mg, daily	n.s. 277 ± 84 n=6

cellular purine nucleotide concentration reduces the feed-back inhibition of phosphoribosyl-pyrophosphate-aminotransferase (PRPP-AT). Additionally, the HG-PRT defect leads to a reduced reutilization of phosphoribosyl-pyrophosphate (PRPP); thus, more of it is available for de novo purine synthesis. The uncontrolled de novo synthesis of purine nucleotides leads to a massive overproduction of uric acid with a rise of the blood level (Table II) and elimination with urine (Fig. 1). The uric acid elimination (in mmol/l per day) was increased in our patients as compared with the control (Fig. 1). This was even clearer for the proportion of the de novo synthetized uric acid, indicated by the cumulative (^{15}N)uric acid excretion. After the reduction of allopurinol we found a gradual decrease of uric acid excretion in the patient (Fig. 1). The proportion of cumulative (^{15}N)uric acid excretion as compared to the cumulative ^{15}N total excretion was not affected and remained high (Fig. 2).

Allopurinol has no uricostatic effect in LNS, since the increased supply of hypoxanthine with the HG-PRT defect cannot be transformed into IMP, and allopurinol cannot be transformed into allopurinol-MP. Therefore, allopurinol therapy affects only the inhibition of xanthine oxidase. A considerable part of the precursors hypoxanthine and xanthine is excreted instead of uric acid due to the inhibition of xanthine oxidase.

Adenine therapy is an alternative to affect the nucleotide concentration and thus, the de novo synthesis. Exogenous adenine is metabolized into adenosine monophosphate (AMP) by means of adenine-phosphoribosyl-transferase (A-PRT), and part of it is transformed into GMP and IMP through the purine nucleotide cycles.

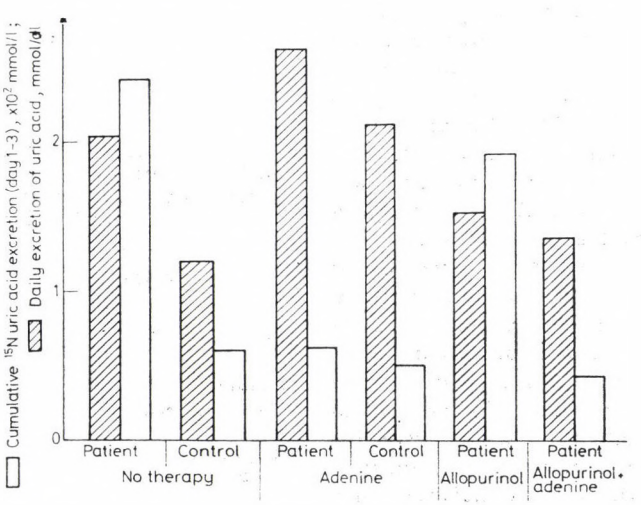

FIG. 1. Daily excretion of uric acid and cumulative ^{15}N uric acid excretion in the urine of a patient with LNS without therapy and on treatment with adenine, allopurinol and a combination of both in comparison with the control

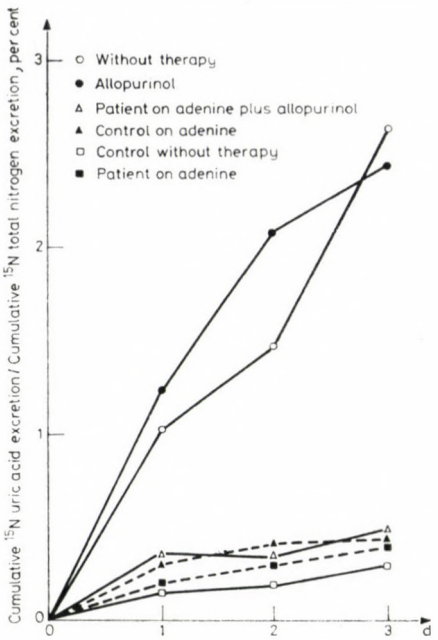

FIG. 2. Quotient of cumulative ^{15}N uric acid excretion to ^{15}N total nitrogen excretion in the urine of a patient with LNS without therapy and on treatment with adenine, allopurinol and a combination of both in comparison with the control

In LNS, the activity of A-PRT is particularly high in the liver, erythrocytes and basal ganglia [16] as well as in HG-PRT deficient fibroblasts [15], due to the stabilizing effect of the increased PRPP concentration on the activity of A-PRT.

After administration of adenine sulphate in a dose of 100 mg/kg body weight daily, we observed an increased elimination of uric acid in the urine of our patient and also in the control subject (Fig. 1). Similarly, the uric acid blood level showed a significant increase (Table II). This rise was induced by that part of adenine which is metabolized directly into uric acid. Since both the cumulative (^{15}N)uric acid elimination (Fig. 1) and the percentual proportion of the total ^{15}N elimination in urine decreased to the values of the control (Fig. 2), an inhibition of adenine of the de novo synthesis of purines may be postulated, and so an inhibition of the de novo synthesis of uric acid in the LNS patient.

Although the actual uricostatic effect originates from adenine, adenine must be applied only in combination with allopurinol. Only on their simultaneous application does the blood uric acid level decrease to normal values (Table II); the quotient oxipurine/uric acid elimination increased from 0.13 to 1.46 in comparison to the administration of adenine alone, and the formation of

nephrotoxic 2,8-dioxiadenine from adenine by xanthine oxidase was prevented [4, 14].

The clinical course was dominated by a progressive neurological picture with dystonic-athetotic infantile cerebral palsy. Whereas the sequelae of juvenile gout could be prevented by allopurinol therapy during the first seven years of life, the cerebral symptoms were not influenced. It is not known by what mechanism the HG-PRT defect leads to such severe neurological lesions [11]. A normal brain function, however, depends on an adequate supply of purine nucleotides [9]. In LNS the synthesis of GMP and IMP through the salvage pathway is reduced; this plays a more important part in comparison with the de novo synthesis in the nerve cell [7, 16]. A decrease of the intracellular concentration of these nucleotides in HG-PRT defects may be prevented by adenine. The activity of A-PRT is increased in compensation [2, 19]. The exogenous intake of adenine leads to an increase of the concentration of AMP and IMP [13], to an inhibition of PRPP-AT and, as we have shown, to a reduction of the de novo synthesis of purine.

