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Termination of anticonvulsive drug treatment and the electroencephalogram

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The value of the EEG in the decision to terminate antiepileptic treatment has been investigated on the basis of longitudinal EEG studies in 433 children and adolescents with epileptic seizure disorders. The data were subjected to uni- and multivariate statistical analysis. Most valuable information concerning the risk of seizure recurrence was derived from (i) the EEG findings obtained just before tapering of medication; (ii) the course of EEG after termination of treatment; and (iii) the maturation of EEG background activity. Thus, the EEG is a valuable tool in the decision of continuation or termination of antiepileptic treatment.

The value of EEG in the diagnosis of epileptic seizure disorders has been established beyond doubt. There are, however, differences of opinion with regard to the usefulness of EEG concerning the choice and especially the termination of anticonvulsive medication. For this reason, a prospective study of the long-term prognosis of childhood epilepsies after discontinuation of anticonvulsive treatment was carried out. In the study, special attention was paid to the EEG findings which will be discussed in the following.

Material and Methods

The study was based on 433 children and adolescents (235 males, 198 females) with various types of epileptic seizures. The age (at the time of discontinuation of treatment) ranged from 2 to 20.5 years. The EEG was carried out with an 8-channel apparatus (Messgeräte Zwönitz) with the use of standard parameters (paper speed, amplification, filter). If feasible, the resting EEG was complemented by hyperventilation and intermittent photic stimulation as well as sleep following sleep deprivation. EEG records were carried out as follows: (1) once or several times before onset of treatment; (2) after initiation of treatment; (3) during treatment, depending on the course (usually in 12-month-intervals); (4) before tapering of medication; (5) during tapering of medication; (6) after termination of treatment at intervals of 4 weeks, 3, 6 and 12 months, then at one-year-intervals.

EEG findings were broken down and categorized as follows: (1) normal EEG; (2) EEG with non-paroxysmal activity (continuous or intermittent rhythmical theta or delta activity with or without a tendency to generalization); (3) EEG with paroxysmal or epileptiform activity (spikes, sharp waves, polyspikes, alone or in combination with slow waves, i.e. forming spike-wave complexes). Marginal or borderline EEG tracings (within very broad normal limits of variability for age) were listed as "normal EEG". All of the acceptable and meaningful EEG data were subjected to statistical analysis using the chi square

test as well as multivariate discriminance and variance analysis. Significance was based on a limit of 1% of probability of error ($p < 0.01$).

The prognostic value of the EEG finding was determined on basis of the recurrence or non-recurrence of seizures after termination of the anticonvulsive therapy.

Results

The EEG findings were assessed with regard to the clinical course.

EEG findings before the beginning of treatment

There was no significant statistical correlation between the EEG before onset of therapy and future tendency to recurrences.

EEG findings during treatment

The difference between these recurrence rates was considered significant but the p value of 0.016 was still within the probability of error.

Intermittent occurrence of epileptiform activity

This implies spontaneous (unprovoked) appearance and disappearance of epileptiform activity in the course of antiepileptic treatment. This criterion appeared to be unrelated to recurrences. The relapse rate was the same (36%) in patients with and without intermittent paroxysmal abnormalities.

Bioelectrical maturation

This term pertains to the normal process of EEG maturation along with advancing age from infancy to adolescence, especially with regard to the frequency spectrum. Presence or absence of normal maturational tendencies during treatment were investigated.

Thus, patients with disturbed maturational development in the EEG proved to have a significantly higher risk for seizure recurrence ($p < 0.001$).

Table V indicates that the presence of epileptiform activity in the EEG prior to discontinuation of anticonvulsants was associated with a significantly increased rate ($p < 0.001$).

The tendency to relapse was similar in all groups and there was no significant difference in the percentage of relapses within the different groups of seizures. The statistical relations between the frequency of recurrence and the presence of epileptiform activity in the EEG prior to discontinuation of therapy in each group of seizures were as follows: absences ($p < 0.01$), tonic-clonic siezures ($p < 0.05$), benign centro-temporal epilepsy ($0 < 0.05$), West and Lennox-Gastaut syndrome ($p < 0.01$), combined seizures ($p > 0.05$). In the other groups a statistical analysis could not be made because the number of the patients was too small and not all variables were available.

There was no significant relation between patients with epileptiform activity in the resting EEG and those with epileptiform activity during activation procedures. The morphology of the paroxysmal discharges (spike-wave-complexes, spikes, sharp waves) and their spatial distribution (gener-

TABLE I

Frequency of relapses in relation to the disappearance of epileptiform activity in the cour seof long term treatment

EEG findings	Number of patients	Patients without relapse	Patients with relapse
Epileptiform activity	60	30	30
No epileptiform activity	257	187	70

The difference between the recurrence rates was highly significant ($p < 0.001$).

TABLE II

Rate of relapses in relation to the disappearance of epileptiform activity along with stabilized seizure freedom

EEG findings	Number of patients	Patients without relapse	Patients with relapse
Epileptiform activity	79	45	34
No epileptiform activity	238	172	66

TABLE III

Rate of relapses in relation to the reappearance of epileptiform activity and their persistence in the course of treatment

EEG findings	Number of patients	Patients without relapse	Patients with relapse
Epileptiform activity	19	4	15
No epileptiform activity	414	272	142

The difference between these recurrence frequencies was highly significant ($p < 0.001$).

TABLE IV

Rate of relapses in relation to bioelectrical maturation

EEG findings	Number of patients	Patients without relapse	Patients with relapse
Normal EEG maturation	289	204	85
No normal EEG maturation	144	72	72

TABLE V

Frequency of relapses in relation to the EEG findings prior to discontinuation of antiepileptic treatment

EEG findings	Number of patients	Patients without relapse	Patients with relapse
Epileptiform activity	83	36	47
No epileptiform activity	350	240	110

alized, focal, diffusely scattered) proved to be an insignificant criterion.

EEG findings after termination of treatment

In 130 patients, the EEG showed persistence, reappearance or first appearance of epileptiform activity after termination of treatment and/or in the ensuing follow-up period. Of the patients, 74 (56.9%) had a relapse. On the other hand, only 83 out of 303 patients (27%) without epileptiform activity in the EEG had a recurrence of seizures. The difference between these groups was significant statistically ($p < 0.001$).

Statistical evaluation of all available EEG data by means of multivariate analysis showed that two prognostic factors were most valuable, a) the EEG findings prior to discontinuation of antiepileptic therapy, and b) the maturational development of EEG background activity in the course of treatment.

TABLE VI

Frequency of relapses with different types of seizure in relation to the EEG findings before discontinuation of antiepileptic therapy (EEG ∅ = EEG without epileptiform activity, EEG + = EEG with epileptiform activity)

Types of seizures	Number of patients	Patients without relapse		Patients with relapse	
		EEG ∅	EEG +	EEG ∅	EEG +
Absences	127	72	7	35	13
Tonic-clonic seizures	124	61	16	29	18
Benign centrotemporal epilepsy	62	34	4	16	8
Combined seizures	40	21	2	13	4
West syndrome	17	15	1	1	—
Lennox-Gastaut syndrome	14	9	1	1	3
Unilateral seizures	12	5	1	6	—
Simple partial seizures	6	4	1	1	—
Complex partial seizures	9	3	2	3	1
Bilateral massive myoclonus	2	—	—	2	—
Unclassified seizures	20	16	1	3	—
Summary	433	240	36	110	47

Discussion

There has been an increasing number of follow-up studies of epileptic patients after termination of anticonvulsive therapy. Thus far, however, only a small number of authors have dealt with the role of the EEG in the decision to terminate drug therapy [1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15]. The results of these investigators differed considerably. According to some [6, 8, 9, 12], the EEG recorded prior to decreasing the doses is of little or no value with regard to the risk of seizure recurrence. Other investigators [2, 5, 11, 15], however, emphasized the prognostically favourable significance of reduced epileptiform activity during anticonvulsive therapy.

Our data showed that the persistence of epileptiform activity in the EEG prior to the intended discontinuation of treatmentd enotes a higher risk of relapses. On the other hand, it should be remembered that a normal EEG does not rule out the recurrence of seizures. This was shown by a relapse rate of 27% of patients with a normal EEG in our material. Similar relapse rates have been reported by other authors [3, 6, 9].

Our data suggested that not only the EEG findings prior to termination of anticonvulsive therapy had an importance but also the course of the EEG findings after termination of treatment. Patients with persistence, re-appearance or first appearance of epileptiform activity in the EEG after anticonvulsive treatment (i.e. in the follow-up period) had significantly more recurrences. This is in accordance with the observations of Förster and Schmidberger [3].

Thus far, there have been no reports concerning wave morphology and spatial distribution of paroxysmal discharges with regard to treatment and relapses. We were unable to demonstrate such correlations. It must be said, however, that statistical analysis was marred by the small number of patients in each group.

In this context, it might be worthwhile to discuss the problems of "EEG-oriented therapy". It must be emphasized that the clinical course is the most important criterion as far as the optimum therapeutic approach is concerned. On the basis of our data, however, it is advisable to aim at both seizure-freedom and EEG without epileptiform activity in the course of longterm treatment. It should be taken into account whether the abnormal paroxysmal EEG was the expression of a per se recalcitrant form of epileptic seizure disorder, or was due to inadequate antiepileptic treatment, or to poor patient compliance. These possibilities should not interfere with analysis of the patient material. It is also well-known that children with absences may show aborted spike-wave bursts in the EEG in spite of seizure freedom; these abnormalities can be eliminated by further increase of the dosage. The intended normalization of the EEG, however, should not be achieved at the expense of undesirable side effects. Optimum treatment

requires much experience and a special "touch", and the intended seizure freedom and side effects must carefully be weighed against each other.

These considerations have led to another important question, i.e. the termination of therapy in patients who have been seizure-free for several years while the EEG has been persistently showing epileptiform activity. According to Doose [1], the presence of "massive EEG abnormalities" does not rule out termination of treatment. This author recommends in such cases an at least five-year-period of seizure freedom and very cautious reduction of drugs. Groh [5] followed such patients in comparison with patients with improved or normalized EEG.

Our study also showed that, in patients with epileptiform activity in the EEG prior to termination of treatment, a seizure freedom of 3 years or longer was associated with a lower relapse rate. Prolongation of the seizure-free period before discontinuation of therapy evidently diminished the risk of recurrences. One is hence tempted to conclude that, even in such problematic cases, termination of therapy is not necessarily contraindicated provided that the duration of seizure-freedom reaches or exceeds a five-year-period. Still, this rule should not be generalized. A responsible and individual approach is needed even though the patient's (or the parent's) request for termination of treatment is taken into consideration.

Further studies will be needed in order fully to assess the risk of relapses. Presumably, there will always be epileptic patients who require much longer treatment periods than the remaining patient population. As it has been pointed out by other authors [5, 13, 15] the prognosis is reflected not merely by the resolution of epileptiform activity but also by the maturational process affecting the background activity. Our longitudinal EEG studies clearly showed that a lack of bioelectrical maturation is associated with a significantly higher relapse rate. This process of EEG maturation, however, may be obscured by the drug-induced slowing of the background EEG activity. In these patients, termination of anticonvulsive therapy resulted in a "de-camouflage" of the EEG and re-appearance of the true background of activity.

Notwithstanding our reservations against strictly statistical analysis of data, our findings and those of the literature suggest that the EEG at the time of termination of treatment represents a useful criterion. If reasonably used and interpreted, the EEG is of great value in the important decision of continuation or termination of antiepileptic therapy.

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Risk factors in childhood diabetes mellitus

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The quotients total cholesterol/HDL-cholesterol and free fatty acid/albumin in serum were examined in various groups of diabetic patients and the changes of the quotients induced by various forms of physical activity were then registered. Both total cholesterol/HDL-cholesterol and free fatty acid/albumin were increased in patients with unsatisfactory control of diabetes. Low albumin levels were only seen in cases with very poor control or in cases afflicted by complications. Increased muscular activity unvariably led to a fall in the value of total cholesterol/HDL-cholesterol while in children with adequate control but performing little physical exercise the quotient of free fatty acid/albumin was low and total cholesterol/HDL-cholesterol was increased. In addition to the quotient of total cholesterol/HDL-cholesterol also the quotient of free fatty acid/albumin is a sensitive indicator of the diabetic condition.

In a previous study [1] we showed by fluorescent angiography (FA) that vascular changes of the retina may occur in the earliest stage of childhood diabetes: in 50% of cases with a duration of diabetes less than 5 years FA demonstrated the presence of microaneurysms. These findings have been confirmed by others [7]. Good control of diabetes, however, markedly reduces the incidence of microaneurysms [2].

In this study we have attempted to answer the question how far the changes in risk factors influencing the onset of angiography are followed by changes in the diabetic condition.

In the pathogenesis of arteriosclerosis an important role has been ascribed to the lipid and lipoprotein status, notably to the high value of the total cholesterol/high density lipoprotein cholesterol quotient (LDL-C/HDL-C) [10]. In addition, changes in haemostasis, due to alterations in the prostacycline system, also play a decisive part in the appearance of vascular phenomena. Prostacycline (PGI_2) inhibits platelet aggregation, and this inhibition is abolished by FFA, which enhances PGI_2 catabolism. Albumin exerts an opposite effect.

In the experiments of Reinila [9] on diabetic rats the value of the free fatty acid/albumin quotient was as high as 2.3—3.3 in the animals affected by vascular changes, 1.4—1.8 in diabetic animals free from blood vessel alterations, and 0.6—1.1 in healthy controls.

To our present knowledge, both increased values of total cholesterol/HDL-cholesterol and FFA/albumin

carry an angiological risk. The value of these quotients has been determined in various groups of diabetic children to see how far the unfavourable circumstances in diabetic control led to an increase in them.

Material and Methods

Group 1 comprised 17 diabetic children prone to acidosis, needing frequent hospitalisation, in whom good control could not be achieved; their FFA/albumin quotient was compared with that of 10 healthy children.

In the second course of studies 10 children with reasonable control of diabetes (daily glucose output less than 20 g, no acetonuria) and 28 children with less satisfactory control (daily glucose output over 20 g, occasional acetonuria) were examined.

The changes in the value of the quotients induced by increased physical activity in a camp were studied in 16 children of Group 3.

The same changes were examined in 28 children going to school, exerting little physical effort and under good diabetic control.

Also, the circadian rhythm of total cholesterol/HDL-cholesterol and FFA/albumin was determined in healthy and diabetic children ($n = 8$).

A total of 107 diabetic children participated in the study, 45 boys and 62 girls, aged between 9 and 15 years. All were treated with monocomponent insulin (Actrapid MC and Monotard). Most of them received insulin twice daily. The main duration of diabetes was 2.7 and 2.58 years, respectively.

Free fatty acids were determined according to the method of Dole and Meinerts [3], albumin was measured by radial immune diffusion, cholesterol and HDL-cholesterol enzymatically, triglyceride by the method of Laurell [6], blood glucose by the o-toluidine method. All results were expressed in mmol/l.

In eight children LDL-cholesterol was determined by Friedwald's formula, from this the changes in LDL-cholesterol/HDL-cholesterol were calculated and compared with those in total cholesterol/HDL-cholesterol. The direction and magnitude of the changes in both quotient values proved to be identical, therefore the lipid status was characterised by the value of the total cholesterol/HDL-cholesterol quotient, in accordance with other authors [10]. In our experience the upper limit of the normal range was 5.5 for total cholesterol/HDL-cholesterol and 1.4 for FFA/albumin.

Results

In the 17 children with poorly controllable diabetes HDL-cholesterol was 0.92 ± 0.31 (mean $\pm$ SD), serum total cholesterol was 7.4 ± 2.7 mmol/l. The quotient FFA/albumin was markedly increased because of a considerably low albumin level (free fatty acid/albumin was 1.53 ± 0.72 in the 17 diabetics while 0.60 ± 0.12 in the healthy controls; serum albumin of the diabetics was 0.48 ± 0.17, that of the healthy children, 0.58 ± 0.12).

As can be seen in Table I, increased FFA and decreased HDL-cholesterol were the most sensitive indicators of poor control of diabetes; as a consequence, both quotients, FFA/albumin and total cholesterol/HDL-cholesterol, showed increased values.

Table II demonstrates that a significant fall in FFA/albumin and total cholesterol/HDL-cholesterol was achieved by a three-week camp offering much physical exercise.

In children who have access to physical activity only during the

TABLE I

Risk factors in healthy controls and diabetic children with good or less satisfactory control

Group	FFA	Albumin	FFA/albumin	HDL-C	Total cholesterol/HDL-C
Controls n = 10	0.344±0.094	0.58±0.12	0.60±0.12	1.29±0.32	3.60±1.03
Diabetic, good control n = 10	0.511±0.099	0.63±0.12	0.80±0.38	1.15±0.23	4.39±0.52
Diabetic, less satisfactory control n = 28	0.897±0.127**	0.61±0.36	1.47±0.58	0.98±0.32	5.98±1.95*

* $p < 0.05$
** $p < 0.001$

TABLE II

Risk factors during camping

n = 16	FFA/albumin	Total Total cholesterol/HDL-C	HDL-C
Before camping	1.35±0.23	5.73±1.50	0.86±0.19
After camping	0.88±0.21**	4.87±1.09*	1.03±0.21*

* $p < 0.001$
** $p < 0.01$

TABLE III

Risk factors during a period of decreasing physical activity

n = 28	FFA/albumin		Total cholesterol/HDL-C
Satisfactory muscular activity	1.30±0.48	$p < 0.001$	5.70±1.40
Decreasing muscular activity (school)	1.08±0.30		7.46±2.50

summer holidays but have much less elbowroom when going to school and whose diabetes could satisfactorily be controlled, a fall in FFA/albumin and a very marked increase in total cholesterol/HDL-cholesterol were observed during a three-month period.

Figure 1 shows the circadian rhythm of the quotients. Total cholesterol/HDL-cholesterol exhibited a

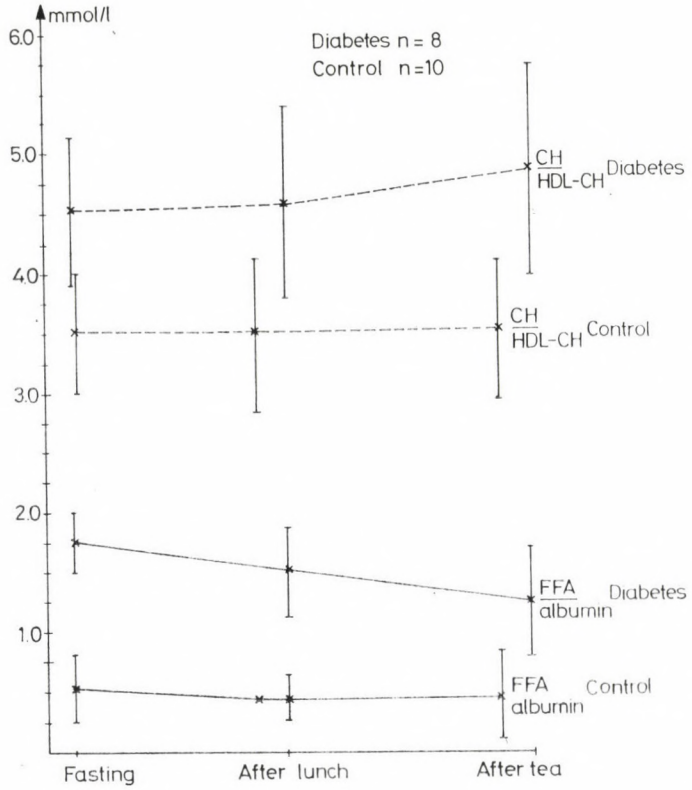

FIG. 1

moderate increase over the day while FFA/albumin a significant fall; this latter phenomenon was due to the decrease of the level of free fatty acids caused by the effect of insulin. Blood glucose was 12.96 ± 1.78, 10.54 ± 5.34 and 11.90 ± 3.97 mmol/l at the corresponding occasions.

DISCUSSION

As a consequence of circadian oscillations the lowest values of total cholesterol/HDL-cholesterol and the highest ones of FFA/albumin could be observed in the fasting subjects. The favourable decrease in total cholesterol/HDL-cholesterol could be achieved by enhanced physical exercise, in addition to good control of diabetes. In case of unsatisfactory physical activity, adequate insulin treatment resulted in a favourable change of FFA/albumin but the mean of total cholesterol/HDL-cholesterol increased in consequence of a fall in HDL-cholesterol. On increased physical exercise, both the FFA/albumin and total cholesterol/HDL-cholesterol ratios decreased. This exercise-induced increase in HDL-cholesterol

could be registered in both healthy and obese non-diabetic subjects [10].

There are few publications on the FFA/albumin quotient although in vitro studies [5, 8] and animal experiments [9] have pointed to its significance in the pathogenesis of vascular changes. Low albumin values accompanied by increased free fatty acid levels can be expected in complicated and poorly controlled cases. In our material, the lowest value, 0.33 mmol/l, has been encountered in a case affected by diabetes complicated by coeliac disease. It is well known that the FFA level in diabetics is a consequence of insulin therapy. In the overwhelming majority of our cases the albumin level was normal and the increase in the value of the FFA/albumin quotient was due to a high FFA level.

In respect to the prevention or postponement of angiopathy or to a deceleration of its progression it may be important to keep the value of the FFA/albumin quotient within normal limits and, doing this, the albumin levels must also be considered.

Risk factors are obviously present in childhood diabetes but it is difficult to judge their role in the pathogenesis of angiopathy. Constitution must here play an important role: FA studies have shown that even the best control of diabetes cannot always prevent the appearance of microaneurysms in childhood.

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Focal nodular hyperplasia of the liver after clomiphene treatment in a young boy

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The case of a 3.5-year-old boy treated unsuccessfully with clomiphene for cryptorchidism is reported. Nine months later focal nodular liver hyperplasia developed. Clomiphene promotes testicular descent by stimulating endogenous production of gonadotropins and their releasing factors. A causal relationship between the hepatic lesion and clomiphene treatment has been supposed. In our case subsequent fertility can only be expected from orchidopexy performed as soon as possible.

Hepatic function must be checked during and after clomiphene treatment also in children.

In childhood, liver tumours rank at the third place after Wilms tumour and neuroblastoma [8]. The most frequent form of liver tumours is hepatoblastoma (40%), followed by hepatocellular carcinoma (20%); adenoma and focal nodular hyperplasia (FNH) are rare (each 2%) in childhood [28]. The youngest reported case with focal nodular hyperplasia of the liver was a girl of four months [20]. Sweeney and Evans [27] described a five-year-old boy treated with a synthetic anabolic steroid for Fanconi anaemia in whom liver adenoma and focal nodular hyperplasia (FNH) developed simultaneously.

We report here on an almost 3 years old boy with bilateral cryptorchidism in whom FNH appeared nine months after clomiphene treatment.

Clomiphene stimulates gonadotropin secretion through the hypothalamo-hypophyseal axis, both by increasing the level of the releasing factors and by a direct effect on the pituitary [18], i.e. clomiphene replaces HCG and LH-RH in the treatment of testicular maldescent and has successfully been applied in cryptorchidism [4]. In gynaecology, clomiphene is used to measure the functional capacity of the hypothalamo-hypophyseo-gonadal system and to induce ovulation [13, 21]; it has also been applied for treatment of male infertility [1, 24]. Borderline carcinoma of the ovarium, due to too high levels of oestrogen provoked by overstimulation has been observed in clomiphene treated women [2]. The drug may play a part in the development of liver tumours: hepatoblastoma was seen in a 15 months old girl whose mother had been treated with clomiphene and follicle-stimulating and luteinizing hormones for infertility before conception [17]. In our case a causal relationship could be anticipated between clomiphene therapy and hepatic lesion.

Report of a Case

G. N., a boy, was born on 26 February, 1980, with 36 weeks gestational age and 2400 g birthweight. His mother was treated because of imminent premature labour with a total dose of 105 mg of allyloestrenol given on seven days in the 35th week of pregnancy. The newborn had prolonged jaundice due to AB0-incompatibility. His bilateral cryptorchidism was detected at birth. At the age of 3.5 years the child still had enuresis. He learned to speak with a considerable lag, he still speaks little and with errors. In December, 1982, he had been treated with 50 mg clomiphene daily over 20 days. By the end of this period his left testicle had become palpable in the inguinal canal, the right testicle could be pulled down from the inguinal canal into the scrotum, but it soon returned spontaneously to the initial site. Physical examination carried out before treatment revealed no abormality except the cryptorchidism. No liver function tests were performed prior to treatment. Nine months later the boy's parents had palpated an egg-sized painless tumour in the epigastric region. The child was admitted to our department. At admission, elevated GOT (135 U/l) and slightly increased serum bilirubin (21.6 μmol/l) were found. Abdominal sonography revealed an echo-rich region 3 cm in diameter in the left lobe of the liver; in addition, the left kidney seemed enlarged, its upper pole appeared to contain a solid structure. This made us to suspect a primary kidney tumour. Infusion urography, however, did not show any renal abnormality. Hepatic metastasis originating from a small Wilms tumour was then suspected and explorative laparotomy was carried out in September, 1983. A tumour 4 cm in diameter was excised in toto from the left lobe of the liver. The left kidney appeared tumour-free at surgery.

The tumour, measuring $7 \times 5 \times 4$ cm, was partly covered by peritoneum and contained a round, hyperplastic area, distinct from its environment but having no own capsule. The tumour was divided by irregular thin streaks into nodules of 0.5—1.5 cm. In the centre of the tumour a necrotic focus 1.2 cm in diameter was found.

The substance of the hepatocellular hyperplasia surrounded by slightly deformed liver tissue with normal trabecular pattern was divided by long collagen streaks which had in some areas a stellate shape (Fig. 1). In other areas the streaks surrounded nodules similar to those characteristic of a cirrhotic liver (Fig. 2). In the broad connective tissue septa lymphocytic infiltration accompanied by bileduct proliferation was seen (Fig. 3). The hyperplastic cells were large, had a polygonal shape, most of them were tightly joined but sparsely they formed pseudoglandular formations consisting of 6—10 cuboid or cylindriform cells (Fig. 4). No signs of atypia or malignancy were seen in the hyperplastic area, rarely binuclear cells could be encountered. No vascular changes were detected in the environment of the well-defined necrotic focus lying in the centre of the tumour-like lesion. The histologic diagnosis was hepatic nodular focal hyperplasia.

After an uneventful postoperative period the child was discharged. Laboratory tests were performed one week and one month after surgery; their results suggested gradual improvement: serum GOT: 45 and 15 U/l, respectively, serum GPT: 58 and 11 U/l, respectively, and serum total bilirubin: 6.5 μmol/l on both occasions.

Discussion

Benign focal nodular hyperplasia of the liver is a rare condition in childhood [25, 28]. The largest series, comprising 61 paediatric cases, collected by Stocker and Ishak [25] revealed a female dominance (72%); 41% of the cases were younger than 5 years. The condition is often accompanied

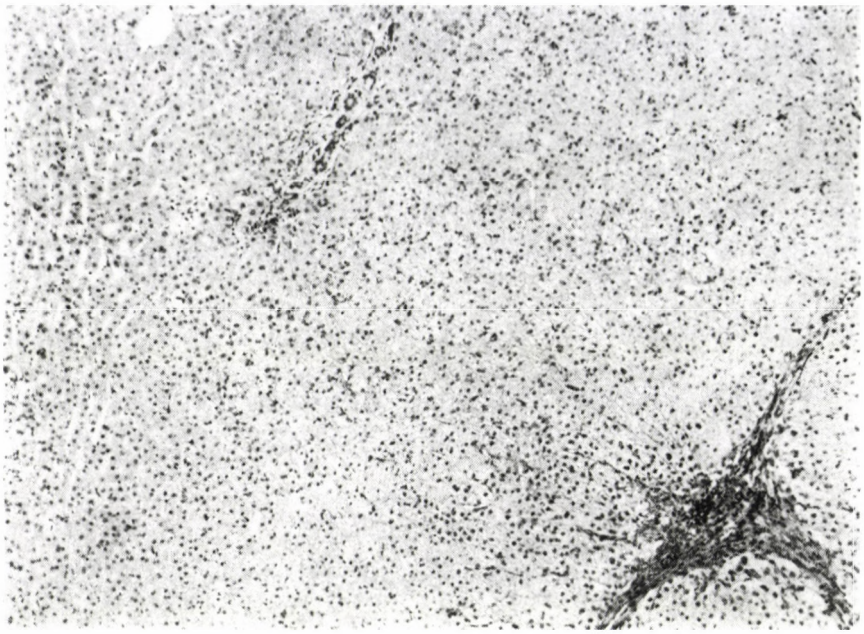

FIG. 1. Focal nodular hyperplasia of the liver, on the left margin hepatic tissue of intact structure. At the right bottom a star-shaped connective tissue streak (H & E, ×63)

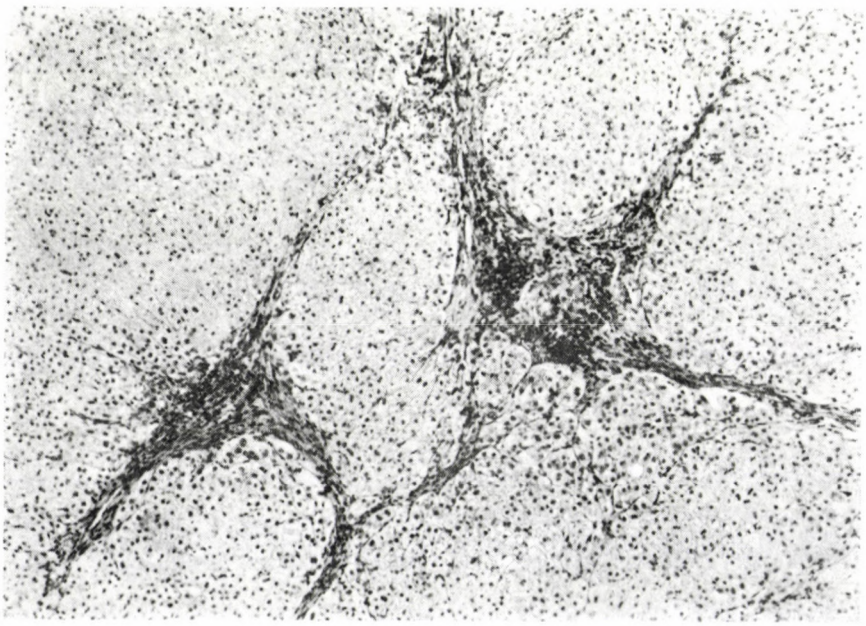

FIG. 2. Cicatrisant streaks with lymphocyte infiltration produce cirrhosis-like nodules within the hyperplastic area (H & E, ×63)

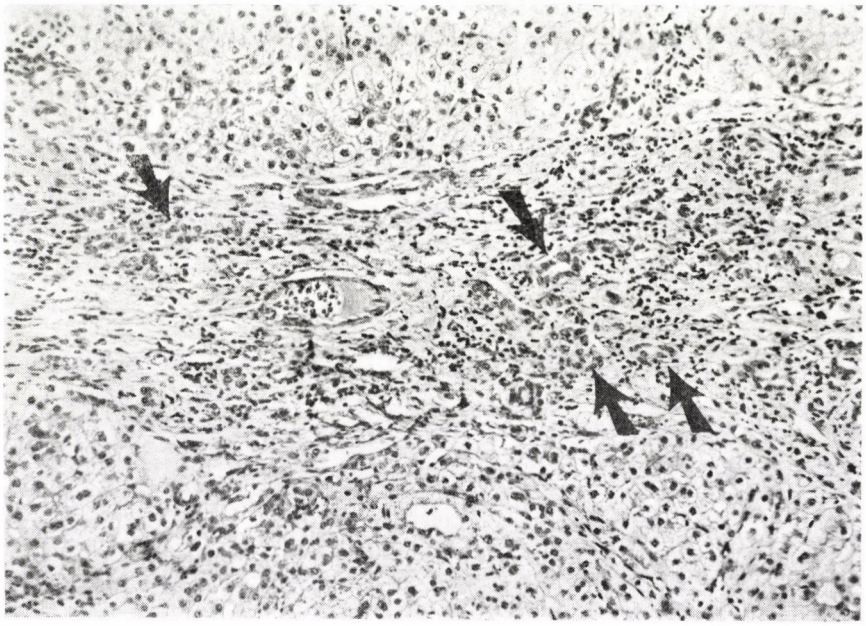

FIG. 3. Lymphocyte infiltration within the broad connective tissue septa, the arrows point to proliferation of bile-ducts (H & E, ×100)

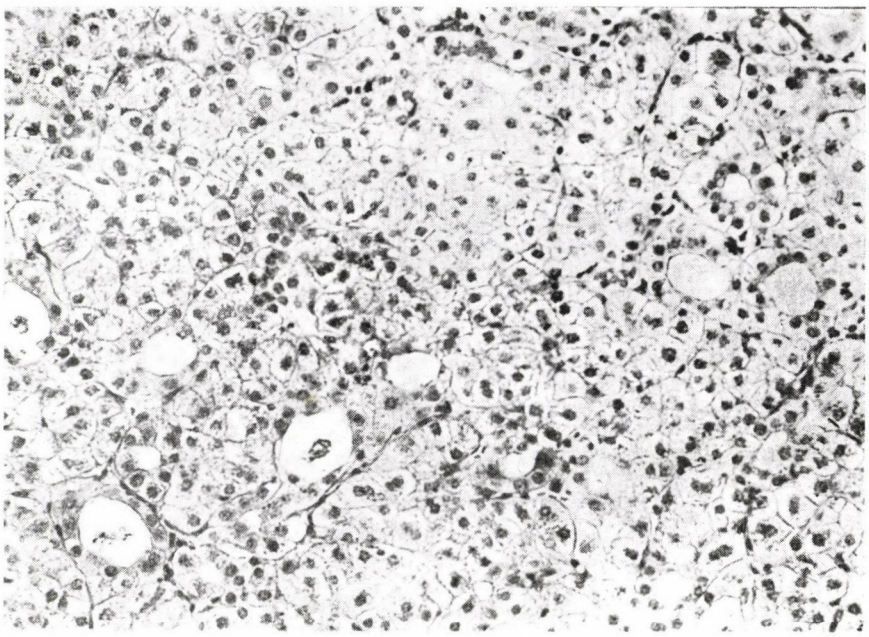

FIG. 4. Pseudoglandular formations of hepatocytes within hyperplastic nodules (H & E, ×160)

by other malformations: multiple teleangiectasis on the arms and legs [10], hypospadiasis, bilateral syndactyly of the toes and bilateral hydrocele, leftsided hemihypertorphy, multiple teleangiectasis over the face and the lips, and umbilical hernia [9]. Some are complicated by a metabolic disorder, type I glycogen storage disease [12]. Mones and Saldana [20] observed in a four-month-old girl Krabbe disease (autosomal recessive demyelinisation of the central nervous system) in addition to nodular regenerative liver hyperplasia. In our case no concomitant disorders could be detected. In cases treated with continuous androgen therapy for Fanconi anaemia the FNH could be ascribed to the drug [6, 27]. In women taking contraceptive pills the relationship between hepatic adenoma [3, 7] or focal nodular hyperplasia [15, 16] and the drug has firmly been established. This factor can, however, be excluded in the aetiology of FNH in childhood. Some authors regard FNH as a hamartomatous malformation [25, 28]. Others underlined the non-tumorous character of FNH by pointing out the structural changes in the blood vessel wall [15, 16, 25] and regarded the hyperplasia as regenerative [20, 26]. Malignant transformation of focal nodular hyperplasia of the liver has never been observed in paediatric patients [8, 25]. Its adequate therapy is surgical excision [25, 28]; no further treatment, cytostatics or irradiation are needed.

Clomiphene is a double-faced synthetic compound with weak oestrogen properties and void of an androgenic effect. Since it binds to the oestrogen receptors of the hypothalamic target cells, the drug deplaces the endogenous hormones with a stronger effect than clomiphene itself, thereby it has a seemingly anti-oestrogen effect as well [29]. As follows from the mode of action of clomiphene [19] the drug can replace HCG and LH—RH in the treatment of testicular maldescent by increasing the level of endogenous gonadotropin and by stimulating production of the releasing factors.

These considerations have led to the introduction of clomiphene in the hormone treatment of testicular maldescent [4]. In addition to an improvement of the testicular position, an increase in the LH and testosterone level have proved the efficacy of the drug [5].

For induction of ovulation, a 5-day course applying a total dose of 250—750 mg has been recommended [13, 21]. For treatment of male infertility daily 50 mg clomiphene on 25 consecutive days was recommended over one to six months, in a total dose of 1250—7500 mg [1]. Paulson [23] and Ross et al [24] reported on favourable effects while Abel et al [1] did not find any difference between the effects of clomiphene and vitamin C. If cryptorchidism is unresponsive to hormone treatment, fertility can only be expected if orchidopexy is performed as early as possible [11].

Hepatoblastoma developed in a 15-month-old girl born to a mother

treated with follicle-stimulating and luteinising hormones plus clomiphene for infertility over one year prior to her pregnancy [17]. As far as we know, this is the only proven case in which clomiphene had an aetiological role in the pathogenesis of a malignant liver tumour.

A role of hormone preparations taken by the pregnant mother in the pathogenesis of liver tumours appearing in her infant has been suspected by some authors [18, 22]. In our own case, the mother had been treated with allyloestrenol; this may have affected the hepatic function of the fetus, resulting in an altered reaction to subsequent clomiphene treatment of the child itself. Hormonal treatment of the mother, even before conception, but especially when administered inadvertently during the first weeks of pregnancy [18, 22] may affect liver function of the fetus and its sensitivity to potentially oncogenic substances.

Clomiphene treatment of cryptorchidism during childhood is based on the experience gathered in the therapy of adult hypofertility [23, 24]: it does induce production of endogenous hormones. However, caution is recommended in case of children as well: liver function has regularly to be checked.

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Antibody dependent cellular cytotoxicity (ADCC)-reaction and an in vitro steroid sensitivity test of peripheral lymphocytes in children with malignant haematological and autoimmune diseases

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ADCC reaction (antibody dependent cellular cytotoxicity), ADCC capacity and ADCC steroid sensitivity examinations were performed in 20 children with tumorous or haematological diseases, 10 children with autoimmune diseases, and appropriate controls, in order to establish the killer function and steroid sensitivity. In the above diseases a study was also made of the correlation of the individual reactions with the duration of steroid therapy.

The two patient groups did not exhibit a significant difference from the controls as concerns the ADCC reaction and ADCC capacity.

In the group of tumorous or malignant haematological diseases the steroid sensitivity behaved in a different way, with sensitivity in 45%, moderate sensitivity in 20%, and steroid resistance in 35% of the patients. Steroid inhibition of the ADCC reaction was significantly decreased in the group of autoimmune patients.

There was no correlation between ADCC reaction and ADCC steroid sensitivity or resistance in either group, and thus the ADCC steroid resistance or sensitivity and lymphocyte killer function proved to be independent. No correlation was found between the steroid sensitivity or resistance and the duration of steroid treatment.

Natural killer (NK) and killer (K) cells are known to play important roles in non-specific antitumorous immunological defence [1, 5, 17]. Explorations of the relationship between K and ADCC effectors [1, 10] have led to the suggestion that NK and ADCC effectors are probably the same. It has been shown that these cells may bear T-cell and/or myelomonocytic markers and that they can be characterized morphologically as large granular lymphocytes which are mostly present in the low-density Percoll fractions [21]. Interferon treatment increases their activity, while mediators such as prostaglandins, steroids and cytophilic immunoglobulins inhibit the activity [12].

Previous publications reported on diminished NK activity or ADCC activity in patients with malignant breast, liver, pancreas or colon tumours, and in melanoma malignum [6, 7, 17, 18, 19].

We have investigated the killer activity of lymphocytes and steroid inhibition of the ADCC reaction in children suffering from malignant diseases. The correlation between the in vitro effect of steroid on the ADCC reaction and the duration of steroid therapy was also analysed.

Patient Material

Steroid sensitivity was measured in the traditional ADCC reaction (number of effector cells in excess) [22] and in the ADCC-capacity (ADCC-C) test (excess of target cells) in 20 children suffering from haematological diseases or tumour (Group I, Table II), 10 children with autoimmune diseases (Group II, Table III) and controls.

Methods

Cytotoxic capacity of lymphocytes (ADCC-C). The essence of the method is as follows. In the event of a high number of target cells the cytotoxic activity of lymphocytes increases, and thus maximum cytotoxic activity (capacity) of the cell population may be measured by titration of the target cells.

A three times washed suspension of freshly taken Rh (D, C)-positive "O" red blood cells was used as *target cells.* ^{51}Cr labelling was performed in the presence of Na-citrate, by the addition of 100 μCi $Na_2{}^{51}CrO_4$ (Amersham; spec. act. 7—8 GBq/mg Cr) for 60 minutes at 37°C. After labelling, the cells were washed 3 times, and the cell count was adjusted to 10^7/ml. The *effector lymphocytes* were isolated on a Ficoll-Uromiro gradient after treatment of the whole blood with colloidal iron powder (GAF, USA).

50 μl quantities of serial 1 : 2 dilutions of the target cells were added per culture in microtiter plates (Greiner), together with 50 μl of a 2×10^6/ml suspension of effector lymphocytes. Thus, while the effector cell count remained constant (10^5 lymphocytes), the target cell count decreased due to the 1 : 2 dilution.

For one test, 50 μl of a 1 : 20 dilution of incomplete anti-D antibody was added, and the volume was made up to 200 μl with 50 μl RPMI medium (Serva) containing 10% calf serum (Herman, Budapest). The cultures were incubated in a CO_2 thermostat for 18 hours at 37°C, and the activity of 100 μl supernatant was measured. The steroid (methylprednisolone) was added together with the supplementary nutrient medium, in a final concentration of 5—10 μg/ml.

For the determination of total activity, measurements were made of the radioactivity of 25 μl labelled red blood cells. The spontaneous activity was given by the count rates for steroid-containing cultures without anti-D antibody.

The means of the count rates for 3—4 parallel samples were used for evaluation. No. of target cells destroyed:

$$\frac{\text{No. of target cells added} \times \text{cytotoxicity}}{100}$$

Steroid sensitivity studies were carried out by means of target cells excess cytotoxic capacity investigations, together with the effector excess traditional *cytotoxic reaction,* ADCC. In the latter reaction all conditions were similar (culture medium, target cell, time of incubation) to those described in the ADCC-C test, the only difference was the effector/target ratio being 10 : 1.

Steroid sensitivity or resistance was measured by the percent inhibition of the ADCC reaction.

ADCC inhibition $<30\%$, resistant to steroid

ADCC inhibition 30—50%, moderate sensitivity to steroid

ADCC inhibition $>50\%$, steroid sensitivity

Results

Table I presents the results of ADCC-reaction and ADCC-capacity examinations in children with malignant tumour or haematological disease or with autoimmune diseases. These two groups of patients did not exhibit significant differences from the controls in the ADCC reaction and the ADCC capacity. In the group with tumour and malignant haemato-

logical disease, the ADCC reaction was markedly decreased, to below 35%, in 4 of the initial 10 cases. The ADCC reaction was above 30% in all children in the autoimmune group, while it proved low (4% and 13%) in two subsequent cases not shown in Table I; a pronounced steroid sensitivity was confirmed in these cases.

Table II details the percentage of the steroid inhibition of ADCC in the two groups, as an indication of the steroid sensitivity or resistance. The clinical diagnosis and the treatment are also listed, with emphasis on whether the children had participated in steroid treatment. Varying results were obtained concerning the in vitro sensitivity in the malignant tumorous and haematological patients: 9/20 displayed sensitivity, 4/20 moderate sensitivity, 7/20 steroid resistance, and thus the group average did not differ from that for the control group. Seven of the 13 steroid-sensitive or moderately sensitive patients had not received steroid before the examination. In the group of autoimmune diseases, a significantly decreased ADCC steroid inhibition was found after prednisolone treatment, as compared to the controls (Table III).

Investigation of a possible connection between ADCC capacity and ADCC steroid sensitivity or resistance of the patients revealed that there was no correlation between the percentage of steroid inhibition of ADCC and ADCC capacity in either the malignant tumorous group or the autoimmune group (Table IV), i.e. ADCC steroid resistance or sensitivity and killer capacity proved to be independent. The Mann—Whitney test did not demonstrate a significant connection between ADCC reaction and steroid sensitivity or resistance in the tumorous group ($r = 0.46$; $p > 0.1$) or in the autoimmune group ($r = -0.21$; $p > 0.1$). Nor was a significant correlation found between ADCC steroid sensitivity or resistance and the duration of steroid treatment for either group (tumorous group: $r = -0.08$; $p > 0.1$; autoimmune group: $r = -0.15$; $p > 0.1$).

TABLE I

Results of ADCC-reaction and ADCC-capacity

Group of patients suffering from malignant tumors and haematological diseases		Group of autoimmune patients		Controls	
ADCC-reaction per cent	ADCC-capacity	ADCC-reaction, per cent	ADCC-capacity	ADCC-reaction, per cent	ADCC-capacity
(n = 10)	(n = 15)	(n = 9)	(n = 10)	(n = 21)	(n = 16)
T = ± 40,2	29783.3	52	34520	41.46	23593.7
S.D. i 23.9	26393.12	15.06	35081	14.06	20396
p > 0.05	(Mann—Whitney's test				

TABLE II

Result of ADCC-steroid inhibition test

Sign	Age year	Sex	ADCC inhibition per cent	Sensitive (2 points)	Moderately sensitive (1 point)	Resistant (0 point)	Clinical diagnosis	Therapy (duration in weeks)
Children's group suffering from malignant haematological diseases or tumors								
U.T.	7	♂	69,5	+			Non-Hodgkin lymphoma (NHL)	—
N.B.	5	♀	57	+			Acute lymphoid leukaemia (ALL)	Prednisolone (3 wk) MTX, Kidrolase, Cytosar, Cyclophosphanide
N.E.	2	♀	10			+	ALL	Prednisolone (8 wk)
B.I.	2.5	♀	29			+	Neuroblastoma	Prednisolone (6 wk) VCR, Cyclophosphamide DTIC
O.I.	5	♀	0			+	NHL	Kidrolase
U.A.	8	♂	17			+	Reticuloendothelioma	VAC
N.B.	5	♂	40		+		ALL	Prednisolone (7 wk) MTX
L.Sz.	4	♀	51	+			Wilms tumour	—
B.A.	4	♀	20.5			+	Neuroblastoma	Prednisolone (5 wk) VAC
Z.K.	8	♀	41.5		+		Neuroblastoma	—
K.L.	10	♂	55	+			ALL	Prednisolone (2 wk)
D.L.	8	♂	42		+		Eosinophil granuloma	Prednisolone (2 wk)
M.P.	3	♂	74	+			ALL	—
M.I.	5	♂	85	+			ALL	—
H.L.	10	♂	15			+	ALL	Prednisolone (12 wk) VCR Cytosar, Kidrolase, MTX, VP 16
P.I.	9	♂	79	+			ALL	Prednisolone (16 wk) MTX, Rubidomycin, Kidrolase
Ö.T.	6 m.	♂	40		+		Hepatoblastoma	—
F.T.	2	♀	80	+			Ependymoma	—
B.H.	4	♀	70	+			ALL	Prednisolone (4 wk) MTX
A.K.	9	♂	15			+	Rhabdomyosarcoma	—
(n = 20	$\bar{X}$ =		44.52	$p > 0.05$ (Mann—Whitney's test)				
	S.D. ±		26.24					
	X =		53.08					
Controls								
(n = 28)	S.D. ±		13.16					

TABLE III

Result of ADCC-steroid inhibition test

Sign	Age year	Sex	ADCC inhibition per cent	Sensitive (2 points)	Moderately sensitive (1 point)	Resistant (0 point)	Clinical diagnosis	Therapy (weeks)
Group of children suffering from autoimmune diseases								
H.L.	15	♂	56.5	+			Aplastic anaemia	Prednisolone (5 weeks) Anapolon
F.J.	9	♂	20			+	Hamman—Rich	Prednisolone (6.5 weeks)
E.É.	6	♀	0			+	Rheumatoid arthritis	Prednisolone (7.5 weeks)
F.E.	13	♀	0			+	Ileitis (Crohn)	Prednisolone (8 weeks) Salazopyrine
Sz.G.	4	♀	35		+		Dermatomyositis	Prednisolone (9.5 weeks)
B.P.	12	♀	61.5	+			ITP	Prednisolone (2 weeks)
K.Cs.	9	♂	48.5	+			ITP	Prednisolone (0.5 week)
V.A.	7	♀	9.5			+	Rheum. arthritis	Prednisolone (4.5 weeks)
R.R.	14	♂	36		+		Rheum. arthritis	—
K.A.	7	♀	4			+	Rheum. arthritis	—
	$\bar{X}$ =		27.1					
	S.D. ±		23.6 (n = 10)	$p < 0.01$ (Mann—Whitney's test)				
Controls								
	$\bar{X}$ =		53.08					
	S.D. ±		13.16 (n = 28)					

TABLE IV

Mathematical evaluation of the ADCC-steroid inhibition test and ADCC-capacity

Group of malignant tumours or haematological diseases	
Correlation between percentual ADCC-steroid inhibition and ADCC-capacity	$r = -0.33$ $p > 0.05$
Group of autoimmune diseases	
Correlation between percentual ADCC-steroid inhibition and ADCC-capacity	$r = -0.15$ $p > 0.05$

DISCUSSION

Children with malignant tumour or autoimmune disease did not exhibit a difference from the controls as concerns the ADCC reaction or the ADCC-C value. The lymphocyte population participating in the ADCC reaction displayed an appreciable overlap with the lymphocytes responsible for the NK reaction. The literature on NK activity in autoim-

mune diseases shows that NK activity is reduced in SLE and rheumatoid arthritis [8, 9, 15, 20], while authors [12] described an enhancement of NK activity. We did not find any killer activity change in either the ADCC reaction or the ADCC-capacity.

Numerous data point to the decreased NK and ADCC activity in adult patients with tumour [4, 6, 7, 17, 18, 19]. In our children with malignant disease or leukosis we found no significant differences from the normal. There was no correlation between killer capacity (ADCC—C) and steroid inhibition of the ADCC reaction in cases of malignant haematological disease or tumour.

The steroid inhibition of ADCC or resistance differed from that of the controls in most cases of malignant tumour, and particularly in steroid-treated cases. ADCC steroid inhibition was significantly decreased in children suffering from autoimmune diseases. As an explanation, the idea emerged that prednisolone treatment during several months caused the in vivo prednisolone-sensitive lymphocyte subpopulation to decrease or their steroid receptors to be blocked; accordingly, further inhibition could not be induced in response to prednisolone in an in vitro test. Another possibility is that steroid resistance may have existed even before prednisolone treatment. Rank-correlation analysis did not reveal a significant correlation between the duration of steroid treatment and the steroid sensitivity or resistance, thus there was no indication of a steroid resistance existing initially in some of the cases. The question arises of whether it is worthwhile to apply prednisolone in all steroid-resistant cases.

Since mainly the IgG Fc receptor-bearing lymphocytes take part in the ADCC reaction, it is conceivable that the interindividual differences in steroid sensitivity may be correlated with a shift in the proportion of the T_G lymphocyte subpopulation.

In patients with cancer [17] and malignant diseases [23], there is much evidence pointing to cellular immunity, that the T cell growth factor (TCGF) is involved in these abnormalities. Grabtree et al [4] presented considerable evidence that glucocorticoids inhibit T cell proliferation by blocking the production of TCGF. Parillo and Fauci [16] found that corticosteroids suppressed NK activity in the human. Similarly, NK activity is known to be reduced in kidney allograft recipients treated with prednisolone [11].

A cytotoxic T cell line could destroy tumour cells only if the TCGF was present in sufficient amounts in the test system[3]. A TCGF level reduced by corticosteroids might be a reason for the lack of effect of T cells in cancer patients. The decreased TCGF production is closely related to tumour progress in patients with cancer [14]. Steroid administration should be considered with regard to the individual patient in the event of an initially decreased T cell function, in order to avoid a further reduction of T cell functions.

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Alloimmune neonatal neutropenia: Clinical observations and therapeutic consequences

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Alloimmune neonatal neutropenia is a rare condition, it must be distinguished from hereditary forms of neutropenia and acquired neutropenia accompanying sepsis.

In a family with four affected newborns, the degree of the disease became more and more severe from the first child to the third child. The third child died of sepsis. After birth of the third child, specific antibodies (anti-NA 1) reacting with the neutrophils originating from the father and the first two children were detected in the mother's serum. No neutrophils were detectable in the fourth child immediately after birth. In this child falling concentrations of diaplacentally transferred antibodies could be demonstrated over 8 weeks after birth. Neutrophil counts returned to normal as the antibodies disappeared. In this newborn, infection could be prevented by the use of a germ-free environment and antimicrobial prophylaxis. The antibody titres could only be lowered by repeated exchange transfusions with Na 1-free blood. White blood cell transfusion only resulted in a short transient effect on neutropenia.

Familial occurrence of neonatal neutropenia is rare. The best-known condition is infantile hereditary agranulocytosis (Kostmann syndrome), transmitted as an autosomal recessive trait, readily distinguishable from other forms of neutropenia by its clinical course and haematological findings. The prognosis of this disorder is grave, most cases die of bacterial infection during early childhood, only few patients survive to adulthood. In peripheral blood persistent granulocytopenia below 0.3 G/1, and an increased number of monocytes and eosinophils can be observed. The bone-marrow is rather characteristic, exhibiting inhibited maturation at the level of promyelocytes and myelocytes.

Benign cyclic neutropenia is characterised by periods of mild febrile infection accompanied by granulocytopenia in peripheral and bone-marrow smears. Between the attacks the patients have normal neutrophil counts. The condition is inherited as an autosomal dominant trait, its appearance is not restricted to early infancy. Familial neutropenia with hypergammaglobulinaemia is also transmitted in an autosomal dominant way with a mild manifestation from the first year of life.

Myelocathexis or chronic agranulocytosis can be distinguished from the above mentioned forms by the bone-marrow finding characterized by inhibited release; accumulation of hypersegmented neutrophile granulocytes can be observed.

Constitutional familial leucocytopenia with partial Pelger—Huet anomaly [7a], also termed myelolymphatic insufficiency, is an X-linked recessive condition, the clinically healthy carriers exhibit nuclear anomalies similar to the Pelger anomaly in their neutrophils.

In addition to these hereditary forms of neutropenia immune neutropenia also occurs, either as alloimmune neonatal neutropenia, autoimmune neutropenia, or as chronic benign neutropenia. Alloimmune neonatal neutropenia is less known to paediatricians and physicians employed in the blood transfusion service; still, it needs extraordinary clinical and laboratory efforts. Thus, description of our own experience and comparison with published findings may appear useful.

Report of Cases

B.M., a woman in the seventh week of her fifth pregnancy, attended a genetic counselling service because her three children had been affected by local and/or septic infection accompanied by granulopenia during the neonatal period. The disease was progressively severe in the children, in fact, the third child died of an infection. In addition, she had a pregnancy aborted in fear of an affected child. She reported that during three of her four pregnancies she had suffered of purulent infections of her toes in the first months and that during the fifth to tenth weeks of her actual pregnancy she had an abscess in the mental region; at no other times had she had suppurative skin disorders.

1.1. Patient M.M. The first child developed purulent rhinitis on his fifth day of life; loss of appetite, vomiting, fever of 39°C, facial pyoderma, paronychia on an index finger and purulent conjunctivitis appeared on the seventeenth day of life. Cultures revealed Staphylococcus aureus. The condition abated by the 28th day of life on treatment with ampicillin, oxacillin and gentamicin. Blood taken on the 18th day of life showed haemoglobin: 12.1 mmol/l; leucocyte count: 7.6 G/l; eosinophils: 0.05; rods: 0.02; bands: 0.01; segmented: 0.02; lymphocytes: 0.86; monocytes: 0.02; large lymphocytes: 0.02. Checking at nine months of age and later revealed normal leucocyte and neutrophil counts.

1.2. Patient D.M. Fever of 38.5°C and lymphadenitis in the left axillary region appeared on the fifth day of life. In spite of ampicillin, oxacillin and polymyxin B therapy, abscess formation necessitated surgery on the tenth day. Bacterial culture revealed Pseudomonas aeruginosa. On the twelfth day of life retroauricular pyoderma, abdominal distension and liver and spleen enlargement appeared. Thoracic X-ray revealed disseminated bronchopneumonia. During the subsequent two weeks a life-threatening condition developed. As a consequence of parenteral nutrition, white blood cell transfusions and antibiotic

treatment, gradual improvement of the septic condition supervened.

Up to the 13th day of life 1—2% segmented neutrophile granulocytes, thereafter young granulocytes appeared, from the 19th day of life segmented granulocytes could also be observed (Table I). Poor cellularity in the bone-marrow, normal B/T ratio among the lymphocytes. Immune globulins, measured on the eighth day of life: IgA: less than 0.01 g/l; IgM: 0.076 g/l; IgG: 1.02 g/l.

1.3. Artificial abortion because of fear of another affected child.

1.4. Patient Ch.M. From the third day of life fever between 38.0 and 39.5°C, from the 6th day of life marked abdominal distension. An abdominal X-ray revealed only distended intestines. Moderate hepatosplenomegaly. Small quantities of mucous faeces. Pseudomonas aeruginosa, Enterococcus and Escherichia coli were cultured from the stools. Blood culture of bacteria and fungi was negative. Enterocolitis was anticipated and parenteral feeding plus antibiotic therapy was introduced. The patient's condition deteriorated. In the lower right quadrant of the abdomen a tender resistance of tomato size appeared in the distended abdomen, the presence of an intraabdominal abscess and diffuse peritonitis was suspected. Oedema, severe oliguria, hyponatraemia, increased direct bilirubin level (total: 188 μmol/l, direct: 154 μmol/l) and serum transaminase activity, thrombocytopenia and an exanthem complicated the picture. The child died of bronchopneumonia on the 45th day of life.

Necropsy revealed chronic fibroplastic colitis with perforation of the transverse colon, chronic diffuse peritonitis, abscess in the right lower quadrant, bronchopneumonia, pleural and pericardial effusion, toxic hepatosis and lipaemic nephrosis.

TABLE I

Haematological findings of Patient D. M.

	Day										
	6	8	12	13	14	15	19	20	22	24	30
Haemoglobin, mmol/l	9.5	10.6	9.8	10.4	10.4	10.0	9.0	10.2	7.0	6.6	6.8
Packed cell volume	0.43	0.50	0.51								
Leucocytes, G/l	5.2	10.0	4.8	11.3	10.8	18.7	35.8	24.8	8.7	5.2	6.4
basophils	—	—	—	—	—	—	—	—	—	—	—
eosinophils	7	7	11	—	6	5	—	3	—	—	5
promyelocytes	4	—	—	3	10	2	1	3	—	—	—
myelocytes	1	—	—	10	9	3	22	24	—	3	4
young	—	—	—	1	33	6	24	5	—	1	1
rods	—	—	3	—	4	4	19	16	24	1	2
segmented	1	1	2	—	—	—	11	16	32	26	21
lymphocytes	36	57	72	72	29	41	14	26	44	58	58
monocytes	44	34	12	7	4	6	9	8	—	10	9
Platelets, G/l	245	65	26		175		230	400	85		43
Reticulocytes, per mil			10						4		

TABLE II

Haematological findings of Patient Ch. M. I.