Attempts of treatment with adenine in LNS were made in some cases [4, 17, 21] but without success and in a few cases therapy had to be discontinued since kidney lesions developed due to the formation of 2,8-dioxiadenine [14]. Therefore, simultaneous administration of allopurinol is indispensable.

Our patient has been given allopurinol and adenine for 18 months so far; no side-effects have been observed during this time and we could observe a clear improvement of her mental and motor development. Thus, therapy with adenine and allopurinol can be recommended in LNS. Still, certain irreversible cerebral lesions could not be changed, and our studies did not answer the question, whether neurological complications may be prevented by an onset of therapy in early infancy.

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Diagnostic Value of C-Reactive Protein in Premature Babies Weighing less than 1500 g

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Quantitative estimation of C-reactive protein was carried out in 34 premature infants weighing less than 1500 g. An increased value proved to be a sensitive indicator of infection. Higher values seemed to be more reliable than low ones. The clinical diagnosis showed a good correlation with CRP positivity and its quantitative value.

Early detection of infection in small premature babies is still a problem in neonatology. Rapid and reliable diagnosis is often difficult, history of pregnancy and the perinatal period, and clinical symptoms still play a decisive role [10]. The latter are not specific, therefore they are not always unequivocal. Differentiation between hyaline membrane disease, connatal pneumonia and intracranial haemorrhage may be often difficult. Many attempts all over the world have been directed to reliable diagnosis (screening programmes, infection workshops).

The mortality rate of cardiopulmonary disorders of low birthweight newborns has much improved; less progress has been achieved in the reduction of the mortality rates of babies weighing less than 1000 g at birth. LaGamma et al [11] have shown that 21 babies among 35 survivors beyond the 5th day of life plus 27 fatal cases had some kind of infection, mostly caused by E. coli, Staphylococcus epidermidis and Staphylococcus aureus. This also points to the importance of new methods in the early diagnosis of infection.

Immunological studies have played here an important part [4]. It has been amply confirmed that the premature baby is able to give an immune response to an antigen in the early postnatal period or even in utero [4, 6]. The so-called acute phase reactions are indicators of the nonspecific immune response. In this study we have examined the diagnostic value of one of these indicators, C-reactive protein (CRP), in premature infants weighing less than 1500 g at birth.

During the last decade many reports on CRP in infected newborns or young infants have been published [1, 2, 3, 5, 8, 15, 16, 24]. Information on newborns below 1500 g is, however, scarce [13]. It seemed reasonable to study this weight group.

Material and Method

During the period from March 1, 1981, to March 31, 1983, CRP was estimated in 34 newborn babies below 1500 g admitted to our department. 22 of them were ill, 12 appeared to be intact. Their gestational age ranged from 24 to 37 weeks, their weight from 600 to 1500 g. Determination of serum CRP was carried out during the first 48 hours after birth. The study was performed in a prospective manner. Allotting to either group, pathological or control, was carried out at the bedside.

CRP was estimated in a Beckman ICS I kinetic nephelometer. The maximum rate of antigen-antibody reaction is proportional to the concentration of human CRP. Time of measurement was 1 minute.

There is no unanimity in the literature as to the normal values. The capillary immune precipitation method, used initially [8] was later deplaced by the more accurate immune diffusion techniques [10, 12]. In 1984, Ewerbeck et al [7] reported on CRP values determined by this method in 100 premature babies weighing between 850 and 2500 g. Kinetic nephelometry is even more accurate and more rapid, the measurement takes 1 minute, therefore it is more useful in the rapid diagnosis of neonatal sepsis. Our patients were all smaller than 1500 g, i.e. this material was more uniform and represented a more vulnerable weight group. Quite obviously, we compared our results with normal values obtained by nephelometry [9], the value of 1 mg/dl was thus taken as a cutting point between normal and pathological.

Results

Tables I and II show some characteristics of 22 sick and 12 intact premature newborns weighing less than 1500 g. The proportion of very im-

Table I

Some data, clinical diagnosis, bacteriological findings and CRP positivity rates of premature babies of less than 1500 g birthweight

	Newborns with intrauterine infection	Controls
Number of cases	22	12
Birthweight, range	710—1500 g	600—1500 g
Gestational age, range	25—34 weeks	24—34 weeks
Sex, boys	10	2
girls	12	10
CRP, positive	20 cases (1.1—418 mg/dl)	5 cases (1.3—2.8 mg/dl)
negative	2 cases (0.3—0.6 mg/dl)	7 cases (0.4—0.7 mg/dl)
Diagnoses	Bronchopneumonia: 13 IRDS+atelectasis: 1 Prematurity: 5	Prematurity: 12
Bacteriological findings	Negative: 9 Positive: 13	Negative Positive
(nose, throat, external ear, umbilicus, stomach)	E. coli and Gram negative bacteria: 6 Staphylococcus aureus and Gram positive bacteria: 7	E. coli

TABLE II

Results of screening tests performed in patients suspect of infection or sepsis

Group	Number of patients	Number of patients with positive finding							
		History	CRP	IgM	C_3	Leukocytes	Bacteriology	Clinical symptoms	Blood smear
Infected	22	17	20	15	13	8	15	19	13
Control	2	5	3	6	2	2	3	7	0

mature babies, weighing less than 1000 g was rather high, there were 12 such infants in the whole material. There were more girls in both groups but in different proportions; in the sick group there were 12 girls against 10 boys, in the healthy group the ratio was 10 : 2. This can be explained by the well-known increased vulnerability and disposition to infection of premature boys. In the affected group an increased CRP value was found in 20 cases out of 22, a very good hit ratio. In the intact group only 5 CRP positive cases were found among 12 babies.