	Day								
	6	8	10	15	18	23	28	34	41
Haemoglobin mmol/l	9.3	8.8	7.6	8.7	8.8	6.3	5.7	6.4	5.8
Packed cell volume	0.48	0.46	0.40	0.46	0.46	0.32	0.31	0.40	0.30
Leucocytes, G/l	4.7	5.2	5.3	4.7	4.0	22.0	36.0	41.0	14.8
promyelocytes	—		7	2	2	6	1	—	—
myelocytes	3		13	2	3	2	1	—	—
basophils	—		—	—	—	—	1	1	—
eosinophils	1		4	6	2	2	3	1	1
young	3		21	12	11	9	—	—	1
rods	10		5	37	51	17	10	27	31
segmented	3		—	23	20	49	52	55	28
lymphocytes	23		40	14	12	11	30	14	35
monocytes	4		4	1	—	2	2	—	4
lymphocytes	3		6						
Platelets, G/l	63	17	80	8	5	30	75	33	40
Reticulocytes, per mil	8		1		6	19	4	11	

Laboratory findings. During the first 14 days of life normal leucocyte counts comprising only 3% segmented granulocytes, 16 and 46% immature granulocytes were found. The granulocyte count increased from the third week of life to 23%, with leucocytosis during the 4th week exceeding 40 G/l, with 50% granulocytes and a shift to the left. From the second week of life the thrombocyte count was between 8 and 75 G/l (Table II). C-reactive protein (8th day): 0.012 g/l, IgA: undetectable, IgM: 0.4 g/l, IgG: 1.120 g/l, rubella antibodies: 1 : 128, CMV (complement binding reaction): negative, toxoplasma antibody: 1 : 16, HB_2Ag: negative.

During and after the fifth pregnancy, clinical, haematological and immunological studies were carried out in the mother and her child and the effect of prophylactic and therapeutic measures was studied.

METHODS

Immunological investigations were carried out monthly in the mother and daily respectively weekly in the newborn baby. Maternal serum was reacted with cell suspensions of the close relatives, with cells of selected and unselected blood donors with known HLA-A, -B, -C and NA 1, NA 2 and NB 1 antigens. The methods used were leucocyte agglutination test of Rood et al [22], indirect immunofluorescence test of Verheugt et al [28] and the lymphocyte toxicity test of Blaschke et al [2].

Blood for exchange transfusion and white blood cell transfusion was selected from donors with blood group 0 d, exhibiting no leucocyte agglutination with the maternal serum. In all available family members blood groups were determined, HLA typing and leucocyte agglutination tests were carried out.

Prior to the birth of the newborn a sterile, germfree environment was prepared according to the usual criteria.

Labour occurred in Dresden, the first steps were performed in the Department of Paediatrics in Dresden (the first two exchange transfusions). Then the child was

transferred to the Second Department of Paediatrics in Berlin-Buch, where the facilities seemed to be more appropriate.

Results

Tests for maternal antibodies. The leucocyte agglutination test revealed agglutinating antibodies against neutrophil granulocytes with a specificity for NA 1, in a titre of 1 : 16 during the whole course of pregnancy. Also, the indirect immunofluorescence test resulted in a positive reaction whenever the test cells carried the NA 1 antigen.

In addition, an antibody specific for HLA—B 17 (57 + 58) was found in a titre of 1 : 2. This antibody could be demonstrated from the eighth month of pregnancy only in undiluted serum; the antibody reacted in the indirect Coombs test on erythrocytes as anti-Bg(b). The immunological findings were described in detail by Leverenz et al [18].

Studies of the family members. The maternal antibody specific for granulocytes reacted only with granulocytes of her children, her husband and his relatives, while with none of her own family members. HLA-typing demonstrated in the first child no haplotype against which the maternal antibody specific for HLA-B 17 (57 + 58) was active although this child had exhibited neonatal neutropenia.

Findings in the newborn. No antibodies could be demonstrated by the lymphocyte toxicity test in cord blood and retroplacental blood. On the other hand, these sera contained the same titre of the granulocyte specific antibody as the maternal serum. The course of the titre is shown in Figure 1.

Delivery. Vaginal irrigations were performed prior to the date of delivery planned for the 38th week of pregnancy. Delivery itself and the first manipulations on the newborn were carried out under germ-free sterile conditions. The newborn baby had a normal appearance. The haematological findings obtained immediately after birth pointed to a complete absence of immature and mature neutrophile granulocytes. The absolute and relative number of eosinophils and monocytes was increased.

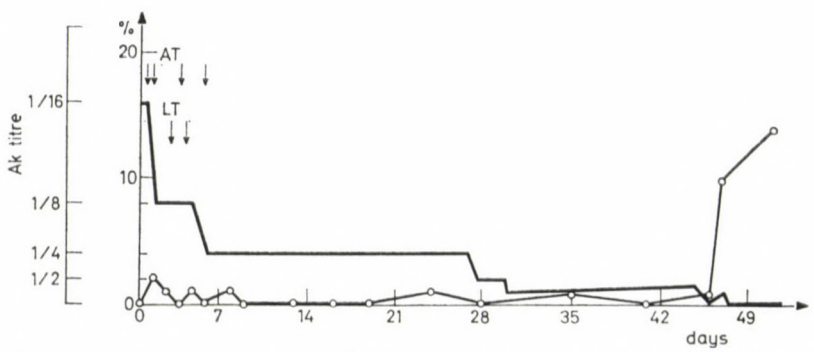

Fig 1. Reactions of neutrophil specific antibodies in the leucocyte agglutination test. ET: Exchange transfusion; LT: Leucocyte transfusion

Care of the newborn. The baby was kept in a sterilized incubator in an isolated room. To reduce the number of enteral germs the newborn was treated daily with 10 mg/kg oral polymyxin B, 150000 IU oral nystatin and 100 mg/kg/day intravenous cephoxitin from the first hour of life. The baby was fed deep-frozen colostrum, then breastmilk originating from a woman who expressed her milk under germ-free conditions. There were no granulocyte specific antibodies in the colostrum of the child's mother but HLA-B 17 antibodies were present in a titre of 1 : 4.

Exchange transfusions using 200 ml/kg heparinized fresh blood (a mixture of packed red cells 0 d and AB d plasma, each free of NA 1 and HLA-B 17 (57 + 58) were started three and 24 hours after birth respectively. One hour after completion of the first exchange transfusion only 2% neutrophile granulocytes could be detected and even four hours after the second exchange transfusion as little as 10% were found. Therefore two additional exchange transfusions were given using fresh blood free of NA 1 and HLA-B 17. Still, the neutropenia persisted unchanged (Table III).

There was, however, a recognizable effect of the exchange transfusions on the antibodies. At the end of each exchange transfusion the antibodies gave only a weak reaction but 24 hours later there was a marked rebound each time. The net effect of the four exchange transfusions was a fall in the titre by two dilutions.

On the third day of life 20 ml/kg leucocyte concentrate, containing $6.8 \cdot 10^9$ leucocytes, 58% neutrophile

TABLE III

The effect of exchange transfusions on leucocyte counts and antibody levels

	Day of life							
	1		2		4		6	
			before	after	before	after	before	after
	immediately after birth	after 1st exchange transfusion	second exchange transfusion		third exchange transfusion		fourth exchange transfusion	
Leucocytes, G/l	6.8	3.6	4.7	2.9	5.5	5.3	13.0	7.2
Blood smear								
myeloblasts	—	0.03	—	0.01	—	—	—	—
promyelocytes	—	0.01	—	0.01	—	—	—	—
myelocytes	0.04	0.02	0.03	0.03	0.01	0.06	0.02	—
basophils	—	—	—	—	—	—	—	—
eosinophils	0.11	0.12	0.05	0.06	0.32	0.17	0.07	0.04
young	0.02	0.01	0.03	0.01	—	0.02	—	0.01
rods	—	—	0.03	0.01	0.01	0.01	—	0.01
segmented	—	0.02	0.04	0.10	0.04	—	0.01	0.02
monocytes	0.59	0.46	0.60	0.40	0.32	0.23	0.31	0.23
lymphocytes	0.24	0.33	0.23	0.37	0.28	0.46	0.50	0.54
large lymphocytes	—	—			0.02	0.05	0.09	0.15
Anti-NA 1 titre	1 : 16	—	1 : 16	—	1 : 8	1 : 4	1 : 8	1 : 4

granulocytes, blood group 0 d, free of NA 1 and HLA-B 17, was transfused over three hours. Unequivocal clinical side-effects were not observed. Obstinate nausea occurred during the transfusion of leucocytes, this was ascribed to oral glucose administration. Oscillations in the respiratory rate accompanied by transient irregularity were related to the sleep/wakeness rhythm. A circumscript skin rash consisting of small patches on the right thorax appeared but rapidly subsided. Two hours after the leucocyte transfusion a marked increase in the neutrophil count was observed but it returned to the previous level by 16 hours after transfusion (Table IV). Fifteen hours after the third exchange transfusion, 36 hours after the first transfusion of leucocytes, a second dose of leucocyte concentrate was applied, 60 ml containing $6.1 \cdot 10^9$ leucocytes with 73% neutrophile granulocytes were transfused. In contrast to the first leucocyte transfusion, the effect of the second one on the blood smear was moderate. There were no marked side-effects during and after this second leucocyte transfusion. Also this time, however, the newborn had nausea, transient irregularities in respiratory rate and 14 hours after transfusion a transient exanthem consisting of large patches appeared on the trunk and some areas of the extremities. Oedema supervened on the sixth to seventh day. Changing skin colour, transient alterations in the respiratory rate were seen. Auscultation over the thorax was normal, a chest X-ray revealed discrete streak shadows in the right lung. Since pneumonia could not be excluded, the therapy initiated shortly after birth was supplemented by Gamma-M concentrate. The above mentioned

TABLE IV

The effect of leucocyte concentrate transfusions on the blood picture

		I					II			
		Before	2	10	16	28	Before	4	12	27
		hours after transfusion					hours after transfusion			
Leucocytes, G/l	Gpt/1	4.0	6.8	4.6	5.5	5.3	see ←	11.1	13	7.2
Blood smear										
myeloblasts		—	—	—	—	—		2	—	—
promyelocytes		—	—	—	—	—		—	—	—
myelocytes		6	2	3	1	6		—	2	—
basophils		—	—	—	—	—		—	—	—
eosinophils		31	9	20	32	17		7	7	4
young		2	1	—	—	2		1	—	1
rods		3	2	2	1	1		7	—	1
segmented		1	32	12	4	—		17	1	2
monocytes		40	19	40	32	23		22	31	23
lymphocytes		17	35	21	28	46		33	50	54
large lymphocytes				2	2	5		11	9	15
Platelets, G/l	Gpt/1	150	184	136	183	92		71	220	50

phenomena were observed during three days, during this period the newborn gained weight in a satisfactory manner. After 6 days there were no pulmonary abnormalities.

During the third week of life high doses of human gamma-globulin 5% made by Institute of Vaccines, Dresden, 1.2 g i.v. on five consecutive days, was applied in order to increase the peripheral neutrophil count since daily checking had revealed 0—1% neutrophile granulocytes and the tests for the maternal antibody were still positive. There was no favourable effect. Similarly, 15 mg of prednisolone divided into two doses, given on the 25th and 26th day failed to increase the neutrophil count. At this time all antimicrobial drugs were stopped. The child gained weight and exhibited no symptoms of an eventual infection. These encouraging events prompted us to continue only the germfree environment.

In the 8th week of life the antibodies could only be demonstrated in undilated serum and they disappeared by the ninth week of life. A corresponding increase in the neutrophil count soon ensued and the child could be discharged during the tenth week of life. Its blood smear was regularly checked, no abnormalities were found up to the eighth month of life, each time a normal leucocyte count and a normal distribution were observed.

The bone-marrow was examined twice. A tibial biopsy was carried out on the eighth day of life, it showed hypoplastic bone-marrow with inhibited maturation of the neutrophile granulocytes. A second bone-marrow specimen was obtained again by tibial punction during the 9th week of life, this time a specimen with high cellularity and exhibiting satisfactory regeneration of granulo- and thrombocytopoiesis was obtained.

Discussion

There is no doubt that the neonatal neutropenia in all of four siblings was caused by an immunological process. Similar observations were published by several authors [8, 17, 19, 24, 25]. In all described families maternal antibodies reacting with the granulocytes of the newborn and the father, respectively, were demonstrated. While the titre in the mother persisted after delivery, a continuous, gradual decrease of the transplacentally transferred antibodies was seen in the children. A concomitant increase in the neutrophil count was then observed, accompanied by healing of the initial local and systemic infections. In analogy to the incompatibilities of the erythrocyte antigen systems, Hitzig and Gitzelmann [8] proposed the term morbus leucolyticus neonati.

Elucidation of the leucocyte antigen-antibody systems was furnished by Lalezari et al (9—16), later on by Verheugt et al [28] and Claas et al. [6]. The earlier clinical observations were then checked in retrospection by new immunological methods, and by now all cases have been fully clarified and the disease is now termed

as isoimmune neonatal neutropenia, as proposed by Lalezari.

As to the clinical picture, a review of 19 cases [15] showed that only four affected babies were first-born, all other patients came from multiparous pregnancies. Also primiparous mothers without any blood transfusion in their history had affected children. A progressively increasing degree of severity, as seen in our cases, has not been firmly established.

The clinical symptoms are variable, in most cases there are febrile local and/or systemic infections during the first week of life. Pyoderma, abscess formation, omphalitis, otitis media, bronchopneumonia, urinary tract infection and sepsis were described but there were asymptomatic cases as well. 2 out of 19 evaluated children died during the second and third week of life, one of pneumonia, the other of sepsis [15].

The fatal outcome of the third child in the family observed by us on the 45th day after birth, at a time when the leucocyte count was already normal, can still be ascribed to the underlying immune process since the disease manifested early and was only prolonged by our treatment.

In alloimmune neonatal neutropenia the spectrum of pathogenic agents comprises mostly staphylococci but also beta-haemolysing streptococci and Escherichia coli. In our own case staphylococcus was demonstrated once and Pseudomonas aeruginosa twice.

The most characteristic haematological finding was a lack or very low number of neutrophile granulocytes, the immature forms included. The finding is present immediately after birth like in the third child of our family. The duration of neutropenia varies from 2 to 15 weeks. At the summit of the local or systemic infections, immature neutrophile granulocytes may appear, further increasing the already high leucocyte count caused by elevation of the monocyte and eosinophile granulocyte counts. All mothers have normal leucocyte counts during pregnancy and after delivery.

The bone-marrow findings are not unequivocal. Increased cellularity with a marked shift to the left in granulopoiesis may prevail; promyelocytes and metamyelocytes dominate while rods and segmented forms are hardly seen. On the other hand, bone-marrow hypoplasia may also be found as was the case in our patient.

It is difficult to evaluate the effect of therapeutic measures described in the literature. In certain cases expectative behaviour and a germ-free environment may suffice since there have been asymptomatic cases. The consequent onset of infections of progressive severity during the first week of life and the fatal outcome in the third child of the family made us suppose that in addition to germ-free care antibiotic and antimycotic treatment supplemented by repeated exchange transfusions was mandatory in the last child of the family.

Our attempt to restitute the neutrophil count by prophylactic leucocyte transfusions has failed. The effect

was so short that the preparation should have been given every day or every second day on a theoretical basis. Since a mean duration of seven weeks can be expected for the neutropenia, an extremely large number of leucocyte transfusions should be applied. This procedure, however, would involve an unjustifiable high risk of immunological complications and other undesirable side-effects. We are thus for a therapeutic indication of leucocyte transfusions. Even incipient local or systemic infection would be such an indication. No side-effects directly ascribable to the leucocyte transfusion were observed, it could, however, not be excluded that the alterations in the respiration rate and the chest X-ray findings were caused by transient pulmonary sludging evoked by the leucocyte transfusion. The course of the disease was not typical for pneumonia. The pulmonary abnormality appeared four days after initiation of the prophylactic antibiotic treatment and all respiratory symptoms and findings resolved without changes. The same holds for the transitory skin phenomena.

By analogy to the alloimmune haemolytic disease of the newborn we expected a drastic fall in the antibody levels after the early exchange transfusion with NA 1 free blood. In spite of the fact that hardly any quantity of antibody was found immediately after exchange transfusion, a marked rebound was observed by the end of the first day following the exchange transfusion. Still, we succeeded in reducing the titre to a level two dilutions lower by four consecutive exchange transfusions. This kind of treatment had no measurable effect on the neutrophil count. Further exchange transfusions might have additionally lowered the antibody level but we felt that for the increasing risk of repeated invasive methods this was not justified. Unfortunately, there are hardly any reports on experience with exchange transfusions carried out in alloimmune neonatal neutropenia, although most case reports mention the necessity and effectiveness of the procedure [7].

Since the exchange transfusions and the leucocyte transfusions have failed, we attribute a major role to germ-free care in the treatment of this transitory but severe disorder.

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D-Penicillamine decreases the H_2O_2 and phenylhydrazine induced lipid peroxidation in the erythrocyte membrane

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The protecting effect of D-penicillamine against hydrogen peroxide and phenylhydrazine induced haemolysis and lipid peroxidation is discussed. It might represent a possible way of action of the drug in some neonatal disorders like hyperbilirubinaemia and retrolental fibroplasia.

In previous studies we have reported that D-penicillamine (DPA) had a significant therapeutic effect in infants with neonatal jaundice [2]. The overproduction of bilirubin is the consequence of haemolysis of different origin in the neonatal period. Lipid peroxidation has been suggested to be a mechanism of membrane damage in a number of red cell disorders leading to haemolysis. Since the susceptibility of red cell lipids to autoxidation is about three times higher in the newborn than in the adult [7], it was of interest to investigate the influence of DPA on lipid peroxidation of red cell membranes.

Goldstein et al. [1] developed a spectrofluorescent assay suitable for demonstrating red cell lipid peroxidation in vivo. In the present work we studied the inhibitory effect of DPA on the fluorescence of lipid containing extracts of red cells peroxidized in vitro by hydrogen peroxide, and in vivo by phenylhydrazine.

Materials and Methods

Chemicals were purchased from Reanal (Budapest, Hungary). All reagents were of analytical reagent grade. DPA (Metalcaptase®) was a gift from Knoll AG (Ludwigshaven, FRG).

Sensitivity of red blood cells to hydrogen peroxide was assessed according to the method of Mengel et al [4].

Lipid peroxidation of red blood cell membranes caused by H_2 in vitro was determined by the fluorescence assay described by Goldstein et al [1]. The same method served for the determination of phenylhydrazine-induced lipid peroxidation of red cell membranes in vivo.

Spectrofluorimetry was performed with a Hitachi—Perkin Elmer MPF 4 spectrofluorometer in the direct mode at 25°C with excitation and emission slits at 5 nm and sensitivity set at 30, an excitation maximum of 360 nm and an emission maximum of 440 nm. For lipid extraction a scaled-down version of the Rose and Oklander chloroform : isopropanol procedure was performed [6].

In vitro experiments

Blood was taken in heparinized syringes from healthy infants. Erythrocytes were washed three times in physiologic saline and made up in 5.0% solution. H_2O_2

haemolysis test was carried out under different conditions as follows.

1. Red blood cell suspension without preincubation with DPA;

2. Red blood cell suspension preincubated with DPA for 10 min;

3. Red blood cell suspension preincubated with DPA for 60 min. The final concentration of DPA was 0.1 mmol/l.

Triplicate samples of 0.5 ml cell suspension were mixed with equal volumes of 5% H_2O_2 in buffer at pH 7.4, incubated at 37°C for 15 minutes and than at room temperature for 2 hours and 45 minutes. Buffer blanks were carried throughout. 100% haemolysis was caused by distilled water. After centrifugation the supernatant was read at 540 nm after conversion of haemoglobin to cyanmethaemoglobin. Per cent haemolysis was calculated as:

$$\frac{E_{samlpe} - E_{blank}}{E_{100\%\ haemolysis} - E_{blank}} \times 100$$

Results of triplicate determinations were averaged.

Animal studies

Female CFY rats weighing 100—140 g were used. The animals were divided into three groups, each of which contained four to five animals.

On the first 3 days the animals in Group I were treated with 200 mg/kg DPA intraperitoneally (i.p.) daily while the animals in Groups II and III were injected with physiologic saline of the same volume.

On the following 6 days 200 mg/kgbw of DPA was administered daily to Groups I and II. The animals in Group III received physiologic saline i.p. instead of DPA. 30 minutes after each injection 20 mg/kg phenylhydrazine was given i.p.

Blood was collected by heart puncture, in heparinized syringes while the animals were under ether anaesthesia. Fluorescence assay was carried out before the start (Group 0) and at the termination (Groups I—III) of drug administration. The assay was routinely done in triplicate.

Results and Discussion

Table I shows the effect of DPA on haemolysis and peroxidation induced by H_2O_2 in a red blood cell suspension. Preincubation with DPA resulted in a significant decrease of both the haemolysis and the fluorescence of chloroform: isopropanol red cell lipid extracts induced by H_2O_2. The fluorescence activity and per cent haemolysis were significantly lower in the erythrocyte suspensions preincubated with DPA. The inhibitory effect depended on the incubation time.

Figure 1 shows the influence of DPA on fluorescence of red cell lipid extracts in rats injected with phenylhydrazine. In Groups II and III the fluorescence activities were higher at the termination of drug administration, than before the treatment. In Group I DPA pretreatment for three days resulted in a decrease of fluorescence, i.e. DPA prevented the phenylhydrazine induced lipid peroxidation. The inhibitory effect was significant statistically.

The mechanism of action of DPA in neonatal hyperbilirubinaemia is complex. DPA exerts an influence on heme metabolism, regulating the activity of heme-oxygenase which is the initial and rate-limiting enzyme in heme degradation. DPA treatment diminishes heme oxygenase activity in the liver of newborn animals, leading to a decrease of bilirubin production [5].

The present study showed that DPA prevents the H_2O_2 and phenyl-

TABLE I

Haemolysis and lipid peroxidation in erythrocyte membrane induced by hydrogen peroxide

Preincubation with D-penicillamine	% erythrocytes haemolyzed	Fluorescence units
None	39.98±3.53	89.00±6.68
10 min	16.17±1.35[a]	56.17±9.11[a]
60 min	11.95±1.32[a]	36.33±7.23[a]

[a] $p < 0.01$ (Student's t-test)

hydrazine induced lipid peroxidation of the erythrocyte membrane. This results in a decrease of haemolysis. It might be another, a direct mode of action of DPA moderating the hyperbilirubinaemia in newborns. Neonatal disorders related to oxygen toxicity, such as haemolytic diseases, retrolental fibroplasia, bronchopulmonary dysplasia, hyaline membrane diseases, have a multicausal aetiology. Their common feature, however, is a free radical induced lipid peroxidation leading to tissue damage. Thus, the decrease of membrane lipid peroxidation under DPA treatment may give an explanation for the beneficial effect of the drug in the prevention of retrolental fibroplasia in very low birthweight infants [3].

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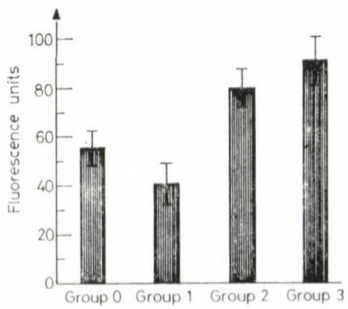

FIG. 1. Phenylhydrazine induced lipid peroxidation in erythrocyte membrane of D-penicillamine treated and control rats. (Details see in text)

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Controlled trial of D-penicillamine to prevent retinopathy of prematurity

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204 infants with birthweights between 751 and 2000 g and 26—35 weeks gestational age (100 treated and 104 control subjects) were enrolled in a prospective controlled trial of the effectiveness of D-penicillamine (DPA) in the prevention of retinopathy of prematurity (ROP). The two groups did not differ significantly in gestational age, birth weight, Apgar scores, the time of exposure to oxygen and in the incidence of PDA or in the number of exchange transfusions and *RBTs*.

Of the treated infants 29, and of the control infants 34 died before the tenth week of life. These cases were not included in further analysis. Patients were subsequently examined and assessed by two ophthalmologists independently, who did not know which babies were receiving DPA. Six of the 70 surviving control infants and none of the 71 surviving treated infants had ROP stage II or graver.

The results suggested that ROP may effectively be prevented with DPA in very low-birth-weight-infants, and that the drug has no serious adverse effects during the neonatal period.

Although retinopathy of prematurity (ROP) or, according to the traditional term, retrolental fibroplasia (RLF) had been described more than 40 years ago [53], it remains an unsolved problem [1, 20, 21, 30, 38, 39, 40, 47, 48]. Cases of ROP still occur today despite improvements in monitoring oxygen therapy [3, 12, 49, 52, 55] and using high preventive doses of vitamin E [14, 18, 19, 22, 29] in the intensive care nursery. The increased survival rate among extremely low-birth-weight infants, combined with additional factors other than excess oxygen, may be responsible for the "second epidemic" of the disease [4, 10, 15, 13, 26, 31, 46].

In our department we have used D-penicillamine (DPA) for the treatment of neonatal hyperbilirubinaemia since 1973 [27, 28]. We have recently reported that DPA therapy in the neonatal period is associated with a marked decrease in the incidence of severe RLF among very-low-birth weight infants [24, 25, 26].

The history of DPA in preventing RLF can be divided into three periods in our department (see Table I). *In the first period* we administered DPA only against hyperbilirubinaemia as indicated in Table I. The original aim of our retrospective screening programme, carried out in the spring of 1979, was to estimate the incidence of RLF during the period 1974—1978. It was surprising that among babies treated with DPA to prevent hyperbilirubinaemia there

TABLE I

The history of D-penicillamine treatment of < 1500 g birthweight neonates in Debrecen, Hungary

	First period (1974—78)	Second period (1979—80)	Third period (1981—82)
Dosage and application	300 mg/kg IV for 3 days	300 mg/kg IV for 3 days	300 mg/kg IV for 3 days + 50 mg/kg IV for 2 weeks
Number of survivals	193	133	152
DPA-treated	61	133	152
RLF	1	1	1
Untreated	132	—	—
RLF	10	—	—

was only one case of RLF, whereas ten out of the 132 babies without such treatment developed severe cicatricial stages of the disease. We then decided that all infants of less than 1500 g birthweight and requiring supplemental oxygen should receive DPA therapy.

During the second period (1979—1980) of DPA treatment there was one case out of 133 surviving infants who developed RLF. This baby received DPA for three days and oxygen therapy for three weeks. We then changed the dosage and duration of DPA administration.

During the third period (1981—1982) a new mode of DPA application was introduced in January. It was, however, still not able to totally eradicate the *occurrence* of RLF, as shown by the data in Table I.

We believe that the above clinical observations provided support for conducting a strictly controlled prospective trial [41] to investigate the prevention of the cicatricial form of the disease (RLF) and the reduction of its acute stage (ROP).

PATIENTS AND METHODS

Subjects

The Newborn Intensive Care Unit (NICU) of the University Medical School, Debrecen, Hungary, is a centre for critically ill newborns. All our patients are born elsewhere but are transferred by ambulance to our Department in the first 24 hours of life. The present study included infants who had been born between January 1, 1983, and March 6, 1984, and had a birthweight between 751 and 2000 g. Gestational age was determined by maternal data and Dubowitz assessment [11].

The infants were nursed in incubators. All prematures had received oxygen therapy for hyaline membrane disease, congenital pneumonia, recurrent apnoea, wet lung, congestive heart failure, aspiration syndrome, or CNS lesion. The infants received uniform care after admission to the NICU. Oxygen therapy was administered by hood, nasal prongs, endotracheal tube, or CPAP ventilation at concentrations sufficient to maintain arterial oxygen tension between 50 and 70 mm Hg. An umbili-

cal-artery catheter was inserted in all infants requiring FiO_2 greater than 0.6, or mechanical ventilation, so that arterial blood gases could be determined. When possible, PaO_2 was continuously monitored by means of transcutaneous (TC)PO_2 electrodes (Radiometer, Copenhagen, Denmark), for intermittent periods. The haematocrit of acutely ill babies was kept under 55% by partial exchange transfusion with fresh frozen plasma. Double volume exchange tranfusion was performed when indicated for either hyperbilirubinaemia or sepsis. The indications of replacement blood transfusion (RBT) were severe anaemia and sepsis, or other conditions requiring prolonged oxygen therapy.

Study design

204 preterm babies of 26—35 weeks gestational age were enrolled in the study. Informed consent was obtained from the parents and the trial was approved by the ethical committee of the University. Reasons for non-enrolment were a significant congenital abnormality in 14 babies, or death before 6 hours of life in 6 babies.

Infants in the study were randomly allocated to control and treatment groups by means of sealed envelopes according to birthweight (751—1000 g, 1001—1250 g, 1251—1500 g, 1501—1750 g, 1751—2000 g).

Infants in the treated group were given daily 3 doses of 100 mg/kg body weight DPA (Metalcaptase^R — Knoll AG, Ludwigshafen, FRG) intravenously (IV) for 3 days. The first dose was administered within 12 hours of birth. Babies below 1500 g continued to receive DPA once daily in a dose of 50 mg/kg body weight IV till the end of the second week of life. Infants above 1500 g were treated with DPA beyond 3 days only in cases where prolonged oxygen therapy was necessary. — We were unable to measure the serum level of DPA in the treated group. No placebo was given to control subjects. Haematocrit, haemoglobin, WBC, platelet count, electrolytes, blood gases, bilirubin, phototherapy and urine analysis were recorded regularly until discharge.

Ophthalmological examination

Examinations were conducted weekly at a set time to ensure regularity of visits to the premature nursery. The infants were first seen by an ophthalmologist (I.H.) at 6 weeks of age. He did not know which babies had received DPA. The pupils were maximally dilated with a mixture of phenylephrine and cyclopentolate. Ocular examination was performed with an indirect (Keeler) ophthalmoscope without scleral depression. Any infant who showed evidence of ROP had regular ophthalmological examination until discharge. Abnormalities were noted and fundus drawings were made. All infants were reassessed before discharge from the NICU. The findings in the acute phase of retinopathy were recorded on basis of the new international classification of ROP [2, 36]. Grading of the cicatricial phase was according to the classification of Reese et al. [44]. The babies were subsequently examined and assessed by an independent ophthalmologist (É.K.) unaware of the previous therapy. The last ocular examination was performed at the age of 6 months.

Statistical analysis

Clinical details were recorded by computer. Comparison between DPA and control groups with respect to baseline characteristics and outcome was carried out using Student's test, Chi square test, Mann—Whitney U and Fischer's exact tests. For all analyses, $p = 0.05$ was taken as the criterion of statistical significance.

Results

Of the infants, 63 died before 10 weeks of age and were not evaluated for the presence of ROP. 141 babies completed the trial; 71 in the DPA group and 70 in the control group.

Infants enrolled in the study are shown in Table II, according to their

TABLE II

Infants enrolled in the study

Birthweight, g	DPA		Control	
	All/survived (per cent)			
751—1000	12/4	(33.3)	10/1	(10.1)
1001—1250	19/11	(57.9)	21/13	(61.9)
1251—1500	35/28	(80.0)	37/26	(70.3)
1501—1750	19/15	(78.9)	23/19	(82.6)
1751—2000	15/13	(86.7)	13/11	(84.6)
Total	100/71	(71.0)	104/70	(67.3)

TABLE III

Baseline characteristics of the study population

	DPA	Control
No of infants	71	70
<1500 g birthweight	43	40
Gestational age, weeks, mean (SD)		
Overall	31.8(2.0)	31.5(2.3)
<1500 g birthweight	30.2(2.0)	30.7(2.4)
Birthweight, g, mean (SD)		
Overall	1488(281)	1474(246)
1500 g birthweight	1299(165)	1310(154)
Male/female	34/37	30/40
Apgar score 1 min. Median (range)	5(1—9)	6(1—9)
Apgar score 5 min. Median (range)	7(3—9)	7(4—9)
Transported from distance of		
<10 km	39	38
10—120 km	32	32
No of infants		
Singletons	59	60
Twins	10	9
Triplets	2	1
Birthweight <10th centile for gestational age	11	13
Delivered by Caesarean section	15	12
Received antenatal dexamethasone	35	38
Received phototherapy	62	68
Received exchange transfusion	15	18
Received RBT	53	50
With severe acidosis (pH <7.2)	31	30
With severe hypercarbia ($paCO_2$ >50 mm Hg)	35	36
With episodes of hyperoxia (paO_2 >100 mm Hg)	21	19
With episodes of hypoxia (paO_2 <40 mm Hg)	33	35
With hyperviscosity	19	18
With sepsis	7	9
With PDA (clinical)	7	7
With haemorrhagic tendency	16	12
Median (range) duration of oxygen treatment (h)	128(27—898)	125(21—860)

birthweight. Table III demonstrates the baseline characteristics of the study population. There were no significant differences between the two study groups in mean birthweight, gestational age, Apgar scores, or with respect to the most important perinatal data which are considered risk factors for ROP and RLF. The two groups did not differ significantly in the duration of oxygen exposure and in the incidence of patent ductus arteriosus (PDA) treated with indomethacin, or in the number of RBT. Only one case of bronchopulmonary dysplasia developed in the control infants. The very low incidence of this disorder among our patients was explained by the fact that only few cases survived long-term mechanical ventilation. Most of the infants were treated with nasal or orotracheal CPAP or oxygen delivered via head hood. No statistically significant difference could be found in the treatment delivered to DPA-treated prematures when compared to controll infants. Exposure to very high ambient oxygen tensions (FiO_2: 0.8—1.0) beyond 48 hours of age was uncommon in both groups.

During the 14 months study period, 6 infants were diagnosed as having ROP stage II or graver during their hospital stay. Both eyes were affected equally. All of these prematures belonged to the control group, so with respect to the frequency of the active phase of the disease, the difference between the DPA treated and the control group was significant statistically ($p = 0.013$) using Fischer's exact test. DPA was found to reduce the incidence of ROP significantly not only in the overall study population but in the infants of less than 1500 g birthweight (DPA: 43/0 *vs* control: 40/6; $p = 0.010$). Table IV reveals that ROP occurred exclusively in neonates with birthweights 1001 to 1500 g. Table IV also shows the most severe stage of ROP according to the

TABLE IV

Frequency of retinopathy of prematurity in the study population

Study population	Normal	Stage of the disease*			
		1	2	3	4
DPA group	71				
Control group					
751—1000	1				
1001—1250	8		2	1	2
1251—1500	25			1	
1501—1750	19				
1751—2000	11				

* Stage 1 — Demarcation line
Stage 2 — Ridge
Stage 3 — Ridge with extraretinal fibrovascular proliferation
Stage 4 — Retinal detachment

new international classification. Infants with ROP had a gestational age ranging from 27 to 31 weeks. It has to be noted that the duration of oxygen therapy in the cases with ROP did not differ significantly from that of the overall study population. All but one had double volume exchange transfusion.

Any infant found to have ROP, whatever its stage, was removed from the study and treated with DPA in a dose of 50 mg/kg bodyweight IV daily for three weeks, and also cryopexy, if it was indicated. The outcome of ROP cases was as follows: 2 infants with stage II and III recovered completely, 3 babies with stage II, III and IV, respectively, developed grade I cicatricial phase, with relatively good vision. Only one case of stage IV developed grade II RLF.

No blindness occurred during the study period.

Discussion

We uphold the view stated by Lucey and Dangman [31]: ". . . RLF is an extremely difficult disease to prevent, treat, or investigate. A disease of this complexity with multiple causes will require very large numbers of infants in any controlled study of a therapy." This sample size, however, would be impractical for any single institution. Our aim was to use the smallest possible sample size yet large enough to have a reasonable chance of detecting any clinically important benefit of the investigated treatment. The number of patients to be included in the study was calculated in advance to be 66 in the control and treated groups, respectively. This assumption was based on an expected ROP rate of 8—10% in infants under 2000 g birth weight [5, 9, 42, 50, 55] and a calculated reduction in frequency of ROP to less than, or equivalent to, 1% in the DPA-treated group ($p_1 = 0.08$; $p_2 = 0.01$; $z = 1.96$) [13]. The likelihood of erroneously rejecting the null hypothesis that the prophylactic administration of DPA had no effect on the incidence of ROP in our patients, was less than 5% with 95% confidence. Thus, the Type I (alpha) error was at the $P = 0.05$ level in our study. After completion of the study, the sample size had a power of 73% for detecting a worthwhile treatment difference if the rate of ROP frequency for the treatment and control groups were 0% and 8.6%, respectively. Thus the Type II (beta) error was at the $P = 0.27$ level. This suggested to accept the hypothesis that DPA protects the eyes of infants against ROP.

When ROP was diagnosed in six infants we completed the blind controlled trial of DPA prophylaxis. Following this the six appeared to have been enrolled in an uncontrolled trial of the effect of DPA plus cryotherapy in progressing ROP. We must make it clear, however, that the latter trial is quite separate from the prophylaxis trial and that the latter results are based on a very small number of patients and have not passed a rigorous test.

How does D-penicillamine act against retinopathy of prematurity?

The aetiology of ROP is now accepted as multifactorial [4, 5, 10, 15, 16, 23, 31, 35, 46]. It may be assumed that the development of ROP is triggered by a number of conditions which may disturb the retinal circulation and result in ischaemic retinopathy with the consequence of vasoproliferation and cicatrisation. Of these factors, the immaturity and the oxygen toxicity, which latter is not equivalent to supplemental oxygen therapy [33], are considered to be the most important.

According to our hypothesis [7, 25, 29, 32, 33, 34], DPA appears to have several modes of action against ROP. The drug scavanges oxygen-free radicals [51] produced by hyperoxia, hypoxia [45] or other conditions frequent in very low-birthweight, sick prematures. In addition, this chelating agent facilitates heme synthesis and inhibits heme degradation in newborn animals. As those enzymes that play an important role in antioxidant defense (peroxidases, catalase) are heme proteins, it is reasonable to conclude that in preventing hyperbilirubinaemia and ROP the mechanism of action of DPA may be identical: the protection of biomembranes against lipid peroxidation. — DPA as a collagen antagonist [8, 37, 43] causes an increase in soluble collagen in soft tissues; intramolecular cross-linkages are blocked, and an absence of intramolecular covalent bonds has been shown. The formation of fresh insoluble collagen is nearly quantitatively inhibited. Furthermore, DPA accelerates the turnover of preexisting insoluble collagen by cleaving intermolecular bonds. Of even more concern is the effect of DPA on the solubility of vitreous collagen [6]. In animals treated with DPA it was found that 47% of the vitreous collagen became soluble after treatment with DPA, compared with 6% in the control group. This observation was confirmed by another experimental study which demonstrated that DPA reduced vitreous proliferation after perforating injury [54]. Consequently, the inhibitory effect of DPA on collagen synthesis may be beneficial also in the process characterized by neovascular proliferation and cicatrisation.

Safety and tolerance of D-penicillamine

In the last eleven years we have given high doses of DPA for some days to more than 3000 term and preterm infants, observing neither acute nor long-term adverse effects nor any late complication during several years follow-up. In spite of this, paediatricians seem reluctant to use DPA in newborn babies probably because long-term administration of the drug in rheumatic arthritis was often followed by unpleasant and dangerous side effects [17].

In our recent controlled trial, DPA therapy was well tolerated by all study infants. No single DPA treated premature was withdrawn because of severe side effects. Mortality rates in the two groups were similar (see

Table II). Including patients who died beyond 10 weeks of age, the rates were 31% and 33.7% for the DPA-treated and the control babies, respectively. There were no significant differences when the data were analysed by cause of death and complications of intensive care. No rash, thrombocytopenia, agranulocytosis, albuminuria and myasthenia, frequently encountered in adults, were observed during the study period. There were no significant differences in treated and control groups with respect to initial weight loss and subsequent weight gain, or weight gain and head circumference corresponding to the six-month adjusted age.

In summary, our results suggest that ROP could effectively be prevented with DPA in very low-birth-weight-infants, and that the drug has no serious adverse effects during the neonatal period. It would be desirable to conduct further collaborative studies by several investigators to recruit large numbers of patients in order to therapeutic effectiveness.

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Addendum

After completion of this manuscript a new case of RLF was diagnosed at our Follow-up Clinic. This infant of 28 weeks gestational age had a birthweight of 850 g. She was born after termination of the trial and so was not enrolled in the study. DPA was administered daily until the 7th day when it had to be withdrawn owing to haematuria which developed in association with sepsis and DIC. She received an exchange transfusion and subsequently RBTs 3 times. At the age of 12 weeks routine ophthalmological examination revealed no change in the eyes. She had then a weight of 1600 g and was returned to the county hospital from where she had initially been referred to us. We lost sight of her until her first visit to our Follow-up Clinic at the age of 6 months. The ophthalmologist diagnosed a severe cicatricial phase of ROP in both eyes.

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Heparin prophylaxis of Henoch-Schoenlein nephropathy

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Eighty eight children with extrarenal manifestations of Henoch—Schoenlein purpura received 120—150 IU/kg heparin in infusion for three days at onset and at relapses. Nephropathy developed in one child (1.1%), whereas renal involvement was noticed in 14 out the 67 control patients with Henoch—Schoenlein purpura (20.9%). The difference was highly significant ($p < 0.001$).

Renal involvement has been reported in 20 to 100% of patients with Henoch—Schoenlein (H—S) purpura. Although this wide range reflects a remarkable variation in its definition, the authors agree that renal involvement is the most serious manifestation of the otherwise benign self-limited disease [1, 5, 6, 7, 10, 11, 12, 14]. Since H—S nephropathy may persist and become chronic leading to renal failure, and no effective therapy is known to alter its course, prevention of renal involvement would be highly desirable.

Considering some observations concerning the pathomechanism of H—S purpura, and the promising experience with anticoagulant therapy [2, 3, 4, 8], we have made an attempt to prevent H—S nephritis by means of low-dose heparin administration.

Subjects and Methods

From October 1, 1973, to August 31, 1984, a total of 154 children (77 girls and 77 boys) aged 3 to 13 years with H—S purpura was hospitalized in our department. The number of patients was higher in the second part of the period, because the referral region was considerably enlarged in 1978.

At admission all patients had had characteristic skin lesions, partly joint symptoms, and in 12 cases abdominal pain and/or melaena for 1 to 7 days. Isolated haematuria (erythrocyte excretion > 10/mm^3 of uncentrifuged urine) was found in 4 cases, haematuria and significant proteinuria (over 1 g/m^2/day) was observed in 3 patients, whereas haematuria + proteinuria + hypertension with decreased GFR occurred in one child. No signs of renal involvement could be detected in the remaining 146 patients.

Out of these from May 1, 1978, 88 children (48 girls and 40 boys) received 120 to 150 IU per kg body weight of heparin IV in 300 ml saline infusion through 2 to 4 hours immediately after admission. This dose was repeated on the 2nd and 3rd day under continuous control of clotting time, prothrombin level, and thrombin time, and the same procedure was performed at relapses. In addition, penicillin and vitamins C and P were administered.

A control group was built from the 49 children (27 girls and 22 boys) with H—S purpura admitted between October 1, 1973, and April 30, 1978, i.e. before the

introduction of heparin prophylaxis, including 5 patients with renal involvement. This group was supplemented by 18 cases (3 girls and 15 boys), including 3 children with initial haematuria and proteinuria, from the period after May 1, 1978, who did not receive heparin for some technical reason.

No significant difference in age distribution, history of previous infection, duration of symptoms before admission, symptomatic therapy and diet was seen between control and heparinized patients. In every case the same vitamin C + P preparation was administered.

Each of the children was followed-up for at least 6 months after the first signs of H—S purpura.

Results

In addition to the 8 children admitted with nephropathy, in 6 patients of the control group macroscopic haematuria and significant proteinuria appeared on the 3rd to 10th day of hospitalization. Thus altogether 14 nonheparinized patients developed renal complications, in 10 of whom complete recovery was found after at most 8 weeks. In 4 children, a mixed nephrotic-nephritic picture was seen, including oedema, massive proteinuria, oliguria, azotaemia and hypertension. Three of these patients were well within 4 weeks, but they had microscopic haematuria and intermittent proteinuria for 2 years after resolution of other symptoms. Chronic renal insufficiency developed in one girl. No signs of renal involvement could be traced at regular long-term urine analyses in the other 53 subjects of the control group.

Similarly, no haematuria or proteinuria developed in 87 out of the 88 children receiving heparin prophylaxis.

The girl with nephropathy in this group showed an unusually severe picture of H—S purpura. The petechiae initially seen on the lower extremities and on the buttocks appeared on the 5th day on the trunk, arms and face accompanied by joint lesions, erythema nodosum, colics and gastrointestinal bleeding. Several relapses followed each other, therefore heparin infusion was repeated 13 times during the first 29 days of her disease. In spite of the increased tendency to multifocal haemorrhages, no haematuria was observed at daily examinations during this period. After the 29th day heparin administration was stopped, and from the 34th day on the classical symptoms of nephritis developed. After nearly three years the child still has intermittent episodes of gross haematuria, proteinuria and oedema with a probably rather unfavourable prognosis.

The cumulative results are demonstrated in Table I. As shown by the figures, a highly significant difference in favour of heparin prophylaxis was found in this material.

No correlation between recurrence rate of skin lesions and nephropathy on the one hand, and between extrarenal symptoms and heparin prophylaxis, on the other hand, could be established.

No complications of the heparin treatment were seen in any of the patients.

TABLE I

Frequency of nephropathy in children receiving heparin prophylaxis and in controls with Henoch-Schoenlein purpura

	Total	Nephropathy developed	
		n	per cent
Controls	67	14	20.9
Heparin prophylaxis	88	1	1.1*

* The difference was highly significant (Fischer's exact test, $p < 0.001$)

DISCUSSION

The frequency of renal involvement in H—S purpura was approximately 20% in our control group, which corresponded to the lower limit of the range found in various surveys. Although recurrence of the original symptoms or development of nephropathy have been reported after an interval of several years [13], it seems unlikely that a significant number of renal abnormalities should appear only after the 6 to 12 months period of follow-up of our patients. This was probably the case also in the heparinized group where only a single instance of nephropathy occurred.

We are aware of the limitations of the present study. The control group did not fulfil the criteria of a double-blind study, neither detailed analyses of haemostasis nor diagnostically important examinations of the complement and immune systems [9] were performed. We could only speculate on the effect and mechanism of the low-dose heparin prophylaxis, which obviously did not affect blood coagulation. The possibility that the pattern of the disease or aetiological factors had changed after the initial five year period could not be excluded, either.

We were, however, fascinated by the significant decrease in the number of renal complications in H—S purpura after heparin infusions. A more thorough investigation of a larger material to determine the possible benefits of prophylactic heparin administration seems to be warranted.

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Reproductive failure in a carrier of inv dupl 1(q21.4→q12)

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High resolution analysis of the early metaphase and prometaphase chromosomes of the father of a child with malformations and mental retardation revealed inv dupl 1(q21.4 → q12). Almost the same was the aberration in the propositus but with a deletion of the band 1q11.2 : 46,XX, inv dupl 1(q21.4→q12)del 1q11.2. This suggested that the malformations and mental retardation in the child were probably due to the microchromosome anomaly in the euchromatin, connected with the heterochromatin block in the father.

Malformations and mental retardation were observed in a child with extreme amounts of heterochromatin lqh+. Although these data contradicted the view of the innocuous effect of this chromosome polymorphism upon the phenotype, there still remained the problem what kind of chromosome variants create phenotype alterations [1, 3, 4, 7]. High resolution G-banding analysis of early metaphase and prometaphase chromosomes might widen the possibilities of the C and Q banding methods in approaching this problem. In this respect it was of interest to study G-banding patterns of the heterochromatin region (lqh+) in early metaphase and prometaphase chromosomes in a man with reproductive failure.

REPORT OF A CASE

The proband VCV, 18 months of age, was a liveborn female child from a second pregnancy. The child was born before term with 970 g weight and 37 cm length.

The first pregnancy ended with a stillborn male fetus and the third one with a spontaneous abortion accompanied by grave metrorrhagia.

At the time of delivery the mother was 24 years old and the father 26 years old. They denied blood relationship.

The weight gain of the child was slow. Clinical examination revealed severe mental retardation, spastic congenital quadriparesis and microcephaly with temporal depression, small forehead and low hairline (Fig. 1). The tongue was large, the teeth were regularly arranged. A high arched palate and bilateral syndactily of the middle two toes were found.

Neurologic examination revealed muscle hypertony, especially in the legs with contractures of the adductors and Achilles tendons.

Psychologic examination showed anxiety and loss of social adjustment.

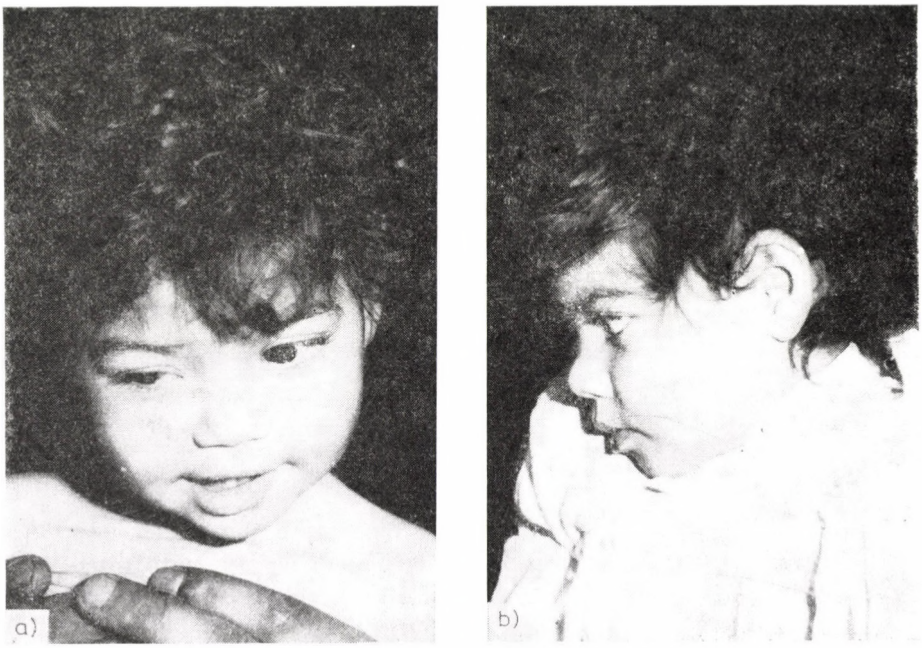

FIG. 1. A and B The proband

Cytogenetic study

Chromosomal study of the parents was performed by means of peripheral blood leukocyte culture. For high-resolution G-banding prometaphase analysis, chromosomes were obtained by a slight modification of the technique of Yunis [10].

The mother's karyotype was 46,XX with normal G-banding patterns. The father's karyotype was 46,XY, lqh+. High resolution G-banding analysis demonstrated an abnormality in one member of the 1st pair of chromosomes: the long arm was 1/3 larger than that of its homologue. The centric heterochromatic region was enlarged and almost doubled, as demonstrated with specific C-banding (Fig 2).

The central part of the chromosome was occupied by a hypochromic band. Thus, the father's karyotype was 46,XY, inv dupl 1(q21.4 → q12) (Fig 3). We suppose that the breakpoint was between lq21.4 and lq21.5. In all stages of chromosomal contraction, clearly demonstrated in the early metaphase and prometaphase, an elongation of band lq11.2 was seen in the derivative chromosome (Fig 4).

Similar was the G-banding pattern of the proband's karyotype, but band lq11.2 was absent in all analysed cells which at 800 G bands differentiation was identified as del lq11.2 (Fig 5). The severe phenotypic anomalies in the propositus probably resulted from an unbalanced rearrangement (karyotype: 46,XX,inv dupl 1(q21.4 → q12)del lq11.2.)

FIG. 2. C-banded 1st chromosome from the father

DISCUSSION

In the father's karyotype, analysis of G-banding of the early metaphase and prometaphase chromosomes revealed inv dupl 1(q21.4→q12). In the proband's karyotype the marker chromosome (lqh+) had lost the band lq11.2, resulting in partial deletion in this region, and affected the phenotype.

Our data correlate with the view that the unusually large heterochromatin variants might affect the phenotype by causing a greater frequency of chromosome rearrangements with genetic imbalance [2, 6, 8, 9, 11].

G-banding prometaphase chromosome analysis in combination with C-banding is a reliable approach to study the problem of chromosomal polymorphism. While the C-banding

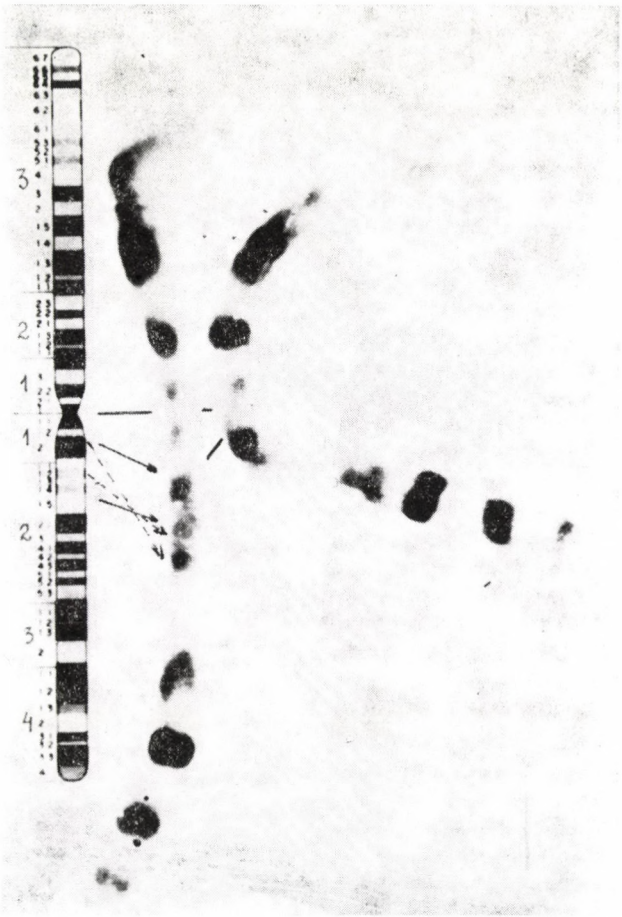

FIG. 3. G-banded 1st chromosome from the father (inv dupl 1/q21.4 q12). Diagram of chromosome illustrating the proposed breakpoints and the resulting inversion [Yunis, 10]

method demonstrates the heterochromatin region as a whole block, the G-banding method of early metaphase and prometaphase chromosomes gives information about the fine structure of these segments.

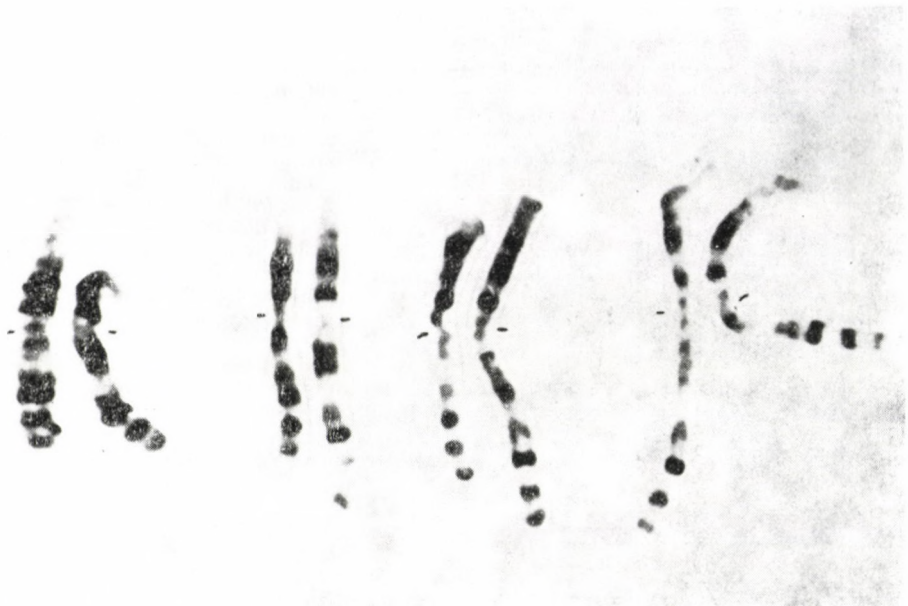

FIG. 4. Early metaphase and prometaphase chromosomes No. 1 of the father after G-banding

FIG. 5. Early metaphase and prometaphase chromosomes No. 1 of the propositus after G-banding

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Dubowitz syndrome

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Four children including two siblings with Dubowitz syndrome are presented. All four were preterm or small-for-dates. On the basis of their symptoms, it is suggested that infantile eczema is not an essential sign of the disorder, whereas the high frequency of hernia, strabism and upward slant of the palpebral fissures is underestimated in the literature.

The congenital disorder characterized by peculiar face, growth deficiency with prenatal onset, infantile eczema and mild microcephaly was initially described by Dubowitz [2]. The syndrome is regarded as a rare autosomal recessive disorder; approximately 26 cases have been reported [12]. Recently we have observed four further children with this syndrome.

CASE REPORTS

The first two patients were siblings. Their parents were healthy; consanguinity, malformations, small stature and skin disease could not be detected in their families.

Case 1. S.C., a girl, was born on the 36th week of her mother's uneventful pregnancy. The placenta was considerably shrivelled. Birthweight was 1640 g, length 43 cm, head circumference 29 cm; each of these parameters were under the 10th percentile of the local growth chart. The ponderal index was 2.06. Because of feeding difficulties the infant failed to thrive. Retardation of psychomotor development was noticed at the age of 10 months, when detailed examinations revealed normal blood chemistry with normal 46,XX karyotype. At that time she had a convulsion without fever.

At the age of 5 years she weighed 11 kg, her height was 94 cm, her bone age 4.5 years. Physical examination showed microcephaly (head circumference, 45 cm), small facies, high sloping forehead, shallow supraorbital ridges, sparsity of hair and eyebrows, hypertelorism, epicanthic folds, broad nasal bridge, hypoplastic alae nasi, low-set ears, small mandible (Fig. 1 A,B). In addition, bifid uvula, bilateral simian crease, 2 whorle (W) 8 ulnar loop (Lu), clinodactyly, wide distance between toes I and II, pes planus, umbilical hernia were noted. Blood and urine chemistry was normal.

The Denver Developmental Screening Test revealed significant delay in each component (gross motor 28

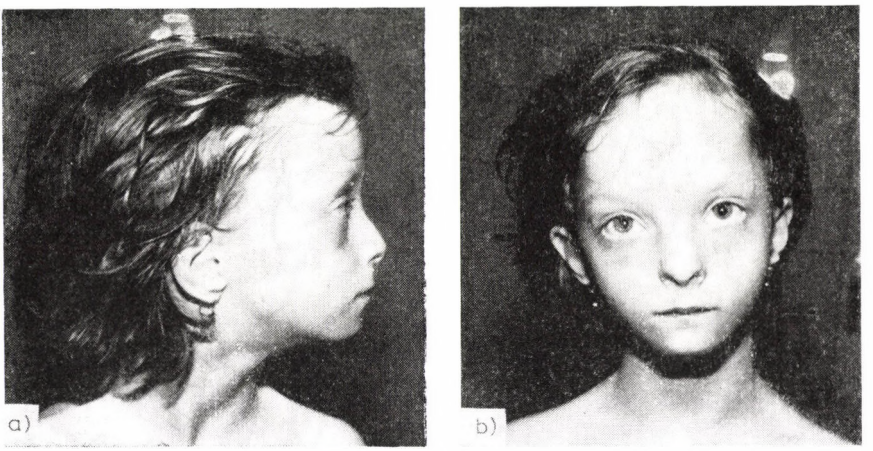


FIG. 1/A—B Case 1 at 7 years of age

months, fine motoradaptive 22 months, language 38 months, personal-social 28 months).