13 sick babies were affected by bronchopneumonia. In the remaining cases the X-rays suggested atelectasis, idiopathic respiratory syndrome or aspiration (see Table I). In these cases the elevated CRP revealed the infective nature of the disease; this was confirmed by further clinical observation and additional laboratory findings. The roentgenogram may not often be useful in clarification of the aetiology [3]. Five premature babies with clinical symptoms and laboratory findings characteristic of infection (they were also treated with antibiotics) were included into the group of infected babies although the site of infection could not be localised.

Bacteriological cultures from the nose, throat, external auditory canal, umbilicus and stomach were performed in all babies. In addition, direct bacteriological evaluation of a smear made of the gastric juice and the buffy coat was carried out. In 8 cases one pathogenic agent was cultured, two in 5 cases. 7 positive outer ear cultures were obtained, an important finding in the bacteriological diagnosis of intrauterine infection. A positive bacteriological culture does not necessarily mean infection. Therefore, a decision for colonisation, contamination or infection was made after considering the results of all tests listed in Table II.

Figures 1 and 2 show the quantitative results of CRP estimations. In Figure 1 the mean of all positive cases included in the analysis (= 7.035 mg/dl) and the highest value after exclusion of extremely high values are indicated. Two very high values were excluded from the analysis: one value of 160 mg/dl — a Shirodkar operation had been carried out in the mother of this baby one month before labour —, and one

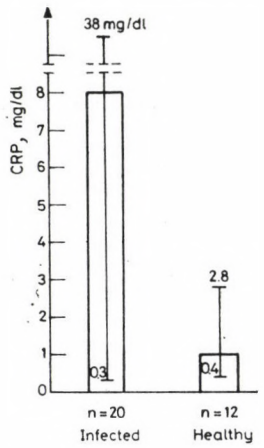

FIG. 1. Mean and range of CRP values in infected and healthy prematures CRP, mg/d

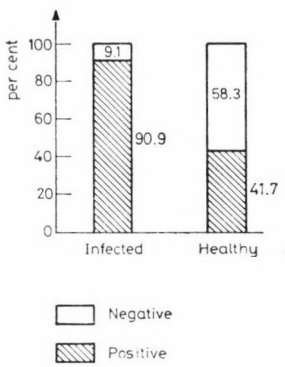

FIG. 2. Percentual proportion of positive and of negative CRP findings in infected and healthy prematures

value of 418 mg/dl, in this case the membranes had ruptured one month before birth. The mean value of intact babies was 1.07 mg/dl. Thus, the degree of elevation within the range of increased values has also to be taken into consideration when evaluating the individual result.

In Figure 2 the percentage of positive and negative results is shown. In the infected group CRP was increased in 91% of cases, in the healthy group in 42%. In view of the high participation of infection in perinatal mortality of premature infants, the risks of "superfluous" treatment are smaller than those of overdue intervention. False positive CRP findings among healthy babies are thus the smaller evil.

DISCUSSION

Table II illustrates our strategy of screening for neonatal sepsis. The relative importance of the exact history and clinical findings is obvious. CRP and quantitative IgM appear to

be the most reliable among the rapid laboratory tests. We examined how far there was a concordance between CRP positivity and the diagnosis. A fairly good correlation between CRP positivity and infection (correlation coefficient = 0.5) and the degree of elevation and infection (correlation coefficient = 0.4) was found. In our present practice, antibiotic treatment is initiated whenever at least three positive findings are present.

Although the CRP level is elevated in most pregnant mothers, the blood of the healthy newborn contains no or negligible quantities of CRP at birth [8, 10, 15]. By the end of the first week there is a slight increase, no pathological levels are, however, attained in healthy infants. It may be concluded that this protein does not cross the placenta. In newborns with premature rupture of the membranes or suspect of being infected, increased CRP values have been found [10, 14]. This speaks for the idea that CRP production can be stimulated as early as in the newborn period.

In the uninfected group positive clinical findings can be encountered. This is striking but not unexpected since a premature baby may be sick without being infected. A good example is intracranial haemorrhage. Rapid diagnosis of infection is a matter of life and death.

Our results suggest that nephelometric estimation of CRP in premature newborns is a valuable step towards early diagnosis of infection and, consequently, to effective treatment.

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Riboflavin (Vitamin B_2) Treatment of Neonatal Pathological Jaundice

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The effect of traditional blue-light and riboflavin combined with blue-light was compared in newborns affected by ABO incompatibility, admitted for exchange transfusion. During the period of preparation for the intervention 14 patients were treated with blue light alone and 14 patients with riboflavin combined with phototherapy. A single dose of 10 mg/kg riboflavin was administered intravenously. The duration of treatment was three hours in both groups. The effect of phototherapy was markedly enhanced by the additional riboflavin, by the end of the 3-hour period a significant fall of serum bilirubin was demonstrated in the 14 patients treated with blue light and riboflavin while in the patients treated with phototherapy alone the bilirubin level continued to rise. There was no difference in the activity of the antioxidant enzymes superoxide dismutase and catalase, and in lipid peroxidation between the groups.

Kostenbauder and Santvordeker [10, 26] have shown that riboflavin sensitises bilirubin against the effect of light, enhancing thus its photocatabolism. This observation led to clinical trials of the drug [6, 7, 19, 22, 31]. Later Meisel et al [19] showed that riboflavin does not affect the albumin-bilirubin binding, i.e. its application carries no risk of bilirubin displacement from its albumin binding. In an earlier paper we reported on the favourable effect of riboflavin in preventing jaundice. In this study we have attempted to demonstrate that phototherapy combined with riboflavin, proven to be effective in preventing hyperbilirubinaemia, is suitable for producing a rapid decrease in the high bilirubin level.

Patients and Methods

The material consisted of 28 term babies affected by ABO incompatibility. Only newborns free of all symptoms but hyperbilirubinaemia, with a bilirubin level above the level of indication for exchange transfusion [24] were selected. The trial was carried out during the 3-hour period necessary for preparing the exchange transfusion. 14 patients were treated with blue light alone, another 14 patients with riboflavin plus phototherapy. Vitamin B_2 (Beflavin, La Roche) was diluted by a three-fold volume of physiological saline and a single intravenous dose of 10 mg/kg was injected slowly. Serum bilirubin was determined at the time of introducing therapy, this value was regarded as the 0-hour level. The determination was repeated after three hours (3-hour value) and the mean values of the two groups were then compared statistically.