At the age of 7 6/12 years she weighed 13.2 kg, her height was 109 cm, head circumference 46 cm.

Case 2. H.C. was the brother of Case 1. He was born in the 40th gestational week with signs of severe intrauterine growth retardation. The placenta was unusually small, birthweight was 2180 g, length 47 cm, head circumference 32 cm, ponderal index 2.11. His appearance was very similar to that of his sister. In addition he had a cleft palate which caused accessory difficulties in oral feeding and an inguinal hernia.

At the age of 8 months he was examined because of somatic and psychomotor retardation; the most frequent inborn errors of metabolism could be excluded, the karyotype was 46,XY.

At 12 months age his weight was 5.7 kg and he was 64 cm tall. He had microcephaly (head circumference 42 cm), sparse hair and eyebrows, high sloping forehead, flat supraorbital ridges, pseudohypertelorism, epicanthic fold on the left eye, mild ptosis, strabismus, mild facial asymmetry. Dental eruption was delayed. He had a complete simian crease on the right and a Sydney line on the left palm with bilateral clinodactyly, 3 W, 7 Lu. According to the Denver test, his performance corresponded to the 7 month level. At that time he had seizures connected with fever.

At the age of 4 years, the growth retardation was conspicuous. He weighed only 9.2 kg, his height was 86 cm, the head circumference 46 cm. He had a high-pitched hoarse voice. No epiphyseal ossification centres were seen on the wrist roentgenogram. The dysmorphic signs were the same as recorded earlier (Fig. 2 A,B). The Denver test revealed a perfor-

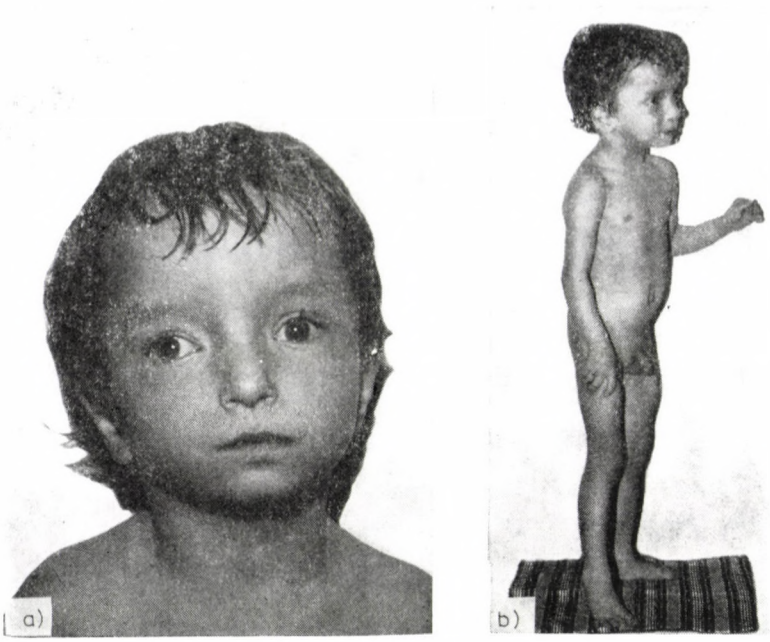


FIG 2/A—B Case 2 at 4 years of age

mance corresponding to 20 to 22 months.

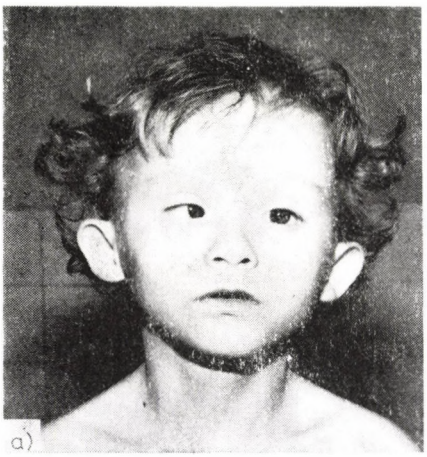
Case 3. Z.L. was a small-for-gestational age newborn boy. The mother was 36 years of age and had already had two healthy twins in her first marriage. As the present husband reported, she had often consumed alcohol during her pregnancy with the proband. No consanguinity, malformation, or short stature were present in the relatives.

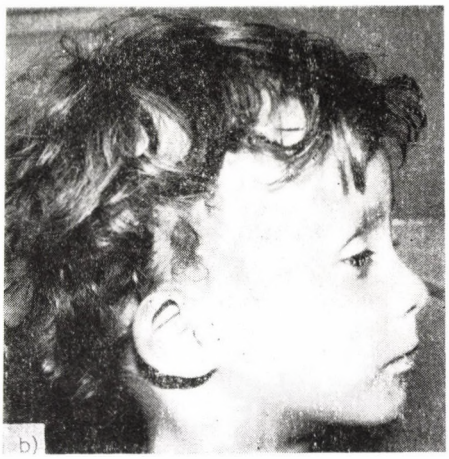
The baby was born in the 36th week of gestation, with 1500 g weight, 39 cm length, and 29 cm head circumference. His cry was high-pitched, oral feeding was extremely difficult.

At 3 years of age he weighed 8.25 kg and was 80 cm tall. At 7 years of age his weight was still only 14 kg, his height was only 111 cm. He had microcephaly (head circumference 47.5 cm), flat occiput, high sloping forehead, sparse hair, flat supraorbital ridges, ptosis with short palpebral fissures, strabism, protruding ears with lack of anthelix and with hypoplastic tragus, pilonidal dimple (Fig. 3 A,B,C). Bone age was 5.5 years. The karyotype was 46,XY; no increase in sister chromatid exchange frequency could be recorded (mean SCE per mitosis = 5.09).

He showed no mental retardation, but had behavioural problems with hyperactivity and short attention span.

Case 4. P.K., a boy, was born after





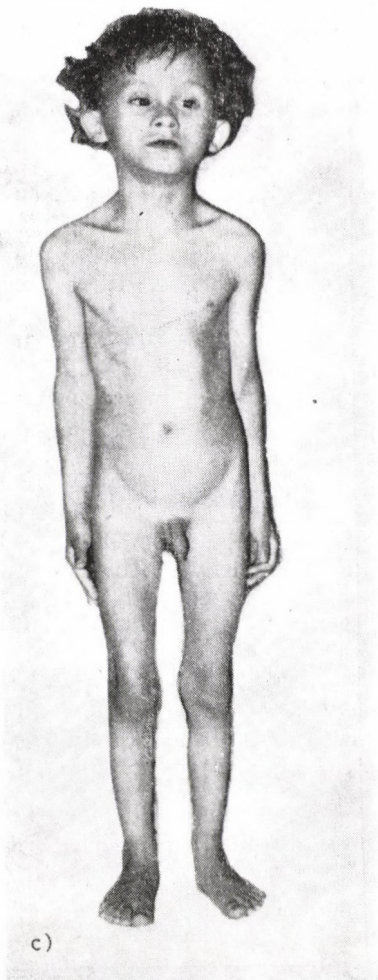


FIG. 3/A—C Case 3 at 7 years of age

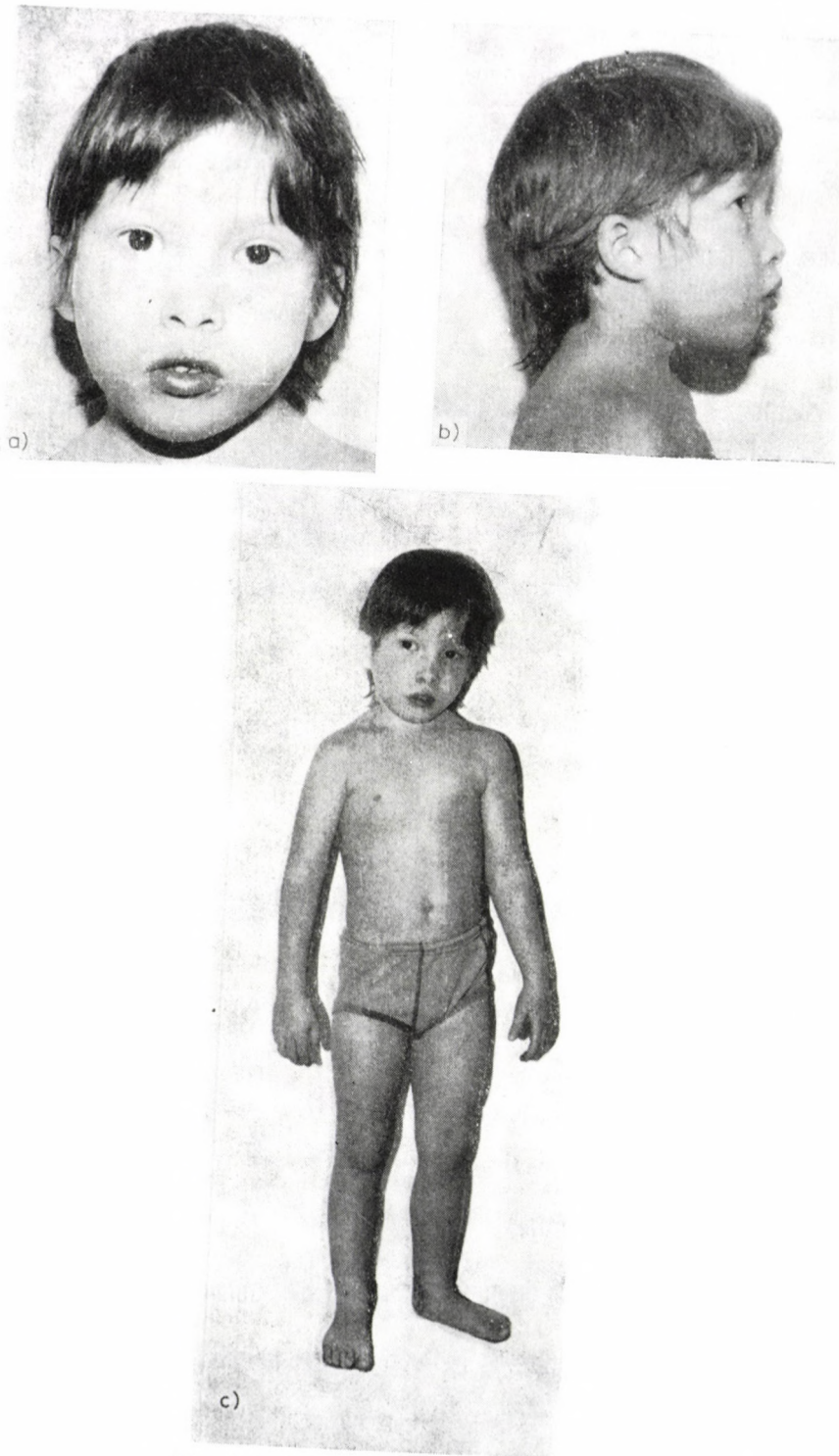


FIG. 4/A—C Case 4 at 5 years of age

TABLE I

Main symptoms of Dubowitz syndrome

Symptoms	Cases in the literature [2, 4, 5, 6, 7, 12]	Present cases			
		S.C.	H.C.	Z.L.	P.M.
		sibs			
Data of delivery					
Placental problems	1/26	+	+	?	?
Preterm birth	5/26	+	−	+	+
Prenatal growth failure	20/26	+	+	+	+
Growth					
Dwarfism	19/26	+	+	+	+
Delayed postnatal weight gain	24/26	+	+	+	+
Microcephaly	25/26	+	+	+	+
Face					
Sloping forehead	21/26	+	+	+	+
Narrow face	9/26	+	+	−	+
Shallow supraorbital ridge	10/26	+	+	+	+
Broad nose	13/26	+	+	+	+
Micrognathia	19/26	+	+	−	−
Eye and periorbital area					
Strabism	4/26	−	+	+	−
Ptosis	16/26	−	−	+	−
Epicanthus	11/26	+	+	−	+
Telecanthus/Hypertelorism	15/26	−	+	−	−
Short palpebral fissures	5/26	−	−	+	+
Slanting palpebral fissures	12/26	+	+	+	+
Incomplete morphogenesis					
Ear	20/26	+	+	+	+
Cleft palate	7/26	+	+	+	−
Gothic palate	7/26	−	−	+	−
Hernia		+	+	−	+
Sacral dimple	5/26	−	−	+	−
Cryptorchidism	5/11		−	−	−
Syndactyly	9/26	−	−	−	−
Clinodactyly	11/26	+	+	−	+
Foot deformity	8/26	+	+	−	−
Retarded bone age	11/26	+	+	+	+
Ectodermal hypoplasia					
Teeth problems (delayed eruption, decay, etc.).	11/26	−	+	+	+
Hair, eyebrow: sparse, thin	18/26	+	+	+	+
Eczema	11/26	−	−	−	−
Dermatoglypha: L_u dominant	11/26	+	+	+	+
Neuro-psychologic signs					
Behavioural problems/Hyperactivity	10/26	+	+	+	+
Mental retardation	9/26	+	+	−	+
Eating and swallowing problems	16/26	+	+	+	+
High pitched cry/Speed defect, delay	12/26	+	+	+	+
Seizures	1/26	+	+	−	−

36 weeks of gestation, with 2420 g birthweight, 44 cm length, 32 cm head circumference, 2.84 ponderal index. He had to be treated repeatedly for loss of appetite and failure to thrive. At 10 months of age he weighed only 7.7 kg, with a length of 66 cm and a head circumference of 45 cm.

At 5 years of age his weight was 15 kg, height 100 cm, and head circumference 49 cm. At this time the following characteristic features were observed: dolichomicrocephaly, sparse hair, hypoplastic lateral part of the eyebrows, upward slant of the somewhat short palpebral fissures, flat supraorbital ridges, epicanthic folds, broad nasal bridge, low-set ears (Fig. 4 A,B). His teeth grew irregularly, exhibited caries and enamel hypoplasia. He had a short neck, mamillary hypotelorism, bilateral inguinal hernia, mild scoliosis, bilateral clinodactyly, simian crease on the right palm (Fig. 4 C). Bone age was 4 years. Serum and urine amino acid chromatography was normal, the karyotype proved to be 46,XY. The Denver test referred to a performance of about 3 years of age. The child had serious behavioural problems including high-grade hyperactivity and speech disorders.

The most important data and symptoms of the patients are summarized in Table I.

Discussion

In accordance with previous observations, intrauterine and postnatal growth retardation were obligate symptoms in our patients. As shown in Fig 5, each of the parameters measured were significantly smaller than expected according to chronological age. The delayed growth of the head circumference seemed to be the most conspicuous sign, while the delay in ossification was less striking.

The small placental weights observed in two cases seemed to be important, but are probably not specific to the Dubowitz syndrome.

As to symptoms, hernia, strabism and upward slant of the palpebral fissures showed a high frequency in our cases in spite of not being mentioned among the specific features of the syndrome [1, 3, 9, 11]. Feeding difficulties were also typical in our patients as well as an abnormal voice. In agreement with Opitz et al [6], we believe that eczema is not an essential sign of the disorder: none of our patients suffered from skin disease. However, the sparsity of hair and eyebrows, and the more or less expressed enamel hypoplasia reflected an ectodermal affection.

In spite of microcephaly the mental retardation of the patients was variable but their behavioural problems were serious in each case. The latter seem to be a remarkable characteristic of the syndrome [8].

From the differential diagnostic point of view, first of all the fetal alcohol syndrome should be considered. Although in our Case 3 the mother had consumed alcoholic drinks during pregnancy, the anomalies of the child differed from those seen in the fetal

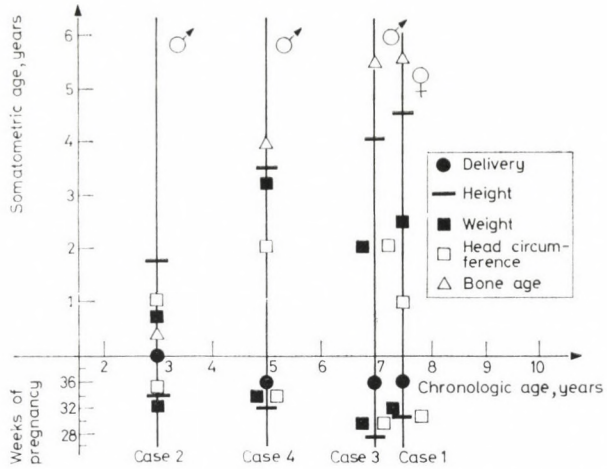


FIG. 5. Anthropometric proportions of our Dubowitz syndrome patients

alcohol syndrome, and just this child had a normal mental development.

All the other syndromes with primordial short stature and mental and behavioural problems could easily be distinguished on the basis of the typical combination of major and minor birth defects.

The present data strengthen the hypothesis of the Dubowitz syndrome being of autosomal recessive inheritance [1, 3, 4, 5, 6, 7, 9, 10, 12]. The parents were apparently healthy and of medium height in each of the families. Cases 1 and 2 were siblings and this too may correspond to an autosomal recessive trait.

Although our findings do not permit an epidemiologic estimation, the fact that 4 cases from 3 families were simultaneously discovered in a town of about 100 000 inhabitants, raises the possibility that the Dubowitz syndrome is not so rare as usually believed [5].

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Book reviews

JK Lloyd, CR Scriver: *Genetic and metabolic disease in Paediatrics.* 324 pages, Butterworths. London 1985. Price: £ 45.00

This book is one of the most fascinating ones that I have come across in the past few years. Sixteen well-known experts, under the excellent editorship of Lloyd and Scriver, summarize in 12 chapters the knowledge on genetic and metabolic diseases which concern the paediatrician.

In the introductory chapter Lloyd and Scriver enlighten the present knowledge and the tasks of the very near future in this field. The last decade brought enormous development in molecular biology, and this achievement must be applied in practice. The most important aim of its use is the prevention of handicaps as well as of mental retardation. This broad area is one of the most urgent problems of paediatricians who are engaged in research, therapy and prevention of genetic diseases.

The 2nd and 3rd chapters deal with basic principles, namely with the genes and the human gene map. In the subsequent ones we can read about the molecular basis of imperfect osteogenesis (which itself is one of the most interesting and exciting sections of the book) as well as about lacticacidaemia, hyperphenylalaninaemia, hyperlipidaemias, etc.

In the following chapters entirely new concepts are presented about the effect of mutation on maternal-fetal metabolic homeostasis. From this point of view completely modern aspects are discussed which broaden the spectrum of placental function and the metabolic consequences influencing fetal development. In the last chapter the biological and biochemical background of the androgen response system is discussed.

Despite the contribution of different authors, all chapters are constructed on the basis of the same principle. The most recent molecular biological methods including genetic engineering are reviewed, followed by the description of conventional diagnostic procedures. Finally, practical conclusions are drawn which help the reader in the therapeutic approach, prevention and genetic counselling.

It is not easy to read this book. Certain basic knowledge should be presumed, and the reader must pay full attention to the text. But making progress, we enjoy it from page to page, and it will be a great pleasure to turn over again the leaves of the book, helping to solve our problems in practice.

Excellent figures and drawings and well arranged clear tables ensure the better understanding. The references are up-to-date and carefully selected. The book should be heartily recommended to all paediatricians and geneticists, especially of the older generation, since the latter can learn first of all "what do we know and what we can do" in the future.

P Kiss

Neonatal and Pediatric Respiratory Medicine. Edited by AD MILNER, RJ MARTIN. 242 pages, Butterworths, London 1985. Price £ 40.00

The editors' endeavour was to give a review on issues of neonatal and paediatric pulmonology exhibiting marked changes or characterized by conflicting views. The following chapters are comprised in the book.

Resuscitation of the neonate (A. D. Milner and H. Vyas). Here the details of asphyxia and its pathophysiological background are offered. In indicating resuscitation, the condition of vital functions (respiration, circulation) and their timely changes are observed rather than the score systems.

Regulation of respiratory muscle activity in infants and children (W. A. Carlo and R. J. Martin). A detailed analysis of function and the role of the individual respiatory muscle groups is given. The clinical aspects of the issue are also touched upon.

High frequency ventilation (I. D. Frantz III). A clear interpretation of terms can be read in this chapter. There are three main types of this treatment: high frequency positive pressure ventilation (HFPPV), high frequency jet ventilation (HFJF), and high frequency oscillation (HFO). The author reviews the characteristics of these methods, the frequencies used and all other parameters involved, and sums up the experience gathered by himself and other authors. In the two first forms the ventilatory volume exceeds the dead space and gas exchange is performed essentially by the traditional way, while with HFO the ventilation volume is smaller than the dead space and the mechanism of gas exchange is different. Recently published data on HFJV are promising but a better characterization of the device itself and controlled clinical trials are necessary. There are as yet few clinical publications on HFO but these are very promising.

Bronchopulmonary dysplasia (E. Bancalari). This chapter is a very nice review of the clinical features, radiological characteristics, differential diagnostics, pathogenesis, therapy and prevention of the disorder.

Cardiorespiratory monitoring in the diagnosis of the sudden death syndrome (J. Hodgman). In spite of great advances in our knowledge of the maturation process of cardiorespiratory functions of young infants, the aetiology of cot death has not yet been clarified; further studies are needed.

Diagnosis and treatment of upper airways obstruction (A. Olinsky and P. D. Phelan). The clinical aspects and details of therapy are fully described in this chapter.

The lungs in immunological diseases (D. M. Roberton). This is a good review on the normal defense mechanisms of the lung and on the non-specific abnormalities of pulmonary defense. Primary and secondary consequences of immunodeficiency are also included as well as conditions characterized by increased proneness to infection.

Bronchial hyperreactivity in children: a clinical review (M. Silverman and N. Wilson). The term of bronchial hyperreactivity refers to the fact that in the asthmathic patient respiratory stimuli provoke a restriction of the bronchial lumen. A good list of indications for measuring bronchial reactivity and a nice review of methods and clinical aspects are offered.

Allergy and infection in cystic fibrosis (R. W. Wilmott). The relative role of allergy and infection in the course of cystic fibrosis is dealt with in this chapter.

Outcome of febrile respiratory diseases in childhood (H. Simpson and J. Y. Q. Mok). Here the authors outline possible links between respiratory diseases of childhood and debilitating respiratory disorders of the adult.

The whole book is an excellent source of up-to-date knowledge and is warmly recommended to all paediatricians interested in the problems of respiration.

J KISZEL

D. WEITZEL, E. DINKEL, M. DITTRICH, H. PETERS: *Pädiatrische Ultraschalldiagnostik*. XV + 306 Seiten mit 310 Abbildungen. Springer-Verlag, Berlin—Heidelberg—New York—Tokyo 1984. Preis DM 138.—

Die gefahrlose, noninvasive Ultrashalluntersuchung hat sich schnell zu einer vielseitig anwendbaren diagnostischen Methode entwickelt, worauf schon die große Zahl der einschlägigen Publikationen und Monographien hinweist. Das vorliegende Buch ist eine ergänzte, überarbeitete und summierende Version der früheren Arbeiten (Morphologische Abdominaldiagnostik im Kindesalter 1982 und Diagnostik intrakranieller Blutungen beim Neugeborenen 1983) dieses über die größten Erfahrungen verfügenden Autorenteams. Es ist eine umfassende Darstellung der pädiatrischen Sonographie in all ihren Anwendungsbereichen und basiert auf etwa 20 000 Untersuchungen, die in einem Zeitraum von 10 Jahren vorwiegend von der Mainzer Ultraschallgruppe durchgeführt wurden.

Das einleitende Kapitel bietet eine kurze Übersicht über Physik, Biologie und Technik des Ultraschalls. In den weiteren 18 Kapiteln wird dann die Anwendung der pädiatrischen Ultraschalluntersuchungen ausführlich behandelt. Ihrer Bedeutung entsprechend werden vor allem die intrakraniellen Untersuchungen besprochen. (Es sei bemerkt, daß der Diagnostik des Hydrocephalus vielleicht zu wenig Raum gewidmet wurde.) Weitere Kapitel erörtern die Untersuchung von Schilddrüse und Thorax. Ein Kapitel über die Sonographie des Herzens ist der Beitrag von Kupferschmid und Lang von der Ulmer Kinderkardiologie. Die Autoren befassen sich mit den sector-scanner und real-time Methoden und auch mit der modernsten Echographie. (Vielleicht hätte in dieser zusammenfassenden Monographie auch die früher gebräuchliche TM-Mode eingehender erwähnt werden müssen.) Bei der Besprechung der Leberdiagnostik werden die morphometrischen Messungen, die zu den einzelnen Krankheitsbilder gehörenden Ultraschallbilder und die Differentialdiagnose von Hepatomegalie tabellarisch dargestellt. Die folgenden Kapitel befassen sich mit der Untersuchung von Gallenblase und Gallenwege, Milz, Pankreas, Magen, Darmtrakt, Nebenniere, Genitale. Eines der umfangreichsten Kapitel ist jenes des Harntraktes und der Niere. Die Diagnostik des stumpfen Bauchtraumas und Tumors ist ein besonders nützlicher Teil des Buches. Der Beitrag von R. Graf über die Morphometrie der Hüftgelenke legt neue Untersuchungsmöglichkeiten vor. In den letzten zwei Kapiteln sind diagnostische Protokolle und die Normalwerte für die einzelnen Organe zu finden. Die sich mit Sonographie befassenden Pädiater und Radiologen werden diese mit besonderem Nutzen verwerten können.

Die Mehrzahl der Bilder ist sehr gut, da aber die früheren Untersuchungen noch mit compound-scan und real-time Geräten erster Generation vorgenommen wurden, die späteren mit digitalen real-time Sektorscannern erfolgten, ist das Bildmaterial ziemlich inhomogen; dies wird jedoch mit Hilfe von ausgezeichneten anatomischen Skizzen überbrückt.

Die Monographie soll als Standardwerk Pädiatern, Internisten und Radiologen empfohlen werden.

G HARMAT

D. MÜCKE: *Herzrhythmusstörungen im Kindes- und Jugendalter*. 208 Seiten mit 55 Abbildungen und 11 Tabellen. Georg Thieme Verlag, Leipzig 1985. Preis M 44,—

In den vergangenen Jahren ist eine ganze Reihe von Monographien über Rhythmusstörungen im Kindesalter erschienen, was mit der Verbreitung der elektrophysiologischen Untersuchungen und deren Adaptation auf das Säuglings- und Kleinkindalter zu erklären ist.

Die vorliegende Monographie der Reihe »Moderne Pädiatrie« besteht aus 8 Kapiteln

und einem Anhang, in dem in einer umfangreichen (24 Seiten), doch übersichtlichen Tabelle der Wirkungsmechanismus, die Indikationen, Dosierung, Kombinationsmöglichkeiten und Nebenwirkungen der verschiedenen antiarrhythmischen Medikamente angegeben sind.

Im ersten Kapitel wird aufgrund von Literaturangaben die Häufigkeit und die klinische Bedeutung und Beurteilung der kindlichen Rhythmusstörungen erörtert. Das zweite Kapitel befaßt sich mit der Physiologie der Reizbildung und Reizleitung und mit der Ätiologie der Reizbildung- und Reizleitungstörungen. In dem dritten Kapitel finden wir unter dem Titel »Systematik und Terminologie« eine Aufzählung der in den 6., 7. und 8. Kapiteln behandelten Störungen. Mit der angeführten Terminologie kann man nicht immer einverstanden sein. So ist z. B. die Einteilung der supraventrikulären heterotopen Störungen der Reizbildung (Vorhofrhythmus, Koronarsinusrhythmus, Atroventrikularrhythmus, Hisbündelrhythmus) nicht zeitgemäß; aufgrund der modernen elektrophysiologischen Untersuchungen werden diese nach ihrer Lokalisation als a) Vorhof- und b) a-v junktionale Reizbildung klassifiziert. Bei a) kann dann genauer zwischen einer Lokalisation im unteren rechten Vorhof (low right atrial), unterem linken Vorhof (low left atrial) und oberen linken Vorhof (high left atrial) unterschieden werden; bei dem a-v junktionalen Rhythmus gebrauchen wir auch heute noch den Ausdruck des oberen, mittleren und unteren a-v junktionalen Rhythmus aufgrund der Lokalisation der P-Welle im Verhältnis zur QRS, wobei es sich nicht um die Verschiedenheit des Reizbildungsortes handelt, sondern um die Differenz der anterograden bzw. retrograden Leitungsgeschwindigkeit. Der laut früherer Terminologie »Sinuskoronarrhythmus« auf dem Oberflächen-EKG entspricht dem EKG-Bild des oberen a-v junktionalen Rhythmus, doch ist die PR Dauer beim ersteren 0.12 sec, beim letzteren kürzer.

Die Klassifikation der a-v Dissoziation in eine einfache- und Interferenz-Dissoziation scheint überflüssig zu sein. Man spricht heute einheitlich über einen a-v junktionalen Rhythmus mit a-v Dissoziation. Leider vermißt man manche wesentliche Rhythmusstörungen: die supraventrikulären Tachykardien (SVT) bestehen nämlich nicht nur aus paroxysmaler SVT, Vorhoffibrillation und -flattern, sondern in ihrem Pathomechanismus, ihre klinischen Erscheinung, Therapie und Prognose grundsätzlich abweichende automatisch-ektopische SVT (Syn: chronisch ektopische, repetitive SVT) und SVT mit a-v Block, -Formen, die nur in ein paar Sätzen erwähnt werden. Es fehlt ferner die Besprechung der mit breiter QRS einhergehenden Formen der SVT und deren Abgrenzung von der Kammertachykardie, welcher Umstand bei der Auswahl der Therapie ausschlaggebend ist.