TABLE I

Data of newborn infants treated with blue light alone, or riboflavin combined with phototherapy

Group of treatment	Mean ± SD		Serum bilirubin, micromol/l		Degree of statistical significance
	Birthweight, g	Age, hours	0 hour	3 hours	
Blue light alone n = 14	3405±467	49.7±28.8	349±77	381±92	NS
Blue light plus riboflavin n = 14	3271±588	50.8±26.7	367±66	280±51	$p<0.01$
Degree of statistical significance	NS	NS	NS	$p<0.01$	

The activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase and the degree of lipid peroxidation (LP) were determined at 0 and 3 hours by biochemical methods [2, 15, 21, 23].

RESULTS

Table I shows the mean bilirubin level of the newborns treated with blue light alone and with riboflavin plus phototherapy, determined at initiation and termination of treatment. It can be seen that the two groups did not differ in birthweight, age at admission and mean initial bilirubin level. The mean bilirubin level was somewhat higher than the initial level in the group treated with phototherapy alone; the difference was, however, not significant. In all cases treated with riboflavin and blue light there was a decrease in the bilirubin level by the end of the 3-hour period. The difference between the mean 0-hour and 3-hour values was significant statistically. There was no significant difference between the two groups in respect of SOD activity (Fig. 1). The mean initial activity of catalase was significantly higher in the group treated with the combination of riboflavin and phototherapy (Fig. 2). LP was similar in the two groups, no significamt difference has been found (Fig. 3).

DISCUSSION

Earlier, a decisive role was attributed to oxidation in the process of bilirubin photocatabolism. It was thought that the energy of light induces formation of singlet oxygen ($^1O_2$), this in turn leads to photoxidation of the bilirubin molecule [8, 16]. It was thus anticipated that the catabolic effect of riboflavin on bilirubin is mediated by its capacity of producing $^1O_2$ [20, 22, 26]. Recent observations have, however, shown that isomeration and not oxidation is the basic factor in bilirubin breakdown [12, 13, 14, 17, 18, 27]. It has turned out that $^1O_2$ formed by the photodynamic reaction plays a minor role, this process may affect the bilirubin catabolism via oxidation of other compounds [3, 5, 11, 26]. In vitro studies have shown that changes in the structure of DNA can be in-

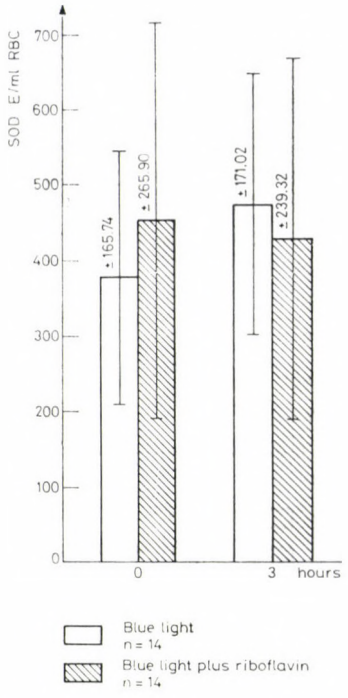

FIG. 1. Superoxide dismutase activity in patients treated with phototherapy alone and with blue light plus riboflavin, before initiation (0 hour) and after completion (3 hours) of therapy

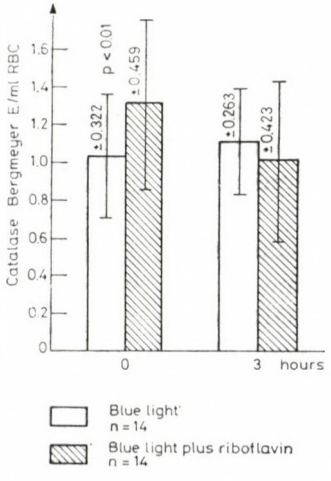

FIG. 2. Catalase activity in patients treated with phototherapy alone and with blue light plus riboflavin, before initiation (0 hour) and after completion (3 hours) of therapy

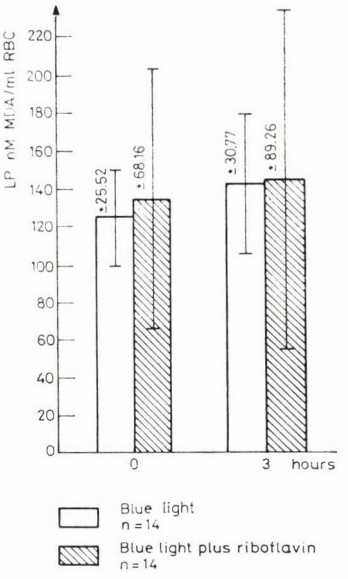

FIG. 3. Lipid peroxidation in patients treated with phototherapy alone and with blue light plus riboflavin before initiation (0 hour) and after completion (3 hours) of therapy

duced by light used in phototherapy [30]. This phenomenon, attributed to the effect of singlet oxygen, can also be seen if irradiation occurs in the presence of bilirubin or riboflavin [9, 25, 28, 29]. This is the reason for warnings of certain authors against the therapeutic use of riboflavin [10, 22, 26, 28, 30, 32]. Others regard phototherapy itself risky [4, 28, 30]. It is also known that exchange transfusion may enhance the toxicity of oxygen [1].

In our material no detrimental effect of riboflavin plus blue light has been demonstrated in SOD and catalase activity or lipid peroxidation. The figures showed that a rapid fall in bilirubin level can be achieved by riboflavin combined with phototherapy during the period of preparation for an exchange transfusion. The method may be useful if prolongation of this preparation period is inevitable.

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Book reviews

M. HAENEY: *Introduction to clinical immunology*. V + 132 pages. Butterworths — Update, London 1985. Price £ 11.95

This book fulfils exactly the task promised in the title: it helps the student of immunology and reviews the knowledge necessary for doctors who have had no contact with clinical immunology recently or since their university studies. This aim has been achieved by a concise text, a large number of instructive colour plates, excellent tables and a few histological pictures. It may also be useful for the teaching staff and for those giving lectures at postgraduate courses on this issue since the book is composed in an outstanding didactic spirit.