Kapitel 4 über Diagnostik behandelt eine Reihe von kardiologischen Untersuchungsverfahren, die bei der Diagnose von Rhythmusstörungen kaum eine Bedeutung haben, während die Schilderung der wichtigsten elektrophysiologischen Untersuchungen sich auf zwei Seiten beschränkt.

Daß das 5. Kapitel über Therapie dem 6. Kapitel über klinische Aspekte, Bewertung, Diagnostik, Therapie und Prognose vorangeht, dürfte nicht ganz günstig sein und daß die therapeutischen Vorschläge in diesen beiden Kapiteln nicht immer im Einklang stehen, wirkt etwas störend. In dem therapeutischen Teil vermißt man ferner die neueren elektrotherapeutischen Verfahren (Overdrive, Vorhofreizung, Transkatheter-Hisbündelablation, cryothermische Ablation). Auf die chirurgischen Behandlungsmöglichkeiten wird nur kurz hingewiesen.

Ein spezielles Kapitel ist den Präexzitations-Syndromen, ein anderes den QT-Syndromen gewidmet; es handelt sich hier um mittels Oberflächen-EKG erkennbare Zustände, die die Gefahr einer Entstehung lebensbedrohlicher Rhythmusstörungen in sich tragen.

Der Wert der nützlichen Zusammenstellung der Pharmaka im Anhang soll aber hervorgehoben werden.

J KAMARÁS

J. HIRSCHBERG, T. SZENDE: *Pathologische Schreistimme, Stridor und Hustenton im Säuglingsalter.* 136 Seiten mit 116 Abbildungen und 2 Schallplatten. Gustav Fischer Verlag, Stuttgart—New York 1985. Preis DM 78,—

In dem vorliegenden Buch werden die Charakteristika der pathologischen Stimmerscheinungen — Schrei, Atmung, Husten —, deren Entstehungsort — Nase, Mundhöhle, Rachen, Kehlkopf, Trachea — und die auslösenden Krankheitsbilder erörtert. Nach der Beschreibung der akustischen Kennzeichen und deren Vorkommen werden jene Krankheitsbilder der Atemwege und des Nervensystems besprochen, die mit pathologischen Stimmphänomenen einhergehen; um nur einige wichtigere hervorzuheben, sollen z. B. Choanalatresie die Pierre Robin, Cornelia de Lange und Hurler Syndrome erwähnt werden, ferner Epiglottitis, Croup laryngis oder Contusio laryngis, tracheale Veränderungen und Erkrankungen der Bronchien und Lunge, usw. Ein Kapitel ist der Spektralanalyse gewidmet.

Auf den dem Buch beigefügten Schallplatten werden mehr als hundert pathologische Stimmerscheinungen angeführt und der entsprechende Text hierzu im abschließenden Teil der Arbeit angegeben.

Das Werk, das als Gemeinschaftsausgabe mit der Akadémiai Kiadó Budapest erschien, darf auf das Interesse von Pädiater und in der Kinderheilkunde tätigen HNO-Ärzte und Phoniater rechnen.

E MIRISZLAY

J. HEINRICH, G. SEIDENSCHNUR: *Praktische Kardiotokographie.* 176 Seiten mit 86 Abbildungen und 31 Tabellen. Johann Ambrosius Barth, Leipzig 1985. Preis M 78,—

In dem vorliegenden unter Mitarbeit von E. Koepcke, H. Hopp und R. Tomczak verfaßten musterhaft ausgestatteten Band werden die modernen theoretischen und praktischen Kenntnisse über die Kardiotokographie zusammengefaßt. Die Autoren haben hier die in anderthalb Jahrzehnten gesammelten Erfahrungen mit dem in der perinatalen Medizin so wichtigen Verfahren, der Kardiotokographie bei der Überwachung von Schwangerschafts- und Geburtsverläufen systematisiert veröffentlicht. Der Aufbau des Buches ist gut, die Abbildungen sind demonstrativ und die Tabellen klar und instruktiv.

Es werden die für den Kliniker wichtigen, den Zustand des Fetus charakterisierenden biophysikalischen, biochemischen und biologischen Parameter, die biophysikalen kardialen diagnostische Verfahren, die Organisation einer zentralen Geburtüberwachung, ferner die klinischen Kennzeichen der Wehentätigkeit, die Nomenklatur und Einteilung der fetalen Herzfrequenzänderungen, die ätiologischen Faktoren der Beschleunigung oder Verlangsamung der fetalen Herzfrequenz, die Ursachen der funktionellen fetomaternellen Zirkulationsstörung, die Plazentainsuffizienz, und deren hämodynamische usw. Ursachen, die bei der Schwangerenbetreuung in Betracht zu nehmenden Risikofaktoren und die praktische klinische Anwendung der Kardiotokographie eingehend besprochen. Einige weitere hervorzuhebende Themen wären die aktuellen technischen Einzelheiten des Verfahrens oder die Indikation der antenatalen und pränatalen Anwendung, die Beurteilung der Befunde und deren therapeutische Folgerungen, Indikationen der fetalen Blutgasanalyse. Probleme, welcher physiologischer Mechanismus sich in den KTG-Zeichen widerspiegelt oder welche Zeichen

pathologisch sind und welche pathophysiologische Vorgänge diese hervorrufen, werden kurz und klar beantwortet. Die physiologische und pharmakologische Regelung der Uteruskontraktion, die uteroplazentare und fetale Zirkulation, die häufigsten ante- oder intranatalen Abweichungen werden auch geschildert, die von verschiedenen Autoren empfohlenen Scores angeführt. Dem Pathomechanismus der Plazentainsuffizienz wurde ein ausführlicher Teil gewidmet und die für bestimmte Schwangerschaftskomplikationen — Infektion, fetale Anämie, intrauterine fetale Retardation und Mißbildungen — charakteristische KTG-Abweichungen behandelt. Abschließend erläutert ein Kapitel die Überwachung der postnatalen Adaptation und die Bedeutung der Kardiorespirographie.

Es muß wiederholt betont werden, daß das ganze Buch, Einband, Papier, Druck und Abbildungen inbegriffen, erstklassig ausgestattet ist. Es kann Gynäkologen, Perinatologen und Neonatologen warm empfohlen werden. B ZSOLNAI

W. HOFFMANN, F. H. HERRMANN: *Neuromuskuläre Erkrankungen.* 156 Seiten mit 54 Abbildungen in 136 Einzeldarstellungen und 14 Tabellen. G. Thieme, Leipzig 1985. Preis 70,— M

Mit neuromuskulären Erkrankungen und Syndromen sind in der Praxis nicht nur der Neurologe, sondern auch andere Fachärzte — Kinderärzte, Internisten, Rheumatologen, Orthopäden, Genetiker und Allgemeinmediziner konfrontiert. Auf die Häufigkeit und Bedeutung dieser Erkrankungen weist schon die heute sozusagen unüberblickbare Menge von diesbezüglichen Literaturdaten hin. Eine ausführliche klinisch-pathologische Einteilung dieser Krankheiten in sechs größere Gruppen wurde 1968 von der World Federation of Neurology ausgearbeitet. Da es sich um eine Vielzahl von Krankheitsbilder handelt, von denen einige als behandelbar und andere heute noch als hoffnungslos betrachtet werden, sollte sich jeder praktizierende Arzt auf diesem Gebiet entsprechend orientieren können. Wir begrüßen deshalb jede Arbeit, die sich mit der Systematisierung, der Zusammenfassung des Kenntnismaterials und mit der kritischen Betrachtung der neuesten Anschauungen des so komplizierten und schwer übersehbaren Gebietes befaßt. Das vorliegende Buch gehört zu diesen Werken. Obwohl die Fragen in ihrer Vollständigkeit wohl nicht behandelt werden konnten, — die Autoren haben sich vorwiegend auf die Besprechung der Dystrophien, Myopathien, spinalen Muskelatrophien und hereditären Neuropathien beschränkt — dürfte das Buch durch die konsequente einheitliche Konzeption, die klare Einteilung, die gute Kennzeichnung der einzelnen Krankheitsbilder und auch durch die reiche Illustration mit entsprechendem Bildmaterial trotz der Kürze aus der Reihe ähnlicher Arbeiten hervorzuheben sein.

Die ersten drei Kapitel befassen sich mit allgemeinen Problemen, dem Wesen und mit der Diagnostik, mit besonderer Hinsicht auf das Kindesalter. In den folgenden Kapiteln werden die verschiedenen Typen der progressiven Muskelatrophie, die einzelnen Formen der myotonischen Muskelstörungen, Myasthenia gravis, endokrin bzw. metabolisch bedingte Myopathien (hier vermißt man die akromegalische Myopathie), die Hauptformen der familiären periodischen Lähmungen und mit Zellanomalien einhergehende Myopathien besprochen. Das Kapitel über die spinalen progressiven Muskelatrophien beruht vor allem auf den im Kindesalter erscheinenden Formen. Fraglich erscheint jedoch, ob die Heraushebung der Charcot—Marie—Tooth Muskelatrophie aus der Nukleargruppe und ihre Einordnung in die hereditäre Gruppe eindeutig gerechtfertigt ist. Ein Kapitel ist der Arthrogryposis multiplex congenita des Säuglingsalters und ein anderes der etwa im Kindesalter beginnenden, doch sich später manifestierenden Myositis ossificans gewidmet.

Das nächste Kapitel erörtert die entzündlichen Myopathien, und abschließend wird über die Sozial- und Rehabilitationsmaßnahmen in der DDR berichtet; bei einem beträchtlichen Teil der Kranken ist ja die Rehabilitation die einzige Möglichkeit.

Der Aufbau der Kapitel ist übersichtlich, was durch die einheitliche Abhandlung der Krankheitsbilder — Definition, Ätiologie und Pathogenese, Klinik, Genetik, ergänzende paraklinische Untersuchungen, Behandlungsmöglichkeiten, humangenetische Beratung — gefördert wird. Die am Ende jedes Kapitels angegebenen Literaturangaben sind gut gewählt und bieten die entsprechende Information über den modernen Wissensstand.

Von den mitwirkenden Autoren — V. Meerbach, W. Hochheim und A. Giese — sind letztere ebenfalls auf pädiatrischem Gebiet tätig, welcher Umstand den Schwerpunkt der Arbeit auf die pädiatrischen Probleme versetzt.

A Szobor

G. Grossmann, H. Dietze, D. Fitzner A. Gerth, W. Schmitz: *Rehabilitationspädagogik Verhaltensgeschädigter*. 112 Seiten mit 16 Abbildungen und 10 Tabellen, Verlag Volk und Gesundheit, Berlin 1984. Preis DM 32,—

In den fünf Kapiteln des Buches werden nach einer kurzen Beschreibung des wissenschaftlichen Gegenstandes und der Aufgaben des Arbeitsbereiches der Rehabilitationspädagogik die Kennzeichnung diagnostischer Untersuchungsbereiche, die Bildung und Erziehung und die Institutionen für verhaltensgeschädigte Kinder behandelt.

Die Ursachen der Verhaltensstörungen können genetisch bedingte Entwicklungsstörungen, prä-, peri- und postnatale Hirnschäden, neurologische oder psychiatrisch Erkrankungen, somatische Krankheiten mit Auswirkungen auf die Funktion des Zentralnervensystems, anomale psychogene Entwicklung, ungünstige Wechselbeziehungen mit der Umwelt sein. Die ungünstigen inter- und intrapersonalen negativen Umstände erfordern dann eine entsprechende pädagogische Kompensation und Korrektur. All diese Gesichtspunkte, ergänzt mit der Erörterung der Charakteristika des auffälligen Verhaltens und Lernvermögens, werden in Kapitel 2. ausführlich besprochen. Das 3. Kapitel ist der mehrdimensionalen Diagnostik gewidmet, wobei die medizinischen und psychologischen Untersuchungen, Erfassung von Verhaltensmerkmalen, von Leistungstempo, Überforderungsreaktionen demonstriert werden und der Verlauf der Zusammenarbeit von Ärzten, Psychologen und Pädagogen geschildert wird. Das 4. Kapitel befaßt sich mit erziehungstheoretischen-neurophysiologischen Aspekten, mit der Konzeption und Methodik des Unterrichtsprozesses bei Verhaltensgeschädigten. Die Wichtigkeit der Wechselbeziehungen von Lehrern und Schüler wird betont, ferner die Möglichkeiten der Familienerziehung und Beratung eingehend erläutert. Kapitel 5 führt dann die Institutionen für die rehabilitative Bildung und Erziehung Verhaltensgeschädigter in der DDR an, wie z. B allgemeinbildende polytechnische Sonderschulen, Sonderschulen mit Ausgleichsklassen, Oberschulen mit Ausgleichsklassen, Sonderklassen in Jugendheimen, Beratungsstellen in Kliniken, usw.

Das Ziel der Autoren war, aufgrund eigener Erfahrungen und literarischen Angaben, zur frühzeitigen Erfassung und dauer haften Kompensation der ungünstigen Persönlichkeitsentwicklung der Verhaltensgeschädigten einen praktischen Wegweiser zu bieten. Den im Schulwesen tätigen Lehrern, Erziehern, Psychologen und Mediziner wird die Arbeit viele nützliche Ratschläge und Impulse vermitteln.

É Szamos

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AETIOLOGICAL STUDIES OF ISOLATED COMMON CONGENITAL ABNORMALITIES IN HUNGARY

by

A. Czeizel and G. Tusnády

In English. 1984. XV + 358 pages, 31 figures, 123 tables. 17×25 cm.
Hardback. $ 29.00/DM 69,—/£ 17.40
ISBN 964 05 3223 9

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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Budapest

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
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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.
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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.

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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.

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THE PATHOPYSIOLOGY OF INFANTILE MALNUTRITION

PROTEIN-ENERGY MALNUTRITION AND FAILURE TO THRIVE

by

Prof. E. KERPEL-FRONIUS M. D.

Emer. Director of the 2nd Clinic of Paediatrics
Semmelweis University Medical School, Budapest
Member of the Hungarian Academy of Sciences

In English · 1983 · 312 pages · 47 figures · 74 tables 17 × 25 cm
Hardcover. $ 28.00/DM 67,—/£ 15.40
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 author 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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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:

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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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Prevalence of minor congenital anomalies in diabetic children

K Méhes[1], G Soltész[2], L Szabó[3], Mária Pap[2], Veronika Meggyessy[1], G Károlyi[1]

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The prevalence of 52 minor congenital anomalies (MCAs) was determined in 111 children with insulin dependent diabetes mellitus (IDDM), and in 111 healthy matched control subjects. The average MCA per person was 1.60 in diabetic children and 0.86 in the controls ($p < 0.001$). The difference was exclusively due to the significantly higher proportion of subjects with 3 or more MCAs in the diabetic group (27.0 versus 9.9%; $p < 0.001$). No specific MCA characteristic of IDDM was found.

Minor congenital anomalies (MCAs) are infrequent structural variations of no medical or cosmetic importance to the affected person. Their diagnostic value rests in their multiple occurrence in the same individual, which points to a prenatal disturbance of morphogenesis in the broadest sense [7, 9]. This is why the study of MCAs has gained increasing interest in teratogenecity research, in mental disorders, and in syndrome identification.

Here we report on a survey of MCAs in diabetic children.

Subjects and Methods

A total of 111 children (50 girls and 61 boys) with insulin dependent diabetes mellitus (IDDM; type I) of the Paediatric Departments of the County Hospitals Győr and Szombathely, and of the University Medical School, Pécs, were examined. Their age varied from 2 to 18 years; duration, course, and therapy were disregarded in this study. An equal number of healthy controls of the same ethnic origin were consecutively matched to the patients by sex and age.

In each subject the presence or absence of 52 MCAs, listed in Table II, was recorded. The diagnostic criteria of Smith [10], Méhes [7], and Holmes (personal communication) were applied. Where possible, objective measurements were performed with tape and caliper [5, 7]. These were made on both sides of paired organs, but when no significant differences were obtained, only the values of the right side were considered at final evaluation. Two standard deviations above or below the mean were regarded as cut-off points for features that were measured. No distinction between unilateral and bilateral occurrence of qualitative features (simian crease, etc.) was made.

Fisher's exact four-field test was used for statistical analysis.

Results

No major malformations or syndromes associated with an unduly high number of MCAs were found in this material, thus all the 111 pairs of children could be included.

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The proportion of subjects with one or more MCAs was 62.2% among the diabetic children and 50.4% among the controls (Table I). This difference was not significant, because the number of subjects with 1 or 2 MCAs was nearly equal in the two groups. At the same time, the occurrence of 3 or more MCAs was significantly more common among the diabetic children (27.0 *vs* 9.9%). This resulted in a high average MCA per subject ratio of 1.60 among the diabetics, in contrast to the 0.86 value of the healthy controls. Although slightly higher frequencies were found in boys, the sex differences were not significant.

The prevalence of individual MCAs is summarized in Table II. The results were first evaluated for boys and girls separately, but since no significant sex difference was found, only the cumulative data are given. As shown by the figures, nearly all MCAs were somewhat more common in diabetic children. However, only epicanthic folds and diastasis recti were significantly more frequent in IDDM. In addition, a moderate but not significant preponderance in diabetic children of clinodactyly and supernumerary nipple may be mentioned.

Discussion

A significant increase in the prevalence of both major malformations and MCAs in infants of diabetic mothers has been firmly established [3, 6]. This is generally attributed to

Table I

Occurrence of MCAs in diabetic children and in matched controls

No. of MCAs		Diabetic children			Controls		
		Girls n = 50	Boys n = 61	Total n = 111	Girls n = 50	Boys n = 61	Total n = 111
0		22	20	42	28	27	55
1		9	18	27	11	19	30
2		5	7	12	6	9	15
3		4	5	9	5	4	9
4		6	5	11	—	1	1
5		3	3	6	—	1	1
6		1	1	2	—	—	—
7		—	2	2	—	—	—
8		—	—	—	—	—	—
Total affected	n	28	41	69	22	34	56
	%	56.0	67.2	62.2	44.0	55.7	50.4
Subjects with 3 or more MCAs	n	14	16	30	5	6	11
	%	28.0	26.2	27.0[a]	10.0	9.8	9.9
Total No of MCAs		76	102	178[a]	38	58	96
MCA per subject		1.52	1.67	1.60[a]	0.76	0.95	0.86

[a] as compared to controls $p < 0.001$

TABLE II

Occurrence of individual MCAs of different pathogenesis in the children examined

	Diabetic children n = 111	Controls n = 111	
Mild malformations			
Preauricular skin tag	0	1	
Preauricular fistula (sinus)	2	1	
Double whorle of the hair	8	10	
Frontal whorle (upswap) of the hair	5	5	
Bifid uvula	1	1	
Alveolo-buccal frenula	2	0	
Cleft lip microform (lip pits)	0	0	
Bifid xiphoid process/short sternum*	7	2	
Supernumerary nipples	7	3	
Umbilical hernia	4	1	
Inguinal hernia	5	3	
Moderate diastasis recti	10	3	$p < 0.05$
Total mild malformations	51	30	$p < 0.005$
Minor deformations			
Prominent forehead	2	1	
Flat occiput	4	1	
Prominent occiput	4	0	
Primitive shape of the ear	5	1	
Earlobe crease	3	1	
Simian crease	6	3	
Sydney line	1	1	
Single flexion crease on finger 5	0	1	
Clinodactyly	7	2	
Sole crease ("vertical")	6	4	
Total minor deformations	38	15	$p < 0.001$
Minor dysplasias			
Haemangioma	2	2	
Large pigmented naevi	3	3	
Café-au-lait spots	11	6	
Total minor dysplasias	16	11	$p > 0.30$
Minor anomalies (phenogenetic variants)			
Small mandible	3	1	
Extra posterior cervical skin	0	0	
Epicanthus (inner epicanthic folds)	8	1	$p < 0.05$
Upward (mongoloid) slant of the palpebral fissures	7	6	
Downward (antimongoloid) slant	0	1	
Short palpebral fissure*	1	0	
Hypertelorism*	3	1	
Hypotelorism*	2	0	
Ptosis	1	0	
Small ears*	0	0	
Asymmetrical size of the ears*	2	0	
Low-set ears*	6	4	
Severe slanting away of the ear from the eye*	2	2	
Short philtrum*	2	0	
Long philtrum*	1	0	

TABLE II (continued)

	Diabetic children n = 111	Controls n = 111	
Small oral opening*	2	0	
Large tongue	0	0	
High-arched palate	7	3	
Wide-set nipples*	3	5	
Sacral dimple	5	6	
Wide distance between toes 1—2	6	2	
Partial syndactyly of toes 2—3	0	0	
Broad and/or dorsiflected hallux	5	6	
Small, hypoplastic hallux	0	0	
Hypoplastic nails	1	1	
Prominent heel	1	0	
Shawl-like scrotal fold	5	1	
Total phenogenetic variants	73	40	$p < 0.001$

* = based on measurements

the teratogenic effect of the impaired maternal glucose homeostasis. However, except for some dermatoglyphic features [1, 2], the occurrence of malformations and MCAs in diabetic children has not been studied, and it is not known whether a familial increase of these features was associated with IDDM. In other words, it is not clear whether the presence of MCAs, and especially of multiple ones, may reflect a certain predisposition to IDDM, similarly to the preliminary findings obtained in families with childhood malignancy [8].

The present results showed an increased prevalence of multiple MCAs at least in some diabetic children. This was due not only to a higher frequency of extreme variants, minor dysplasias, and deformations, but also to that of "malformation-type" minor anomalies or "mild malformations" such as supernumerary nipples. Could this be confirmed on a larger material, it would be necessary to reconsider our present view on the relation of metabolic disorders to dysmorphology [11]. It should, however, be stressed, that neither a specific individual MCA nor a typical combination characteristic of IDDM was found in this material.

We think that our findings deserve reexamination in other genetic/demographic settings. In particular, detailed family investigations would be needed to determine how far the occurrence of multiple MCAs was related to susceptibility to IDDM, and which individual MCAs or which combinations of them are of predictive or diagnostic significance.

ACKNOWLEDGEMENT

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Limited joint mobility in diabetic children: a risk factor of diabetic complications?

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Limited joint mobility (LJM) was detected in 24 of 55 children with type I diabetes. Among children with longer duration of diabetes (5 years) 15 of 25 had LJM. Ten of these 15 also had preclinical diabetic retinopathy. LJM, impaired respiratory function and early retinopathy together were detected in 8 patients. Three children had early retinopathy without LJM but two of them also had impaired respiratory function. The findings were not related to the degree of metabolic control. These results confirm the great importance of connective tissue changes in childhood diabetes with respect to the early development of diabetic microvascular disease.

It has been shown that diabetes mellitus is accompanied by widespread biochemical, morphologic and functional abnormalities of collagen and elastin [3, 11, 15]. Previously we reported impaired respiratory function in diabetic children, probably due to an abnormal elastic behaviour of the lung [12], and diminished vital capacity has been observed in a diabetic boy suffering from joint contractures [1]. Recently it has been suggested [14] that limited joint mobility affecting mainly the proximal interphalangeal joints of the hands indicates an increased risk for early development of microvascular complications in childhood diabetics. Earlier, we found a significantly higher prevalence of early retinopathy in diabetic children with limited joint mobility than in patients without joint limitation [9]. Simultaneously, an increased prevalence (36.5%) of joint limitation was observed in patients with type I diabetes who had a significantly higher incidence of retinopathy than patients with normal joint mobility [7].

The aim of the present study was to investigate the association of limited joint mobility, impaired respiratory function and preclinical diabetic retinopathy in childhood type I diabetes mellitus.

Patients and Methods

Fifty five insulin-dependent children and adolescents were evaluated whose diabetes had become clinically apparent before the age of 14 years. Their age ranged from 5 to 18 years (mean 13.3 $\pm$ 5.1), the duration of diabetes ranged from 1 to 10 years (mean 4.5 $\pm$ 2.6). They had no history of previous lung disease or atopy and none of them smoked cigarettes. All patients were free from manifest proteinuria.

To demonstrate limited joint mobility, the patient attempts to approximate tightly the palmar surface of the inter-

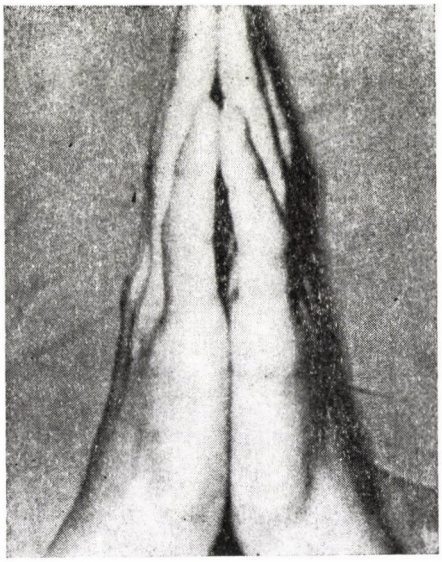

FIG. 1. Limited joint mobility in a diabetic child stage II

phalangeal joints of both hands (Fig 1). Joint contractures were classified in three stages according to Grgic et al [6] as follows. Stage I: unable to make contact with some portion of one finger of each hand; Stage II: unable to make contact with two or more fingers of each hand; Stage III: joint limitation of all fingers of each hand, plus in some larger joints (wrist or elbow).

Respiratory function data of all but five subjects were measured with the patient seated in a constant volume whole body plethysmograph (Fenyves-Gut, Basle). All lung volumes were quoted at BTPS (Body Temperature Pressure Saturated) conditions. Predicted values were taken from Polgár [13].

Fluorescein angiography was performed in patients having had diabetes for more than 5 years. Signs of preclinical retinal microangiopathy were evaluated according to Brooser [2].

The degree of metabolic control was estimated by repeated measurements by the Boehringer test of haemoglobin A_1c in each individual.

Student's t test was used for statistical analysis.

RESULTS

Limited joint mobility was detected in 24 patients; 15 were classified as stage I, 9 as stage II. Clinical data of patients with normal joint and with limited joint mobility are shown in Table I. Duration of diabetes and the mean insulin dose differed significantly between the two groups ($p < 0.01$ and $p < 0.05$, respectively). The latter finding would be expected in view of the longer duration of diabetes. No statistical differences were apparent between the two groups in age at diagnosis, in sex distribution and in the level of metabolic control.

The increased frequency of joint limitation with longer duration of diabetes is also shown in Fig 2. The shortest duration of the disease in any patient with joint limitation was just over one year. Most of the chil-

TABLE I

Clinical data of diabetic patients

	Patients with normal joints (n = 31)	Patients with limited joint mobility (n = 24)	Significance
Age at onset of diabetes, years	8.9 ± 1.8	9.3 ± 2.0	N.S.
Duration of diabetes, years	2.9 ± 1.7	6.5 ± 1.9	$p < 0.01$
Hb A_1, per cent	10.8 ± 1.4	11.3 ± 2.1	N.S.
Daily insulin dose, units/kg/day	0.87 ± 0.1	1.12 ± 0.2	$p < 0.05$

Results are mean ± SD

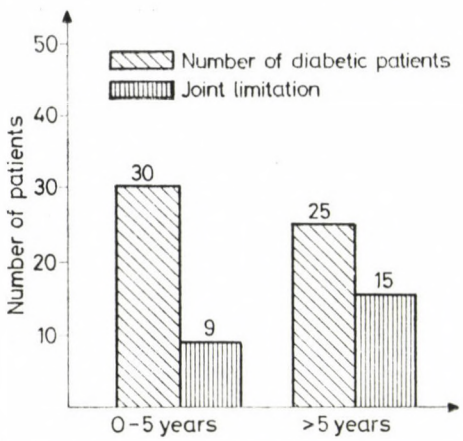

FIG. 2. Frequency of joint limitation according to duration of diabetes. Number of patients is indicated above the columns

dren were unaware of any abnormality of their hands and so it was not possible to evaluate exactly when the joint limitation had started. The 30 patients with a duration of less than 5 years included 9 patients with joint limitation; the 25 patients with a duration of more than 5 years included 15 with joint limitation.

The frequency of preclinical retinal microangiopathy in patients who had diabetes for more than 5 years is shown in Fig 3. Among the 10 children without joint limitation, 3 had microvascular abnormalities, while among 15 children with limited joint mobility, 8 had preclinical retinal changes.

Pulmonary function data in diabetic children with and in those without joint limitation are shown in Table II. All values except for the data of airway resistance are expressed as per cent predicted, as determined by the subject's height and age. There were significant differences between the two groups in total lung capacity (TLC), in airway resistance (Raw), and in peak expiratory flow measured at 50% of forced vital capacity (PEF50%). TLC and PEF50%

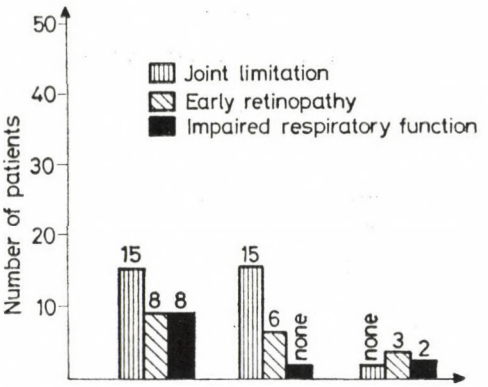

FIG. 3. Frequency of early retinopathy and impaired respiratory function, according to presence of joint limitation in patients with diabetes for more than 5 years. Number of patients is indicated above the columns

TABLE II

Comparison of pulmonary function data in diabetic patients with joint limitation and those without it (values are mean ± SD)

Patients	Age, years	Duration of diabetes, years	VC per cent pred.	TLC per cent pred.	Raw, cm H_2O lit/sec	PEF per cent pred.	PEF 50 per cent pred.
With joint limitation (n = 24)	16.1 ±1.2	6.4 ±0.8	92 ±4	88 ±4	3.1 ±0.2	95 ±5	90 ±3
			N.S.	$P < 0.05$	$P < 0.01$	N.S.	$P < 0.05$
Without joint limitation (n = 26)	11.4 ±0.9	3.1 ±0.6	99 ±5	100.5 ±3	2.0 ±0.1	102 ±8	112 ±7

VC = vital capacity
TLC = total lung capacity
Raw = airway resistance
PEF = peak expiratory flow
$PEF_{50\%}$ = peak expiratory flow measured at 50% of forced vital capacity
per cent pred. = per cent predicted value

were significantly lower ($p < 0.05$), Raw was significantly higher ($p < 0.01$) in patients with limited joint mobility.