The book begins with a description of immunoglobulin structure, intrauterine and extrauterine immunoglobulin production and of factors influencing the blood levels of immunoglobulins. There is a nice chapter on the disorders caused by monoclonal immunoglobulin production including a sensible differentiation between Waldenström macroglobulinaemia and multiple myeloma. The allergic diseases are represented according to the role of pathological IgE production in their pathogenesis. There are separate chapters dealing with the role of the complement system and immune complexes in particular diseases. In the present reviewer's opinion, the description of conditions based on selective IgA deficiency is too short even for such a concise review and a more detailed differentiation would have been preferable. The author keeps some distance from the gamma-globulin preparations administered intravenously in antibody deficiency although up-to-date preparations may be lifesaving in sepsis. It is true that the very high expenses of such a treatment make questionable the use of intravenous preparations in the sustained therapy of immune deficiency but it is still desirable to apply them in short term treatment of septic conditions of newborn babies and young infants. Most textbooks mention the Nezelof syndrome among the immune deficient conditions of cellular origin; this entity is missing in the book. The chapter on disorders of neutrophil functions contains instructive figures, the review of immunological aspects of autoimmune diseases is genuinely constructed so that it is condensed to 16 pages, figures and tables included.

The appealing appearance of the book is very useful in teaching. It can be recommended to anyone needing a concise introduction to the bases of clinical immunology which can be learnt in little time with little energy expenditure and for little cost.

E CSERHÁTI

J. M. GERRARD: *Prostaglandins and leucotrienes: Blood and vascular cell function*. 336 pages. Marcel Dekker Inc., New York 1985. Price $ 78.—

This book is a spendid review of the large literature on prostaglandin and arachi-

donic acid metabolism in particular types of tissues and cells; it has been badly needed up to now.

The reasonably short general introduction is followed by a description of prostaglandin metabolism as the general activation system of the cell; the role of the arachidomic acid cascade triggered by any damage to the cell is underlined. There is an up-to-date biochemical analysis of the reactions involved in calcium flux and calcium activated enzymes. Also, the equilibrium of the cAMP and cGMP systems is discussed here although there is only a hypothetical link between them. A special chapter deals with the mechanisms liberating fatty acid from free fatty acid substates for prostaglandin biosynthesis: phospholipases, diglyceride kinases, etc. Their inhibitors are also discussed. Similarly, there is a short description of the biochemical properties and inhibitors of the general arachidonic acid cascade consisting of cyclooxygenase, peroxidases, thromboxane synthetase, prostacyclin synthetase and lipoxygenases.

The bulk of the book consists of a detailed analysis of the prostaglandin metabolism of the particular cellular blood elements, platelets, erythrocytes, neutrophils, basophils, eosinophil granulocytes (treated in separate sections), monocytes, lymphocytes, endothelial cells and smooth muscle cells of the blood vessel wall. Of course, knowledge is most extensive on the platelets and endothelial cells, but much new information on the prostaglandin and leucotriene metabolism of lymphocytes and basophilic granulocytes has been put down in a hitherto undescribed system. This system is a coordinated network of leukotriene cytotoxicity, arachidonic acid derivative inhibition of lymphocyte activation elicited by T-cell mitogen and inhibitors of these reactions. In the section on endothelial cells each individual effect of endogenous metabolites and metabolites produced by lipoxygenase and cyclooxygenase of other cells are discussed and the reader gets insight into the knowledge on specific inhibitors of each particular function. Similarly, there is an apart analysis of smooth muscle cell functions for each organ system, since the renal, pulmonary, cerebral and coronary blood vessels each have a particular pattern of prostaglandin production, physiology and regulation.

The third part of the book deals with the role of prostaglandins in cell-to-cell interrelationship: in haemostasis, thrombosis, vascular wall damage, inflammatory processes, in defense mechanisms against tumours, viruses and bacteria, in cellular hypersensitivity reactions and in production of the individual cells. All these sections review the whole topic but the regulatory role of prostaglandins and leucotrienes is underlined.

Finally, there is a brilliant chapter on perspectives of new types of drugs acting specifically at certain points of the above described systems. The book ends with a small glossary of the most important definitions of the field.

This book is useful both for beginners and for experts of this important and rapidly developing branch of science.

G Blaskó

Mechanisms of gonadal differentiation in vertebrates. Eds: U. Möller, W. W. Franke. VI + 121 pages with 84 figures. Springer Verlag, Berlin—Heidelberg—New York—Tokyo 1983. Price DM 78.—

The topic of the present issue is one of the oldest questions of mankind, that of the origin of males and females. It was the subject of an EMBO-workshop held in Freiburg i. Br. (FRG) in 1982. The twenty lectures that have been delivered by the most outstanding experts of the field are contained in this booklet.

The lectures are grouped into six chapters. 1. Evolution of sex chromosomes (Becak, Schmid, Fredga), 2. Function of sex chromosomes (Epstein, Goodfellow, Fraccaro), 3. Sex-specific DNA sequences

(Davis, Cooke, Jones, Ohno), 4. Morphological aspects of gonadal differentiation (Wartenberg, Paranko), 5. Gonadal sex reversal (Reinboth, Scheib, McLaren), 6. Morphogenetic factors in gonadal differentiation (Müller, Wolf, Wachter, Simpson).

The contributors have approached these subjects from various aspects, viz. molecular genetics, cytogenetics, biochemistry, endocrinology, immunology and morphology. Specific results and methods are described in detail, some of which are especially remarkable. Such presentations were Fraccaro's "Chromosome abnormalities and gamete production in man", Cooke's "Structure and evolution of human Y chromosome", Wolf's "X-linked genes and gonadal differentiation" and Wachtel's "On the nature of H-Y antigen".

The volume contains the most current knowledge in connection with gonadal differentiation; it is indispensable for specialists dealing with the subject.

S GARDÓ

Schock. Herausgegeben von G. RIECKER. Band 9, Teil 2 des Handbuches der Inneren Medizin. XIV+432 Seiten mit 120 Abbildungen. Springer Verlag, Berlin—Heidelberg—New York—Tokyo 1984. Preis DM 190.—

Wie es gleich im Vorwort des Herausgebers betont wird, will dieses Buch die in der 1960 erschienenen Arbeit "Schock und Kollaps" von E. Buchborn niedergelegten und heute noch gültigen Konzepte nicht wiederholt darstellen. Das bedeutet, daß der Leser die im Säuglings- und Kindesalter am häufigsten vorkommenden, die nach Dehydration, durch Blutverlust oder nach anaphylaktoiden Reaktionen entstandenen Schockformen vergebens suchen wird. Demgegenüber findet man eine ganz ausgezeichnete Beschreibung des kardiogenen Schocks (blutig gemessener systolischer arterieller Druck unter 80 mm Hg, Herzindex unter 2 l/min/m^2 und Pulmonalkapillardruck über 15 mm Hg mit metabolischer Azidose, Hypoxie, Hyperkapnie und hohem Laktatspiegel), bei dem nebst der üblichen Therapie auch die chirurgischen Maßnahmen (akuter Verschluß des Ventrikelseptumdefektes, akuter Mitralklappenersatz, Bypass-Operation) besprochen werden. Im von Bussmann (Frankfurt) geschriebenen Kapitel über die Therapie der akuten Herzinsuffizienz sind die guten Ergebnisse mit Nitroglycerin und Nitraten z. B. Isosorbidnitrat und Natrium-Nitroprussid und Kalziumantagonisten z. B. Nifedipin, äußerst beachtenswert. Erstklassig ist die Beschreibung der Niere im Schock — Schockniere, wobei die wohlbekannten erschreckenden Statistiken von Pilgrim (1982) mit 82% Letalität nach Trauma, 90% nach hämorrhagischer Pankreatitis, 100% nach Thorakotomie usw. darauf hindeuten, daß diese übliche Komplikation vorgebeugt (Mannitol, Diuretika, Furosemid) und so früh wie möglich behandelt (Dialyse, Dobutamin, Vasodilatoren) werden muß.

Ein zusammenfassendes Kapitel über das ARDS (Adult Respiratory Distress Syndrome, Wet Lung), die respiratorische Insuffizienz, die besonders nach dem Vietnamkrieg bekannt wurde, ist eine ziemlich kurze, aber musterhafte Übersicht dieser zu oft tödlichen Komplikation, wobei die vorwiegend symptomatische Therapie (Volumenkontrollierte Beatmung mit großem Atemzugvolumen und positiv-endexpiratorischem Druck) wenig helfen kann. Von der üblichen Kortikosteroidtherapie wird die Letalität nicht oder nur ganz unwesentlich beeinflußt. Ein Kapitel bespricht die Nutzen und Gefahren der Volumenersatztherapie, wobei auch die heute noch im experimentellen Zustand befindlichen Mitteln (Stärke-, Fluorocarbonemulsionen usw.) und Apparate (Micropore-Filter, G-Suit) besprochen werden. Zuletzt kommt eine Differentialtherapie des Schocks in dem Intensivmedizin genannten Kapitel, das die gebräuchlichen Mittel und Maßnahmen kurz zusammenfaßt.

Das Buch gehört in die Bibliothek von jedem sich mit Schockzuständen befassenden Mediziner, ob er sich mit innerer Medizin, Chirurgie oder aber mit Kinderheilkunde befaßt und ganz besonders Anästhesisten und anderen, die an Intensivstationen arbeiten.

PV VÉGHELYI

W. FROMM, R. DEGENHARDT: *Rehabilitationspädagogik für Sehgeschädigte*. 143 Seiten mit 29 Abbildungen und 13 Tabellen. Verlag Volk und Gesundheit, Berlin 1984. Preis: M 39,—

Das Buch wurde von auf diesem Gebiet in der DDR anerkannten Fachautoritäten in der Reihe Beiträge zum Sonderschulwesen und zur Rehabilitationspädagogik (Band 41) verfaßt.

Einleitend werden der Begriff Rehabilitationspädagogik, die Kennzeichnung von Blindheit und Sehschwäche sowie die historische Entwicklung der Pädagogik für Sehgeschädigte kurz behandelt. Das folgende Kapitel befaßt sich mit den Ursachen der Schädigungen, wie z. B. Mißbildungen, entzündliche und degenerative Erkrankungen, Brechungsfehler, Schielen, Verletzungen, und deren Häufigkeit. Ein Kapitel ist der Persönlichkeitsentwicklung von sehgeschädigten Kindern, deren Wesensmerkmale, ferner den Aufgaben und der Richtung der Entwicklung von kognitiven und motorischen Funktionen gewidmet. Das ausführlichste Kapitel befaßt sich mit dem pädagogischen Prozeß. Hier werden die rehabilitative Bewegungs-, Sinnes- und Spracherziehung und die Einflußnahme auf den emotionalen — volitiven Bereich besprochen. Es werden dann die speziellen Erziehungs- und Unterrichtsinstitutionen erörtert und auf die Wichtigkeit der frühen, schon im Vorschulalter beginnenden, zu Selbständigkeit verhelfenden Erziehung hingewiesen. Der sich mit dem Schulunterricht der Sehgeschädigten befassende Teil erstreckt sich auf die Besonderheiten, angefangen vom Lese- und Schreibprozeß, Modellieren bis zum naturwissenschaftlichen- oder Sportunterricht bei Blinden und Sehgeschädigten. Es wird auch über die Elternberatung und Familienerziehung bei diesen Kindern berichtet. Weitere Themen des Buches sind die Berufsberatung und Berufsausbildung oder die Rehabilitation der Späterblindeten (in der DDR arbeiten etwa 5000 Blinde). Ein eingehendes Kapitel bespricht das heutige Angebot von Hilfsmitteln für Sehgeschädigte sowohl für den Beruf als auch für die Freizeit. Schließlich findet man Information über die Struktur, Aufgaben und Einrichtungen des Blinden- und Sehschwachen-Verbandes in der DDR sowie über die einschlägigen Rechtsgrundlagen.