The frequency of retinal microangiopathy and altered respiratory function according to the presence of joint limitation in subjects with diabetes of more than 5 years duration is shown in Fig 3. Limited joint mobility was detected in 60% of these patients. Preclinical retinal microangiopathy, disturbances in respiratory function, and joint limitation together have been shown in 32% of patients. Three children had retinal microangiopathy without joint limitation but two of them also had impaired pulmonary function.

Discussion

Limited joint mobility, mainly involving the small joints of the hand, is a common manifestation of childhood onset type I diabetes [6]. Its prevalence varied from 8.4 to 36% in different diabetic population [6, 7, 14]. We found an even higher prevalence (43%) in our study [9].

Although the exact aetiology of limited joint mobility in diabetes is unknown, increased cross-link formation with accumulation of inflexible collagen has been demonstrated in induced diabetes in animals [4]. It is always difficult to extrapolate from animal studies to human disease but it does appear that collagen structure and biosynthesis are abnormal, particularly in the skin of human diabetics [5].

Recently, Lyons and Kennedy [8] investigated the glycosylation of skin collagen from diabetic patients with and without limited joint mobility. Although their results do not support the hypothesis that non-enzymatic glycosylation of collagen would play an important part in the development of joint limitation in diabetes, the possibility that subsequent degradation of the ketoamine link may play a role remains to be investigated [8].

Abnormalities in lung elastic behaviour due to the widespread elastin and collagen abnormalities in diabetes have been shown in 11 young men suffering from type 1 diabetes [16], and earlier we observed impaired pulmonary function in diabetic children [12]. In our present study significant differences were found in three parameters of pulmonary function between patients with joint limitation and those without it, i.e. total lung capacity, airway resistance and peak expiratory flow measured at 50% of forced vital capacity. Although such abnormalities in lung elastic behaviour are, in some respects, similar to those that occur during normal aging [17], their presence in childhood without lung disease is extremely rare, and it seems that the observed changes are due to alterations in tissue elasticity. The relation between limited joint mobility and the early development of microvascular complications in type I diabetes has been postulated by Rosenbloom et al [14] and has been confirmed by other reports [7, 9]. These findings suggest that the alterations of periarticular connective tissue are related to changes occurring in the microvasculature [14]. Retinopathy is a sequel of diabetes mellitus that causes significant morbidity in the form of visual loss, however, describing the association of retinopathy with the degree of metabolic control, the age of onset and duration, and genetic factors, have been contradictory [10].

Therefore, detection of limited joint mobility in diabetic children would appear to identify patients who are exceptionally at risk for the development of diabetic retinopathy.

In conclusion, our data support the concept that limited joint mobility is another chronic complication of diabetes developing prior to retinopathy,

nephropathy and neuropathy [8]. On the other hand, abnormalities in lung elastic behaviour could also be an evidence of connective tissue changes in diabetes.

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Pneumococcal Infections During Childhood: Serotyping of Pneumococcal Strains from Forty-six Childern

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The results of serotyping of forty-six strains of pneumococci isolated from children aged 3/12 to 14 9/12 years and diagnosed as having pneumonia, meningitis, primary peritonitis, otitis media, lymphadenitis, osteomyelitis, bacteraemia and conjunctivitis are presented.

The results of serotyping and the frequency distribution of the detected serotypes were compared to the particular diagnoses established and the age at which the subjects involved had become ill. Questions of epidemiology and possibilities of immunoprophylaxis are discussed. Finally, the occurrence of pneumococci that are resistant to antibiotic agents are discussed since an isolated strain was found to show reduced susceptibility to penicillin G.

In spite of present day possibilities of therapy with antibiotics, infections with pneumococci (Streptococcus pneumoniae) still present major medical problems [27]. This particular fact and reports describing the occurrence of strains of pneumococci that were observed to be resistant to penicillin and other antibiotic agents [2, 13, 14] resulted in problems studied intensively in the last years.

There are few recent studies of results of typing of pneumococci isolated from diseased children [22]. This paper presents initial results of our studies of the subject.

Patients and Methods

The studies conducted by us included strains of pneumococci that were isolated between April, 1980, and January, 1984, from patients at the Children's Hospital and Clinic of Paediatric Surgery, Karl Marx University at Leipzig. The strains were obtained from blood cultures, cerebrospinal fluids (CSF), pleural and peritoneal fluids, ear and eye swabs, as well as abscesses and bone aspirates. Preliminary identification was carried out by bile solubility testing. Resistance determinations were made using the standard agar diffusion test [37].

The strains were freeze-dried and transferred for typing to the Laboratoire de Bactériologie-Virologie de l'Université Lyon I, France.

The capsular swelling reaction was used for serological determination with the aid of antipneumococcic sera (Statens Serum Institute, Copenhagen, Denmark; 9 pool sera, A through I, 46 type or group sera). The Danish nomenclature was used for the designation of types and groups. The clinical and laboratory data were considered for the arrangement or classification of diseased children in diagnostic groups. In one child where pneumococci were detected in the blood culture it was not possible to obtain significant evidence of local mani-

festation of the infection; that is why it was diagnosed as bacteraemia.

For statistical considerations, use was made of the U-test of Wilcoxon et al [31].

Results

A total of 46 strains of pneumococci could be typed. Table I shows the relations between clinical diagnosis and the various materials examined, with blood cultures, CSF and pleural fluids accounting for the majority. The diagnoses were in order of frequency: pneumonia (2), meningitis (14), primary peritonitis (4), otitis media (2), lymphadenitis (2), osteomyelitis (2), bacteraemia (1), and conjunctivitis (1). The seasonal distribution of the diseases is shown in Fig 1. From the data presented in this Figure it is apparent that the incidence showed a marked increase in the period from January to April and was less pronounced in the period between August and October. As far as pulmonary infections are concerned, the month of July showed the same morbidity rate as the month of April. For children with pneumonia, sex distribution showed a male to female ratio of 1.5:1. For children with meningitis the ratio was 1:1. For all the diseased children the ratio was in the order of 1:1.2. The median values for the age at which children had become ill were 37 months for children with pneumonia, 15 months for children with meningitis, and 24 months for all of the diseased children.

The age at which children fell ill ranged from 3/12 to 14 9/12 years. There was a significant difference in age distribution between children with pneumonia and those affected with meningitis ($\alpha = 0.05$). Of the children with pneumonia, six were two years of age or less (of a total of twenty), of those affected with meningitis, ten were two years of age or less (of a total of fourteen). And of all the diseased children, twenty-three were two years of age or less (of a total of forty-six).

Ten different serotypes were observed in our material. Serotypes of

Table I

Relationship between clinical diagnosis and kind of material examined (with presence of pneumococci detected)

Material examined	Clinical diagnosis					
	Pneumonia (N = 20)	Meningitis (N = 14)	Peritonitis (N = 4)	Osteomyelitis/ arthritis (N = 2)	Other diseases* (N = 6)	Total (N = 46)
Blood culture	9	3		1	2	15
CSF		11				11
Pleural fluid	11					11
Ear swab					2	2
Eye swab					1	1
Abscess aspirate					1	1
Bone aspirate				1		1
Peritoneal fluid			4			4

* = Lymphadenitis, otitis media, conjunctivitis, bacteraemia

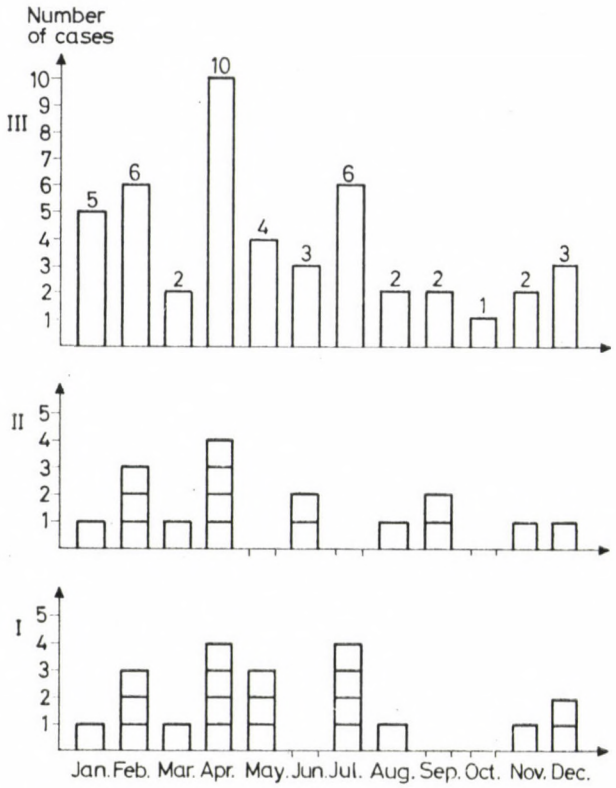

FIG. 1. Seasonal distribution of 46 pneumococcal infections. I = pneumonia, II = meningitis, III = all patients studied

pneumococci are shown in Fig 2. Table II shows a breakdown of serotypes according to the clinical diagnosis. Table III shows the distribution of serotypes in relation to age. Type 6 took the first place in the group of children aged 3 months to 2 years, it was not present in children aged 2 years to 4 years, and it appeared once only in patients over 4 years of age.

Type 1 was dominant in the latter group of patients; it took the first place in children aged 2 years to 4 years, and was not present in the group of youngest patients. Whereas type 14 was strongly represented in the two lower age groups, it was absent in the children over 4 years. The widest spectrum of isolated serotypes was found in the children aged 3 months to 2 years.

Of the 46 strains of pneumococci one strain showed, in the agar diffusion test, only a 12 mm zone of inhibition when penicillin G was used in a test dose of 3 I.U. Checking yielded the following minimum inhibitory concentrations: penicillin G, 0.125 μg/ml; ampicillin, 0.032 μg/ml;

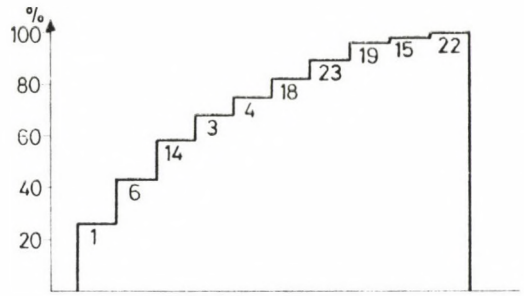

FIG. 2. Cumulative percentages of pneumococcal serotypes cultured from 46 children between April, 1980, and January, 1984

TABLE II

Frequency of occurrence of serotypes of pneumococci in various diseases (N = 46) during childhood beyond the neonatal period

Serotype	Pneumonia (N = 20)	Meningitis (N = 14)	Peritonitis (N = 4)	Osteomyelitis/ arthritis (N = 2)	Other diseases* (N = 6)	Total (N = 46)
1°	7	1	4			12
3°	4				1	5
4°	1	1			1	3
6°	3	3		1	1	8
14°	3	2			2	7
15		1				1
18°		3				3
19°	1	1		1		3
22		1				1
23°	1	1			1	3

* = Lymphadenitis, otitis media, conjunctivitis, bacteraemia
° = Serotypes which are, either directly or in form of subtypes, represented in a 14-valent vaccine (Pneumovax®)

TABLE III

Frequency distribution of serotypes of pneumococci in relation to age

Rank order	Patients							
	Total number (N = 46)		Three months to <2 years (N = 23)		Two years to <4 years (N = 11)		>4 years (N = 12)	
	Type	(Number of strains)	Type	(Number of strains)	Type	(Number of strains)	Type	(Number of strains)
1	1	(12)	6	(7)	1	(3)	1	(9)
2	6	(8)	14	(4)	14	(3)	6	(1)
3	14	(7)	19	(3)	3	(2)	3	(1)
4	3	(5)	3	(2)	4	(1)	22	(1)
5	4	(3)	4	(2)	18	(1)		
6	18	(3)	18	(2)	19	(1)		
7	23	(3)	23	(2)				
8	19	(3)	15	(1)				
9	15	(1)						
10	22	(1)						

oxacillin, 1.00 μg/ml. Evidence for beta-lactamase production was not obtained. The strain had been isolated from a child with otitis media and mastoiditis, and proved to be serotype 4. The child, before admission to our hospital, had been treated for an extended period with oxacillin in another hospital.

Discussion

A detailed description of the history of pneumococcal research was presented by Austrian [4]. The fact that pneumococci isolated from normal carriers and diseased subjects show polysaccharide capsules soon proved of particular interest. The different capsules provide the basis for classification into serotypes of which the total number is at present eighty-three (3).

Pneumococci that are merely present on the mucous membranes show, in so far as the frequency distribution of serotypes is concerned, a different pattern than those isolated in the case of invasive infections [24.] Acquisition of antibodies without clinical infections by pneumococci is appropriately explained by stimulation through homologous antigens (i.e. pneumococci carried in the nasopharynx) and cross-reacting antigens of other bacteria found on various body surfaces, e.g., Klebsiella spp., E. coli and some types of streptococci [16]. Results of both clinical and experimental studies provided ample evidence for the central position occupied by the spleen in the defense against pneumococcal infections [7, 15]. This is considered to explain the special disposition to infections by pneumococci of morphologically or functionally asplenic patients [7, 27]. Further dispositional factors for pneumococcal infections in infancy [surveys of which are given in 3, 7, 27] are: anatomical defects in the cranial region, sickle cell anaemia, malignancies (particularly Hodgkin disease), primary or secondary immunodeficiencies.

In cases of childhood pneumonia, pneumococci have been reported to be the most frequent causal agents [27]. As agents causing bacterial meningitis of childhood they generally are second or third in frequency [20, 27]. During childhood, infections by pneumococci occur above all in the first two years of life [22, 28]. As far as the principal types of manifestation during childhood are concerned, children with meningitis seem to be of the lowest average age.

From a detailed consideration of the observations analysed here it is reasonable to assume that, in the period under review, the diagnosis of pneumococcal pneumonia could be established just too infrequently. Detection of the presence of pneumococci in blood cultures and pleural fluids, respectively, reflects only part of the diseases since not all cases of pneumococcal pneumonia showed a simultaneous occurrence of bacteraemia and/or pleural involvement and because suitable material was not or could not be obtained in each case or a microbiological diagnosis was not made

until after the administration of antibiotic agents [3].

In our hospital, too, pneumococci take the third place as meningitis causing agents. As to the age at which the patient had fallen ill, our observations are in agreement with those reported in the literature. We, too, were able to find that the average age of children with meningitis, which is fifteen months, is markedly lower than that of children affected with pneumococcal pneumonia.

In the literature available to us, authors reported a more or less pronounced predominance of the male sex [27]. In our children such a predominance was shown only by pneumococcal pneumonia, whereas children with meningitis showed a substantially balanced ratio. The predominance of girls in the overall result might be considered as being due chiefly to the proportion of cases of primary peritonitis, a disease which occurs almost exclusively in girls during childhood.

In studies on the seasonal distribution of diseases produced by pneumococci, some authors observed a substantial increase in months with lower temperatures [22, 27]. Our results only showed a less frequent occurrence of pneumococcal infections between the months of August and October.

As a rule, determination of the serotypes of isolated strains of pneumococci was dispensed since the advent of antimicrobial chemotherapy. Interest in serotyping again arose in the last fifteen to twenty years. Reasons therefore can be summarized as follows [9, 19, 27, 32].

- Pneumococci continue to be causal agents of common and occasionally serious infections.
- Their importance for old and disposed patients is on the increase.
- There are not only penicillin G-resistant, but multiple resistant pneumococci.
- Production and controlled application of vaccines for the prevention of pneumococcal infections is desirable and feasible.
- There are indications of varying frequency distributions of major serotypes which differ in different geographic regions and, possibly, over larger periods of time and therefore require programmes of continuous surveillance to be instituted.

From the large number of previous studies on the frequency of occurrence of different serotypes in invasive infections it is evident that only about twenty types or subtypes can be regarded as being of special importance [6, 8, 9, 11, 12, 28, 29, 33]. They give the following series according to the frequency of occurrence:

Types 6, 14, 19, 23, 9, 15, 17, 18 [Gray et al, 23]

Types 14, 19, 6, 18 [Broome and Facklam, 8]

Types 19, 14, 23, 18, 4 [Weisholtz et al, 36]

Types 19, 6, 23, 14, 3, 18, 4, 9, 7, 1 [Gray et al, 22].

Special importance has been attached to type 14 in childhood [8, 23];

in our material, too, type 14 was frequent in the lower age groups, whereas it could not be found beyond four years of age. Type 1 is the most common with pneumonia and it is the only type that was identified in all of the cases of primary peritonitis. As regards pneumonia, in some reports [33] type 1 took the first place. Our observation of the frequent occurrence of type 6 in children under two years of age as compared with older children has been found also by other authors [22, 27].

On the basis of a large number of studies of the prevalence of serotypes of pneumococci responsible for invasive infections, some immunoprophylactic vaccines were developed containing the polysaccharides of the most important types (surveys are given in 3, 5). For example, there is a clinically tested and certified 8-valent [32], a 14-valent [1, 10], a 17-valent [18], and more recently a 23-valent [5, 28] vaccine. Of these, the 14-valent vaccine (Pneumovax®, MSD) found widest application in the USA [1] and later in other countries [3, 26, 35]. It contains the polysaccharide substance of types or subtypes 1, 2, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19F, 23F and 25 and, according to the studies cited, it is capable of covering effectively some 70 to 80% of all serious pneumococcal infections occurring in North America and Europe, since these are caused by corresponding or closely related serotypes. The serotypes found in diseased children correspond better to those contained in the vaccine than to the serotypes isolated from adults [22, 27, 28, 33]. Also, among the 46 strains of pneumococci which were isolated by us there are only two (namely, types 15 and 22 occurring once each, both isolated from children with meningitis) that did not correspond nor were related to any of the types contained in the vaccine. This means a level of agreement of approximately 96%.

As to the age of children, opinions are divided in certain points [for surveys 7, 21, 26, 28, 30, 32]. The most highly endangered age group, children up to two years of age, cannot probably be considered to come into question for immunization. In the case of splenectomy, it would be necessary that the inoculation be performed some four to eight weeks prior to the proposed surgical intervention; otherwise, vaccination would have to be performed at the earliest one or two weeks after the intervention.

Children with Hodgkin disease should, if possible, be vaccinated before therapy is started. Since a protective effect could not convincingly be demonstrated for the majority of indications, some authors [21, 30] recommend that immunization after splenectomy should be combined with antibiotic (e.g. penicillin) prophylaxis for three to five years and that these children be examined by the attending physician whenever they have a febrile disease.

It is of crucial importance that the resistance of pneumococci to antibiotics, and more particularly to penicillin, be most carefully controlled. Obviously, there are marked differ-

ences in resistance in different geographic regions [2, 13, 25, 34, 39]. In Europe, the situation seems to be favourable [12, 35, 38] although some papers [10, 14, 17] reported the occurrence of resistance or of reduced susceptibility. Of the 46 strains isolated by us only one proved to be of reduced susceptibility to penicillin G; in a child that had received oxacillin for a prolonged period of time.

Summing up, the following conclusions have been drawn.

- The distribution of serotypes of the strains of pneumococci isolated from different age and different disease groups should be surveyed on a broad basis.
- The resistance to antibiotic agents proposed to be used for therapeutical purposes should carefully be determined in every case, it being advisable for the results obtained to be included in a Chemotherapeutic Resistance Surveillance Program.
- The group of subjects up to two years of age should be included in a program undertaken with a view to arriving at a better understanding of the immune response to polysaccharide antigens and enabling new approaches to be adopted prior to inclusion in immunoprophylaxis of this particularly endangered group of patients.

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Influence of protein intake and liver function on acid base balance in premature infants

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In 43 patients with late metabolic acidosis (LMA) the factors promoting LMA were investigated. Postnatal adaptation was disturbed in all cases, in 35 patients acidosis developed after introduction of formula feeding. Whereas no differences were observed in renal function (urine volume and renal molar excretion) between acidotic and non-acidotic patients, there was a significantly higher concentration of bile acids in serum (26.1 $\pm$ 9.6 *vs* 98.6 $\pm$ 21.6 μmol/l), a significantly increased fractional volume of stools (8.2 $\pm$ 1.3 *vs* 11.4 $\pm$ 1.9% of intake, and higher faecal fat excretion (26.5 $\pm$ 5.2 *vs* 39.1 $\pm$ 6.6% of faecal weight) in LMA patients than non-acidotic formula-fed infants.

It is suggested that impaired postnatal development of liver function caused by severe disturbances of postnatal adaptation (respiratory distress, persistent fetal circulation, sepsis) is one of the most important factors in the pathogenesis of LMA. Thus, liver function should be checked before a protein intake surpassing that of a breastfed infant is introduced. Concentration of the serum bile acid level seems a reliable marker of LMA.

Since its first description, late metabolic acidosis (LMA) is considered a result of a temporary disproportion of renal H^+ elimination capacity and dietary intake of nonvolatile acids [16–20, 22, 25]. As a rule, LMA can be observed during formula feeding, especially if the protein intake is high [29]. In spite of this, only Schwartz et al [25] estimated the renal elimination capacity during LMA but they failed to find significant differences between acidotic and non-acidotic infants.

The metabolic response to formula feeding is characterized above all by a loading of liver function. The higher protein intake during formula feeding results in hyperaminoacidaemia and increased amino acid losses as well as in nonphysiologic cholestasis [7, 26]. It was therefore attempted to estimate the influence of postanatal development of liver function on the occurrence of LMA in premature infants appropriate for gestational age (AGA).

Patients

All admitted AGA premature infants who developed LMA between 1st of February, 1980, and 31st of August, 1984, were subjected to study. A total of 8 patients developed LMA during human milk (HM) feeding (Group 1), and in 35 patients LMA occurred on changing HM to formula (Group 2). To compare these patients, Group 3 included AGA premature infants without any perinatal problem fed

TABLE I

Data of infants studied (M ± SD)

	Patients with late metabolic acidosis		Controls	
	Fed human milk (Group 1)	Fed formula (Group 2)	Fed human milk (Group 3)	Fed formula (Group 4)
Gestational age, weeks	30.3 ± 1.8	30.9 ± 1.8	31.3 ± 1.6	32.9 ± 1.9
Weigth at birth, g	1413 ± 299	1798 ± 328	2072 ± 391	1812 ± 359
Length at birth, cm	40.6 ± 1.9	42.7 ± 2.2	44.7 ± 2.6	42.9 ± 2.3
Postnatale age, days	25.3 ± 4.9	20.8 ± 6.2	21.0	29.4 ± 4.9
Number	35	8	54	11

native HM from the first day of life and studied on the 21st day of life, and in patients of Group 4 nutrition had been changed from HM to formula (1.7 g protein/100 ml) during the third week of life without developing LMA. The data of the studied infants are summarized in Table I.

METHODS

The diagnosis of LMA presupposed a poor weight gain and a base excess of more than −5 mmol/l after the 7th day of life. At the same time, cardiac decompensation as well as bacterial infectious diseases had to be excluded. In all patients the clinical course including postnatal adaptation, development of body weight, and the concentration of bilirubin in serum on the 3rd day of life were determined. In selected patients of Groups 2, 3 and 4 we estimated in addition the

amount of urine and faeces;

fat excretion in stools gravimetrically by chloromethanol extraction;

osmolality of urine;

renal total and alpha-amino-nitrogen excretion by Kjeldahl and ninhydrin method;

serum concentration of alpha-amino-nitrogen and bile acids by the method of Senger et al [26].

For statistical analysis Student's *t*-test was applied.

RESULTS

As compared to the corresponding control group, patients with LMA were characterized by severe complications during the first days of life (Table II) which caused the significantly ($p < 0.01$) longer time of total parenteral nutrition as shown in Table III. The maximum postnatal weight loss was significantly ($p < < 0.05$) higher and the birthweight was reached later ($p < 0.01$) in both LMA groups than in the corresponding control groups (Table III). On the 3rd day of life the serum bilirubin concentration was significantly ($p < < 0.01$) elevated in LMA patients compared to patients without LMA (Table III).

Comparison of patients suffering from LMA during HM feeding with those fed formula (Tables II, III), the groups with HM feeding was characterized by a lower gestational age, lower birthweight, a higher incidence of severe disturbances of postnatal adaptation, a longer time of total parenteral nutrition, and a lower base excess.

TABLE II

Clinical findings

	Patients with late metabolic acidosis		Controls	
	Fed human milk Group 1	Fed formula Group 2	Fed human milk Group 3	Fed formula Group 4
Apgar-score below 4	1	13	0	0
Apgar-score 4 to 7	0	9	9	3
Respiratory distress syndrome	3	3	0	1
Sepsis	3	4	0	0
Persistent fetal circulation	1	5	0	1
None	0	2	45	6
Number	8	35	54	11

TABLE III

Cumulative protein intake and protein intake on days LMA, duration of postnatal total parenteral nutrition (TPN), weight gain, and serum bilirubin level on the 3rd day of life and acid–base balance on the day of LMA (M ± SD)

	Patients with ate metabolic acidosis		Controls	
	Fed human milk Group 1	Fed formula Group 2	Fed human milk Group 3	Fed formula Group 4
Protein intake:				
cumulative (g/kg/d)	1.97 ± 0.36	2.05 ± 0.39	1.98 ± 0.38*	2.09 ± 0.41**
Day of LMA (g/kg/d)	2.32 ± 0.40	3.98 ± 0.47	2.31 ± 0.41*	3.81 ± 0.51**
Duration of TPN (days)	6.2 ± 2.9	3.1 ± 1.9	7.1 ± 2.8 (hours)	1.9 ± 1.9
Postnatal weight loss (per cent birthweight)	7.1 ± 3.1	6.8 ± 2.6	4.2 ± 2.5	5.4 ± 2.9
Day reaching birth-weight	22.6 ± 6.8	19.7 ± 6.6	8.2 ± 4.2	10.3 ± 5.1
Serum bilirubin level (μmol/l)	248.6 ±23.9	217.9 ±49.6	154.0 ±49.3	181.4 ±48.2
pH	7.31 ± 0.028	7.29 ± 0.034	7.36 ± 0.031*	7.34 ± 0.039**
Base excess (mmol/l)	−7.6 ± 1.2	−8.8 ± 1.1	−1.1 ± 1.2*	−2.9 ± 1.3**

* referred to the 21th day of life
** referred to the 1st day of formula nutrition

Urine volume decreased during the change from HM to formula feeding ($p < 0.01$), but there was no difference between LMA patients and patients without LMA (Fig 1). Renal molar excretion as well as renal nitrogen losses were significantly higher ($p < 0.01$) in formula fed patients than in infants fed HM (Fig 2), but there was no differences between acidotic and non-acidotic patients.

Whereas no differences in renal molar excretion could be observed between patients fed formula with

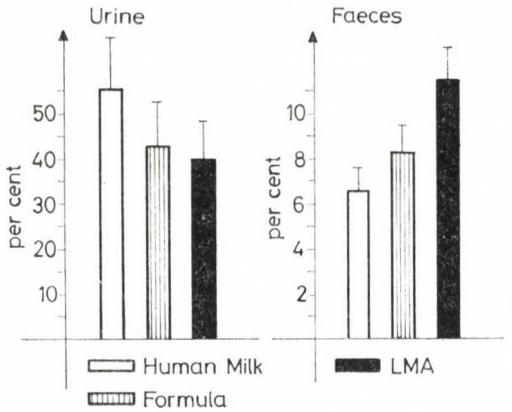

FIG. 1. Volume (M $\pm$ SD) of urine and stools are percentage of intake in patients fed human milk (Group 3), in patients without acidosis after change from human milk to formula (Group 4), and in patients fed formula with late metabolic acidosis (Group 2)

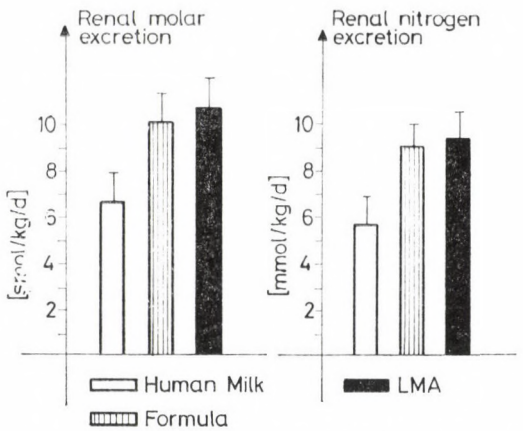

FIG. 2. Daily renal nitrogen and molar excretion (M $\pm$ SD) in patients fed human milk (Group 3), in patients after change from human milk to formula without acidosis (Group 4), and in patients fed formula with late metabolic acidosis (Group 2)

(Group 2) and without (Group 4) LMA, there were significant differences ($p < 0.05$) in faecal volume, as shown in Figure 1. The faecal excretion of fat was lowest in Group 3 ($18.1 \pm 3.4\%$ of stool weight) and highest in Group 2 ($39.1 \pm 6.6\%$ of stool weight). In Group 4, fat excretion was more than in the group fed HM but less than in the formula fed LMA group ($26.5 \pm 5.2\%$ of stool weight).