Demonstrative Abbildungen und Tabellen fördern die Orientierung, ein reiches Literaturverzeichnis die entsprechende Information im Thema.

Das Buch enthält zahlreiche anregende Gedanken und Vorschläge, die bei der Weiterentwicklung der Rehabilitationsarbeit mit Nutzen verwertet werden können.

CS KOVÁCS

W. PLENERT, W. HEINE: *Normalwerte*. Untersuchungsergebnisse beim gesunden Menschen unter besonderer Berücksichtigung des Kindesalters. 6., völlig überarbeitete und ergänzte Auflage. 518 Seiten. Verlag Volk und Gesundheit, Jena 1984. Preis M 45,—

Die früheren, zwischen 1966 und 1978 erschienenen Auflagen dieses Buches sind in den medizinischen Laboratorien gut bekannt. Bei dem vorliegenden Band handelt es sich um die sechste Auflage.

Die Mittel der biochemischen Analyse verfeinern sich in einem fort, folglich vermehren und modifizieren sich auch ständig unsere Kenntnisse. 1980 wurde die SI Maßeinheit eingeführt, die Konzentrationen werden in der Quantität der Substanz (mol) und nicht in ihrer Menge (gramm) angegeben; bei einigen Parametern hat sich

dies äußerst problematisch erwiesen. Die Verfasser haben die Anwendung der SI Maßeinheit konsequent durchgeführt, in vielen Fällen wurde aber das Ergebnis in der früheren Maßeinheit hinzugefügt, was eine besonders wertvolle Hilfe bietet.

Das Buch gliedert sich in folgende Kapitel; 1. Biochemische und physikalische Werte im Blut, Plasma und Serum; 2. Hämatologie; 3. Harn; 4. Liquor; 5. Duodenal- und Magensaft; 6. Stuhl; 7. Speichel, Schweiß und andere Körperflüssigkeiten; 8. Funktionelle Größen und Funktionsprüfungen; 9. Somatisches Wachstum; 10. Psychische Entwicklung; 11. Bedarf an Nährstoffen, Vitaminen und Mineralien; 12. Physikalische und chemische Einheiten, und 13. Literaturverzeichnis.

In den ersten sieben Kapiteln werden Quantität, Konzentration, Aktivität und Verteilung von mehreren Hundert Substanzen angeführt, deren Anhäufung oder Verminderung pathognomonisch sein kann. Bei manchen Substanzen hängt der normale Bereich von der angewandten Methodik ab, so daß in diesen Fällen (z. B. Hormone) auch auf die Meßmethode (Chromatographie, Fluorimetrie, RIA usw.) hingewiesen wird. Ein besonderes Verdienst der Arbeit ist die große Anzahl von Labordaten hinsichtlich des Säuglings- oder Kindesalter, bei denen sich die Parameter für die Normalwerte ja verschiedenartig gestalten. Das ausführlichste achte Kapitel (156 Seiten) befaßt sich mit den verschiedenen Funktionsprüfungen, welche bei der Differentialdiagnostik von entscheidender Bedeutung sein können. Die Kapitel 9, 10 und 11 bieten dem Pädiater grundlegende zahlenmäßige Angaben. Im 12. Kapitel sind die wichtigsten zur SI Einheit nötigen Daten und Umrechnungsfaktoren zusammengefaßt, wobei die in den vergangenen Jahren erschienenen zahlreichen, oft sinnverwirrende SI Publikationen etwas klargestellt werden. Das die Arbeit abschließende Literaturverzeichnis umfaßt mehr als 900 Angaben; bedauerlicherweise stammt die Mehrzahl der Zitate aus den Jahren 1960—70, so daß man die neuesten Resultate vermißt (bei einem Teil des Schrifttums wurde auf die alphabetische Reihenfolge verzichtet, bei einer nächsten Auflage wäre das zu korrigieren). Dennoch wird sich das Buch für Kliniker, Chemiker, Physiker und Biologen sehr nützlich erweisen.

A Szabó

A. Dittmer, H. Seipelt; *Arzneiverordnung für das Kinderalter*. 3., völlig überarbeitete und neu gestaltete Auflage. 236 Seiten. G. Fischer Verlag, Jena 1984. M 22,—

Die vorliegende dritte Auflage des vor 12 Jahren erstmals publizierten Büchleins ist in einer völlig überarbeiteten und neu gestalteten Form erschienen.

Der erste Teil bietet eine theoretische Zusammenfassung des Wirkungsmechanismus, der Applikationsformen, Verteilung, Resorption-Elimination und Dosisberechnung der Medikamente.

Im zweiten Teil wird die konkrete Anwendung der Pharmaka besprochen. Zuerst wird die Antibiotikatherapie behandelt, wobei nach der Indikationsstellung — um nur einige Abschnitte herauszugreifen — die lokale Anwendung, Resistenz, Nebenwirkungen usw. erörtert und abschließend mit einer Dosierungstabelle demonstriert werden. Die folgenden Abschnitte sind der Immuntherapie, Glukokortikoidtherapie und der antiphlogistischen Therapie gewidmet. Weitere Abschnitte befassen sich mit der Fieberbehandlung, Beruhigung, Dämpfung, mit der Krampfbehandlung, dem Hirnödem, diabetischen Koma, mit der Infekt- und Enteritisbehandlung und antimikrobieller Therapie. Schließlich werden der Flüssigkeits- und Elektrolytersatz, die Azidosetherapie, die Anwendung von Kardiaka und Hypertensiva und die Behandlung vom akuten Asthma bronchiale-Anfall erläutert. Der Arzneimitteltherapie bei Neugeborenen ist ein eigenes Kapitel gewidmet.

Die ausführlichen demonstrativen Dosierungstabellen, in denen die Präparate in

alphabetischer Reihe, mit Substanz- und Handelsnamen, mit entsprechender Alters- und Körpergewichtverteilung und eventuellen besonderen Hinweisen angegeben sind, bilden den größten Teil des Buches. Ein Arzneimittel- und Sachwortregister verhelfen den Leser zur raschen Orientierung.

Zusammenfassend soll betont werden, daß sich das Büchlein, daß vorwiegend aufgrund des Arzneimittelverzeichnis 1981 der DDR zusammengestellt wurde, sich besonders dort für den in Praxis tätigen Arzt als sehr nützlich erweisen wird.

K SCHMIDT

E. SCHMIDT-KOLMER (Herausgeber): *Kinderkrippen — Krippenkinder*. 232 Seiten mit 32 Abbildungen und 42 Tabellen. Verlag Volk und Gesundheit, Berlin 1984. Preis M 39,—

Das Buch, Werk einer aus elf Mitgliedern bestehenden Autorenkollektive, ist das Ergebnis einer sechsjährigen Zusammenarbeit von bekannten Krippenexperten der DDR, CSSR und UdSSR. Der Gegenstand der Zusammenarbeit war, Methoden zur vergleichenden Beurteilung des Bedingungsgefüges in den Krippen der drei Länder zu bearbeiten und die schon früher publizierten Kontrollmethoden der DDR (Zwiener, Schmidt-Kolmer) und der UdSSR (Frucht, Pantjuchina, Petschora) zum Vergleich der Entwicklung von Krippenkindern anzuwenden. Das Ziel der Zusammenarbeit war ferner die Schaffung eines Methodeninventars für die Objektivierung und die vergleichende Einschätzung der Wirkung des Bedingungsgefüges der Einrichtungen auf die Entwicklung der Kinder.

Das Buch enthält eine Reihe grundsätzlicher Ergebnisse zu materiellen und personellen Bedingungen in typischen Einrichtungen der drei Länder und deren Vergleich miteinander. Was die vergleichende Entwicklungskontrolle betrifft, da deren Fragen in engem Zusammenhang mit dem Inhalt und der Methode der Erziehungsarbeit der Krippen stehen, wird der Leser nicht überrascht, daß die Ergebnisse und ihr Vergleich miteinander die Gleichheit bzw. die Unterschiede der erzieherischen Zielsetzungen und andere landesübliche Besonderheiten widerspiegeln.

Judith FALK

10th European Congress of Perinatal Medicine

Organized by the Gesellschaft für Perinatale Medizin der DDR in cooperation with the European Society of Perinatal Medicine

Dear Colleague,

We invite you to the 10th European Congress of Perinatal Medicine, which will be held in Leipzig, German Democratic Republic, August 12—16, 1986.

We ask you for assistance, especially to the formation of the scientific program. The current collection of topics may be completed or changed by your proposals.

We hope to welcome you at this congress not only for the importance of the papers and discussions, but also for the warmth with which your friends and colleagues will receive you.

Cordially yours,

Klaus Jährig
Chairman

Hans-Joachim Woraschk
Secretary General

Correspondence:

Frauenklinik
POB 63
DDR — 4010 Halle (Saale)

ACTUAL PROBLEMS IN PAEDIATRIC SURGERY

Proceedings of the 7th Congress of the Hungarian Paediatric Surgeons, Budapest, August 25-28, 1982

edited by

T. VEREBÉLY

In English. 1983. XII + 332 pages. 136 figures. 24 tables. 17×25 cm
Hardcover $36.00/DM 85,— ISBN 963 05 3392 8

Four main topics were dealt with at the Conference attended by paediatric surgeons from many countries of Europe and from overseas. Papers in the first section deal with special diagnostic examinations in paediatric surgery. The second topic, errors and mistakes in childhood traumatology, was chosen because in the last decades fatalities resulting from trauma have become the most frequent cause of death in the affluent countries. The third domain, relating to the surgical conditions of neonates leading to respiratory syndromes, was necessitated on account of recent improvements in the therapy of these cases. The fourth section includes freely chosen topics in connection with paediatric surgery.

AKADÉMIAI KIADÓ
BUDAPEST

MINOR MALFORMATIONS IN THE NEONATE

by

K. MÉHES

In English. 1983. 132 pages. 39 figures and photos, 12 tables, 13×29 cm.
Paperback $6.00/DM 14,— ISBN 963 05 3010 4

The book deals with the incidence and relevance of easily recognizable dysplasias and measurable extreme variants of local growth in the nonate. Such minute deviations, innocent as they are in itself, have always been considered as "Leit-Fossils" of a hidden major anomaly. However recent surveys of neonate populations by the author in Hungary and by others in the United States have substantiated their frequency and relative significance. Based on his examinations, the author recommends a simple score for screening purposes considering six easily recognizable dysplasias together with the family history and the gestational age. Preliminary results in 10.000 routinely scored, unselected newborn babies showed that every sixth with an elevated score proved to be afflicted with a significant congenital anomaly.

AKADÉMIAI KIADÓ
BUDAPEST

The VIIIth Congress of ESCO (European Sterility Congress Organisation)

will be held in Budapest, Hungary beeween September 27–30, 1987.

Information on scientific matters: Prof. Dr. R. Gimes
H-1088 Budapest
Baross u. 27.

Information on congress organisation: Congress Bureau MOTESZ
VIIIth ESCO
H-1361 Budapest
P.O. Box 32.

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INSTRUCTIONS TO AUTHORS

Acta Paediatrica Hungarica publishes articles on clinical observations and original research of paediatric subjects and related fields. Articles are subject to editorial revision. Two complete copies of the manuscript including all tables and illustrations should be submitted. Manuscripts should be typed double-spaced with margins at least 4 cm wide, 25 lines to a page, 50 character spaces to a line. The first page should include the title, authors' names and name of the institution where the work was done. On a separate sheet an abstract of not more than 200 words should be supplied; it should not contain abbreviations or references.

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.

Examples:

Royer P: Metabolism and action of vitamin D in the fetus. Acta Paediatr Hung 25.161, 1984

Erlandsen SL, Meyer EA (eds): Giardia and Giardiasis. Plenum Press, New York 1984

Detter JC: Biochemical variation. In: Textbook of Human Genetics, ed Fraser O, Mayo O, Blackwell Scientific Publications, Oxford 1975, p. 115

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