During formula feeding, the serum alpha-amino-nitrogen concentration increased significantly as compared to Group 3 ($p < 0.01$), but there were no differences between formula fed acidotic and non-acidotic patients. In

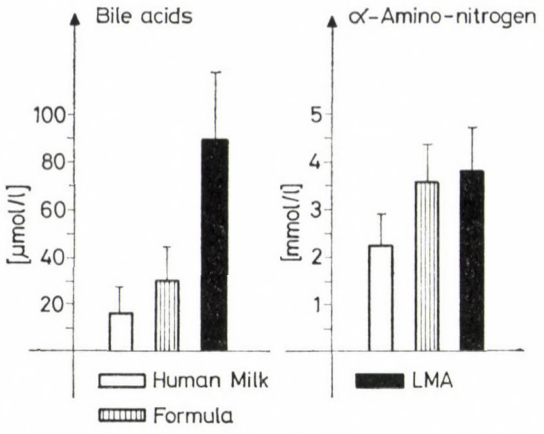

FIG. 3. Concentrations of bile acids (µmol/l) and alpha-amino-nitrogen (mmol/l) in serum (M ± SD) in patients fed human milk (Group 3), in patients after change from human milk to formula without acidosis (Group 4), and in patients fed formula with late metabolic acidosis (Group 2)

contrast, the serum bile acid concentration was significantly different ($p < 0.01$) between acidotic and non-acidotic formula fed patients (Fig 3).

DISCUSSION

LMA is usually described to result from an insufficient renal function related to dietary non-volatile acids [16–20, 22, 25], but the present results seem to confirm the data of Schwartz et al [25] showing that renal function in LMA patients is in the range normal for both gestational and postnatal age [1, 6, 9, 16]. In contrast, the limited liver function seems to be a more important factor in these patients: elevated serum bile acid levels, higher volume of stools and increased faecal fat excretion may be a result of an aggravated neonatal cholestasis. The results of Blitzer et al [4] showing an influence of high amino acid concentrations in the extracellular space on hepatocellular bile acid uptake may explain these findings.

It is remarkable that in all patients with LMA, postnatal adaptation was disturbed. This may lead to liver dysfunction in different ways, as follows.

Hypoxia of hepatocytes changes the cellular metabolic situation by decreasing protein synthesis as a basic condition of postnatal development [15]. As a clinical consequence of such disturbances, it is well-known that total parenteral nutrition leads to hepatocellular dysfunction and not only during the first days of life [3, 5, 14, 21] and, in addition, to an insufficient stimulation of gut hormones which are important factors in postnatal development of the gastrointestinal tract [2, 14]. Bacterial toxins may also have a role in the disturbed development of liver function [8]. Thus, damage of hepatocytes may explain the finding that the same elevation of serum amino acids in both

formula fed groups resulted in a significantly higher increase of serum bile acid concentration in the LMA group (Fig 3) and that during HM feeding the development of LMA is based on a disturbance of liver function. The same was observed by Svenningsen and Lindquist [29] while other authors presumed that in newborns LMA had no connection with postnatal disturbances [16–18, 20, 22].

The observed effects of non-human protein on bile acid metabolism cannot directly explain the acid-base disturbance. The decreased digestion of food may lead to excretion of bases and this may be related to LMA.

Despite the high interest in problems of newborn nutrition, publications related to LMA are scarce. Some investigators demonstrated metabolic acidosis during formula feeding without description of LMA as a disease entity [17, 24]. On the other hand, the absence of LMA is regarded as a sign of good quality nutrition [10, 11, 27, 28]. From the present data it seems that the acid-base balance reflects the quality of nutrition as well as the postnatal development of liver function. It is concluded that for low birthweight infants a protein intake of more than 2.5 g/kg/day should be prescribed only with a good liver function. The serum concentration of bile acids seems to be a good marker to estimate the metabolic situation of the liver. Concentrations of more than 40 μmol/l are signs of cholestasis, and the substitution of HM for formulas containing more protein than that of HM cannot be recommended.

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Concentration of tumour markers CEA, AFP, alpha and beta subunits of hCG in cerebrospinal fluid in children with inflammatory diseases of the central nervous system

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Carcinoembryonic antigen (CEA), alphafetoprotein (AFP) and alpha-beta subunits of human chorionic gonadotropin (hCG) were determined by radio-immunoassay in CSF of 83 children presenting some central nervous disease and compared to the corresponding values obtained in 88 children without neuroinfection. CEA and alpha hCG were absent in the CSF of children without neuroinfection. CEA and alpha hCG levels in CSF higher than 0.0 ng/ml were regarded as elevated. In patients with inflammatory process of CNS, CEA values were positive in 9% (maximum 8.0 ng/ml) and alpha hCG in 4% (maximum 5.0 ng/ml). AFP in CSF ranged from 15.0 to 49.0 ng/ml in children without neuroinfection, and from 0.0 to 100.0 ng/ml in patients with inflammatory diseases of the CNS. As a normal upper limit of AFP in CSF, 53.2 ng/ml is suggested; in 31% of the patients with inflammatory diseases of CNS the AFP level was elevated. The normal upper limit of beta hCG concentration in CSF was regarded as 0.4 ng/ml; in 12% of the patients with viral meningoencephalitis the beta hCG level in CSF was slightly elevated, it ranged from 0.5 to 3.0 ng/ml.

The usefulness of serum CEA, AFP and hCG as tumour markers and as an indicator of the response to treatment is well established. Subsequently, elevated serum levels of tumour markers were also found in patients with nonmalignant diseases. Several authors observed CEA and AFP activity in body fluids other than plasma [2, 4] and increased CSF levels of CEA in patients with intracranial tumour [7, 8, 9, 12, 13], and preventive and therapeutic properties of AFP were demonstrated in experimentally induced allergic encephalomyelitis.

The present study was designated to determine the concentration of CEA, AFP and alpha and beta subunits of hCG in CSF of children with inflammatory diseases of the CNS.

Material and Methods

CEA, AFP and alpha and beta subunits of hCG were determined in the CSF of 83 children aged from 2 to 14 years, presenting signs of some CNS affection and compared to the corresponding values of 88 healthy children of the same age. Samples were obtained with lumbar puncture from hospitalized patients, without CNS affection. The CSF was not centrifuged. Simultaneously, blood samples were obtained by peripheral venipuncture, centrifuged at 2500/min for 5 minutes and held at −18°C until use (maximum two months). CEA and alpha and beta subunits of hCG were determined by double-antibody radioimmunoassay and AFP level by single-antibody radioimmunoassay with final separation by polyethylene glycol of bound and unbound antigens. All RIA kits were supplied by Isotope Production and Distribution Centre, Swierk, Poland.

Patients were grouped as follows.

Group A. 19 children without inflammatory diseases of the CNS. Their CSF was normal, the indication for its analysis were febrile convulsions during acute respiratory tract infection or severe vomiting suggestive of neuroinfection;

Group B_1. 6 patients with purulent meningitis;

Group B_2. 49 patients with viral meningoencephalitis. In this group the level of tumour markers in CSF was determined several times.

Group C. 26 patients examined after three weeks treatment of viral meningoencephalitis, when the CSF was free of inflammatory signs.

Statistical significance between means was estimated by Student's *t*-test (level of significance, $p = 0.05$).

Results

Plasma concentration of tumour markers in healthy children

In the 88 healthy children the normal value for plasma CEA and AFP was at 2 SD above the mean. The plasma CEA level was less than 4.1 ng/ml (mean 0.83 ± 1.6 ng/ml) and the plasma AFP level less than 12.2 ng/ml (mean 3.18 ± 4.47 ng/ml). The mean alpha hCG was 0.12 ± 0.44 ng/ml, and the beta hCG level 0.80 ± 0.15 ng/ml. The upper normal limit of alpha and beta hCG levels was at two SD above the mean; thus, levels of alpha hCG higher than 1.0 ng/ml and beta hCG higher than 0.4 ng/ml were regarded as elevated. Between 0.0–1.0 ng/ml were 98% of the results for alpha hCG and between 0.0–0.4 ng/ml were 95% of the results for the beta hCG.

Plasma concentration of tumour markers at the time of lumbar puncture for CSF analysis

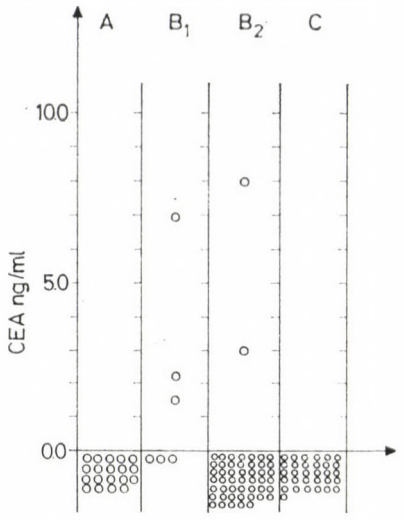
In 3/19 children of the A group plasma CEA levels were higher than 4.10 ng/ml. In 5/55 patients with neuroinfection plasma CEA values were elevated (maximum, 12.5 ng/ml). In 3/26 patients during the recovery period, plasma CEA values were elevated, ranging from 4.2 to 6.0 ng/ml.

Plasma AFP values higher than 12.2 ng/ml were found in 2/19 patients of the A group, and in 7/49 patients with viral meningoencephalitis. In Group C, plasma levels were normal and ranged from 0.0 to 3.2 ng/ml.

In all 83 children, the alpha hCG concentration was not higher than 1.0 ng/ml. Only in 3/49 patients with viral encephalomeningitis was the beta hCG concentration higher than 0.4 ng/ml. Simultaneous elevation of plasma CEA, AFP, alpha and beta hCG levels was not observed in patients with neuroinfection.

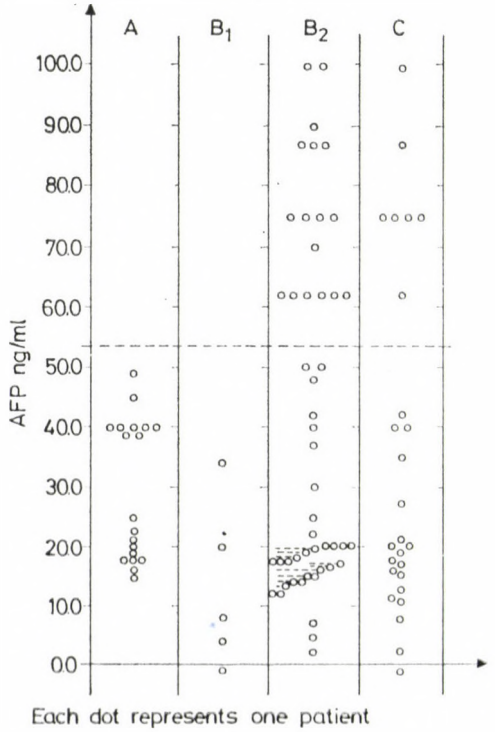
CSF concentration of tumour markers

The results of CEA, AFP, alpha and beta subunits of hCG assay in CSF are shown in Figs 1 to 4. In the results obtained from patients without neuroinfection there was no CEA. Five of 55 patients with inflammatory process had positive CEA values (maximum, 8.0 ng/ml), while during recovery the CEA values were negative. (All CEA levels in CSF higher than 0.0 ng/ml were regarded as elevated.) In patients without neuroinfection AFP values ranged from 15.0 to 49.0



Each dot represents one patient
A. children without symptoms of neuroinfection
B_1. patients with purulent meningitis
B_2. patients with viral meningoencephalitis
C. patients in convalescence after viral meningoencephalitis

FIG. 1



Each dot represents one patient

FIG. 2

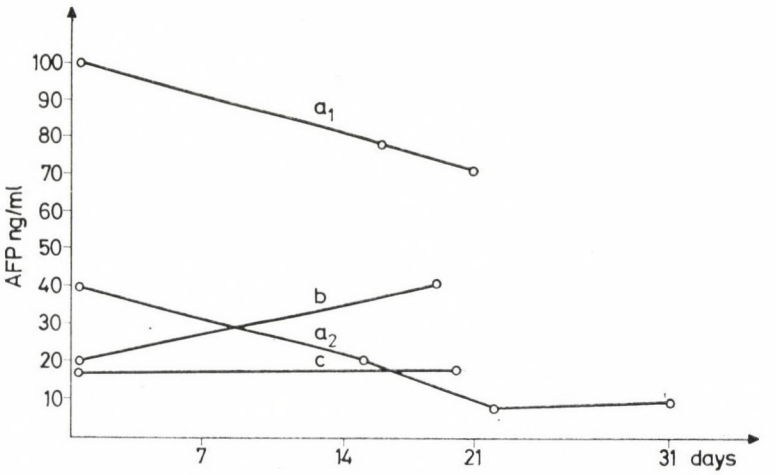


FIG. 3

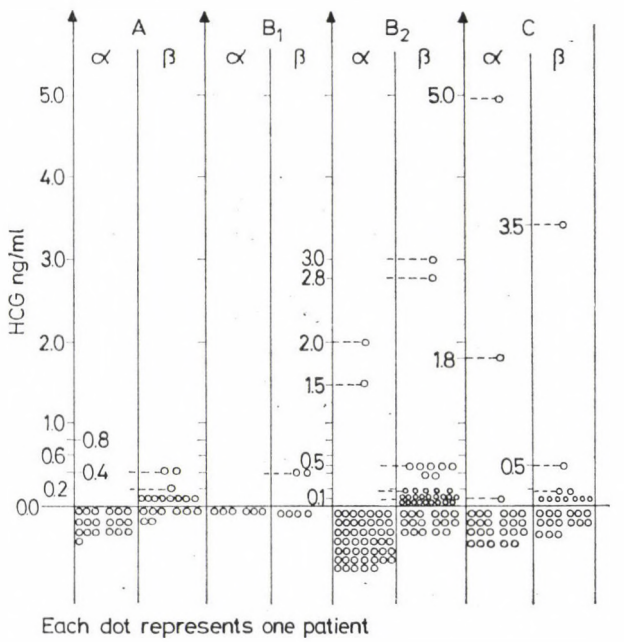


FIG. 4

ng/ml, with a mean of 29.74 ± 11.71 ng/ml. The normal upper limit of AFP in CSF was regarded as 53.20 ng/ml ($\bar{x}$ + 2 SD). In patients with purulent meningitis the AFP level in CSF ranged from 0.0 to 34.0 ng/ml, in patients with viral meningoencephalitis from 2.0 to 100.0 ng/ml. The mean AFP level in the whole B group was 40.5 ± 29.13 ng/ml, by 10.76 ng/ml

higher than that of the normal CSF. In group B, 34.7% of the patients had elevated levels. The mean CSF AFP level during recovery was 35.45 ± 29.11 ng/ml, 5.05 ng/ml lower than with acute inflammatory process but 5.71 ng/ml higher than in the control group A. Statistical differences between the groups were not observed ($p < 0.05$).

In patients without neuroinfection the alpha subunit of hCG was not detectable in the CSF. Two of 49 patients with viral meningoencephalitis and 3/26 patients in the recovery period had positive alpha hCG values ranging from 0.1 to 5.0 ng/ml. The mean beta hCG level in CSF without inflammatory process was 0.095 ± 0.12 ng/ml while its normal upper limit ($\bar{x} + 2$ SD) was set at 0.40 ng/ml, as 100% of the results of group A were between 0.0 and 0.40 ng/ml. In 7/49 patients with viral meningoencephalitis and in 2/26 patients during recovery the beta hCG level in CFS was higher than 0.40 ng/ml, ranging from 0.5 to 3.5 ng/ml.

Serial tumour marker assays in CSF

The level of tumour markers in CSF was determined repeatedly in some patients with viral meningoencephalitis during or after treatment. The initial level in one patient was 3.0 ng/ml and dropped to 0.0 ng/ml during recovery. In the second one with an initial CEA of 8.0 ng/ml we were unable to obtain further values, and in the other patients with meningoencephalitis no CEA was detectable. Figure 3 shows serial CSF AFP levels of patients with neuroinfection; in 15/26 the level decreased during recovery (curves a_1 and a_2 in Fig 3). In 8/26 patients the control AFP levels were higher than at onset of the disease (curve b in Fig 3). There was only a single patient in whom the 18.90 ng/ml AFP level did not change (curve c in Fig 3). All positive alpha and beta hCG levels decreased to 0.0 ng/ml during recovery.

No relationship was found between plasma and CSF levels of CEA and alpha and beta hCG in meningoencephalitis, using correlation analysis and linear regression. Each level seemed to be an independent variable. The same analysis for AFP yielded a positive correlation.

Discussion

Some biological markers have previously been detected in serum and CSF in patients with various intracranial tumours [2, 6, 7, 8, 9, 11, 12, 13, 14, 15]. In some of these, a correlation was observed between the concentration of tumour markers and the clinical data [2] in that in 6 patients with verified intracranial germ-cell tumour the AFP and beta hCG profile for a given tumour correlated with the histological diagnosis [2]. Other authors [6, 8, 12] suggested that the CEA level was of value in the differential diagnosis of primary and metastatic brain tumours, but until now the clinical significance of these biological markers has not sufficiently been proven. It would be useful to

determine CSF fetal-neoplastic antigens both in healthy individuals and in patients with non-neoplastic diseases of the CNS. As there are difficulties in analysing the CSF of healthy subjects we have determined some immunological markers in non-tumorous CSF to receive comparative values for further studies in neoplastic patients.

Suzuki and Tanaka [14] considered 0.5 ng/ml as the upper limit for CEA in normal CSF. In the present study, CEA and alpha hCG were not detectable in CSF obtained from patients without neuroinfection. Our results suggested that the upper normal limit of CEA and alpha hCG in CSF was 0.0 ng/ml. Only in 9% of patients with inflammatory diseases of the CNS was the CEA level elevated, ranging up to 8.0 ng/ml, and the alpha hCG level was elevated only in 4% of them, ranging up to 5.0 ng/ml. The mean beta hCG level in CSF in patients without neuroinfection was 0.95 ± 0.12 ng/ml and in only 12% of the meningoencephalitis patients was it slightly elevated, ranging from 0.5 to 3.0 ng/ml. AFP in CSF obtained from patients without neuroinfection ranged from 15.0 to 49.0 ng/ml, and from 0.0 to 100.0 ng/ml in children with inflammatory disease of the CNS and in 31% of the patients with neuroinfection.

AFP has a non-specific immunosuppressive effect on both the cellular and humoral immune response [3, 5, 9, 10]. Abramsky et al [1] assumed that AFP may prevent experimental allergic encephalomyelitis. In our study we have found elevated AFP levels in CSF that subsequently declined during recovery in patients with neuroinfection. It is possible that AFP is involved in immunoregulative mechanisms during neuroinfection. This hypothesis requires further investigations.

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Bronchial secretions and bronchial mucosa in children with cystic fibrosis: comparison of bronchoscopic, biochemical, bacteriological, microscopic and ultrastructural findings

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In children (mean age 12.1 ± 2.9 years) with cystic fibrosis, 44 bronchoscopic examinations were done under general anaesthesia with muscle relaxation using a Friedel type ventilation bronchoscope. The endoscopic picture of the mucous membranes was compared with the state of the bronchial secretions, its bacteriologic findings and content of acid mucopolysaccharides and DNA fibres (semiquantitative estimations). In all patients biopsy of the mucous membrane (central part of the bronchial tree) was performed for light and electron microscopy. The degree of reddening, swelling of the mucous membrane and hypersecretion was in some agreement with the intensity of the cellular infiltration and the production of pus (microscopic investigation). Secondary ultrastructural changes were detected in nearly all children, consisting of cellular oedema, swelling of mitochondria, dilatation of the endoplasmatic reticulum, protrusion of cells and fusion of cilia, enlarged intercellular spaces, thickening of the epithelial basal membrane, increased number of goblet cells, microtubular abnormities of the cilia, lesions of the apical cell membrane with loss of cilia and microvilli. These ultrastructural changes were not correlated with the above-mentioned signs of inflammation.

Except from deaths in the neonatal period due to meconium ileus, lung lesions are the major factors contributing to morbidity and mortality of cystic fibrosis (CF). The chronic pulmonary alterations in CF start from the trachea and the main bronchi [14]. Therefore, the aim of the present study was to obtain more information about the changes of the bronchial mucosa and their relation to the bronchoscopic picture and the characteristics of bronchial secretions. The study continues our earlier bronchologic studies in CF patients [7, 8, 9, 22].

MATERIAL AND METHODS

The subjects were 22 children, 14 boys and 8 girls from 7 to 17 years of age, with slight to moderate cystic fibrosis ascertained by repeated sweat tests, who were for years under our outpatient treatment. All these patients underwent bronchoscopic investigations under general anaesthesia with barbiturates and muscle relaxation with succinylcholine by Friedel's ventilation bronchoscope [10, 20]. The children's parents and in most cases the patients themselves gave informed consent to the examination.

The endoscopic picture (reddening and swelling of the bronchial mucosa, localized or generalized hypersecretion) was recorded. Samples of secretions were in-

vestigated by bacteriology and thin secretion films stained with acridine-orange dye for detection of DNA fibres (Bürgi method [3]) and with toluidine blue [21] to show the content of acid mucopolysaccharides (aMPS). In all patients biopsy of the mucous membrane was done at the central part of the bronchial tree with the aid of a special forceps. The specimens were kept in buffered 8% formalin for light microscopy or immersed in 3% glutaraldehyde-cacodylate buffer for 2 hours and post-fixed in 1% osmium tetroxide for 2 hours for electron microscopic investigation.

Results

From the clinical point of view, the severity of CF was estimated with Shwachman's score [14]: 10 patients had scores from 95 to 100, and the remaining 12 patients had values between 75 and 94.

The bronchoscopic picture showed in 35 of 44 examinations a slight (15), moderate (23) or severe (1) reddening of the bronchial mucosa, 34 times a swelling of the mucous membranes, in 34 of 44 examinations a slight (20) or heavy (14) localized hypersecretion and 16 times a generalized secretion. The secretions were in 20 cases purulent and in 14 cases seromucous.

These data were taken together into an own bronchoscopic score with a 13 stage scale; 4 times the finding was normal (score 1), 22 times slight inflammatory changes (score 2 to 5), 17 times moderate (score 6 to 9) and in 1 patient severe (score 11) alterations were observed (Fig 1).

The bacteriological examination showed 13 times sterile secretions, 26 times one strain of bacteria and 5 times a mixed bacterial infection. Staphylococci were found most often (25 times), other bacteria were Haemophilus influenzae, pneumococci, enterococci and Staphylococcus epidermidis (each of them found in two

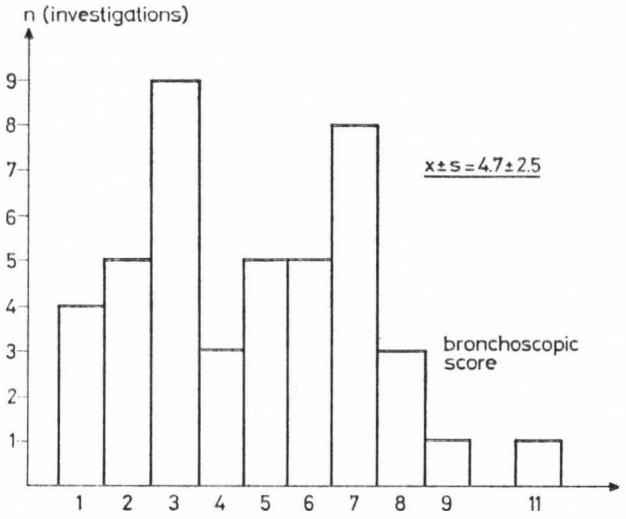


Fig. 1. Distribution of bronchoscopic score (degree of endobronchial inflammation) in 44 bronchoscopic examinations in children with cystic fibrosis. See text for details

cases) and Gram-positive cocci (one case). Pseudomonas aeruginosa was never detectable in these patients.

Semiquantitative evaluation of DNA and aMPS in the secretions was given in an 8 stage scale from 0 to +++ with intermediate stages.

From the findings the following correlations were calculated (r = coefficient of correlation):

clinical severity (Shwachman's score)/endobronchial inflammation (own bronchoscopic score) —r = 0.32,

DNA fibres in bronchial secretions/bronchoscopic score — r = 0.31, aMPS in bronchial secretions/bronchoscopy score — r = 0.34 and DNA fibres/aMPS in bronchial secretions — r = 0.59.

The highest (worst) mean bronchoscopic scores were recorded in children with staphylococci in the bronchial secretions (Fig 2), so the mean content of DNA fibres and aMPS was higher in these children than in those with sterile secretions or other bacteria found in it.

If the biopsy material was too small for both light and electron microscopy, we gave priority to the latter. In 21 of 44 examinations was the material sufficient for histological

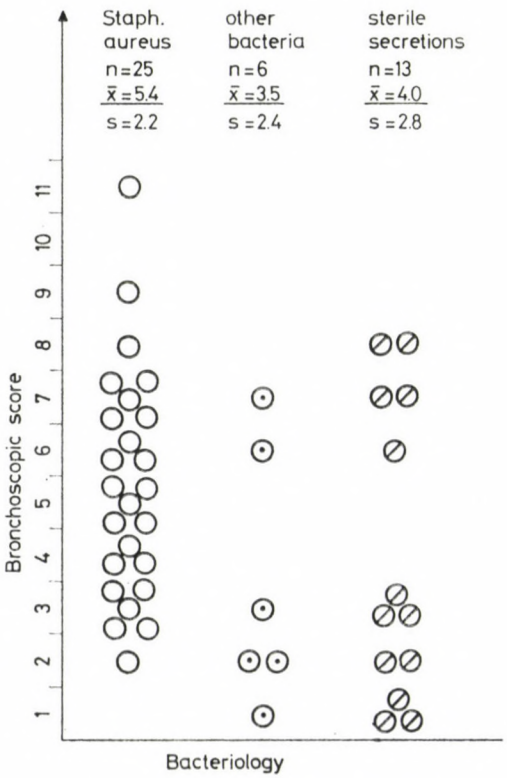
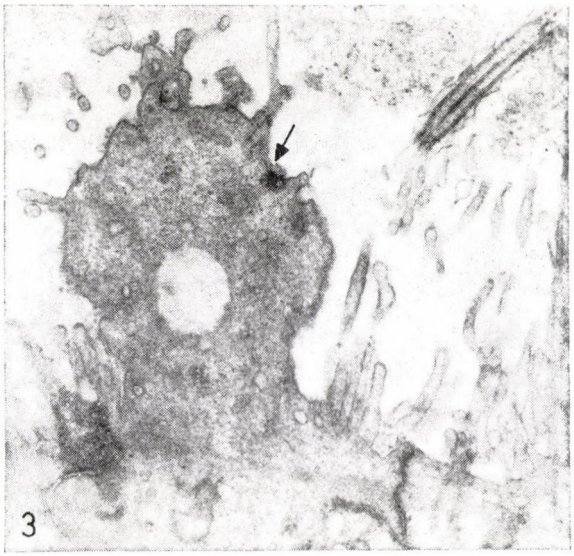


FIG. 2. Bacteriological findings of the bronchial secretions in 44 bronchoscopic examinations of children with cystic fibrosis, compared to the bronchoscopic score (Fig. 1)

examination and only in 16 cases could a clear decision be given about the degree of inflammation of the mucosa. We found twice a severe, 8 times a moderate and 3 times a slight chronic round cell infiltration and in 3 cases no inflammatory activity. With the exception of 3 children, the moderate or severe chronic inflammatory alterations were found in those



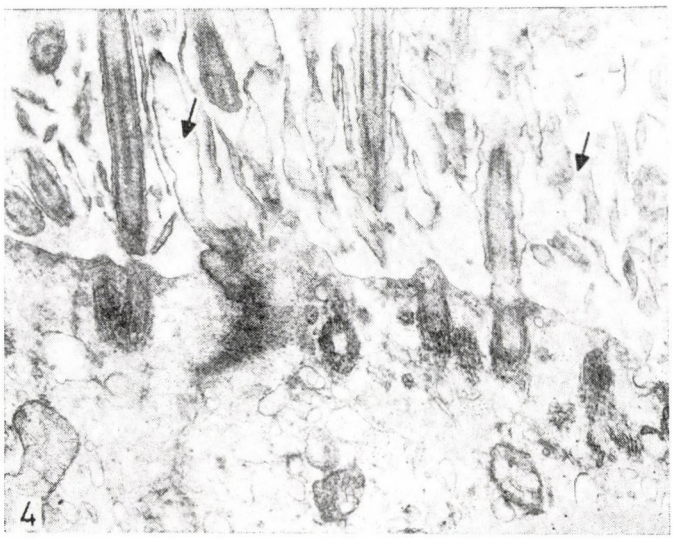


FIG. 3. Transmission electron micrograph of ciliated epithelium in a child with cystic fibrosis, showing protrusion (↓) of a cell with local destruction of cilia and microvilli. Magnification, 1:28 000

FIG. 4. Longitudinal-sectional profile of microvilli with degenerative changes (swelling and decay ↓). Magnification, 1:36 000

who showed a high degree of inflammation (i.e. high bronchoscopic score). Only in one case was the material sufficient for evaluation of the submucous glands. These showed signs of dyscrinia.

The material sufficed for ultrastructural investigation in 34 of 44 cases. Predominantly we evaluated the ciliated epithelium. The situation was as follows: no important changes (9 times), ciliary fusion with or without transposition of microtubuli (27 times), swelling and/or defect of the ciliary membrane (36 times) and cellular oedema (31 times). In more detail, the ciliary epithelium displayed

cellular oedema with swelling of mitochondria and dilatation of the endoplasmatic reticulum;

protrusion of cells (Fig 3) with alteration of cilia and microvilli (Fig 4);

enlargement of intercellular spaces;

penetrating inflammatory cells (lymphocytes and granulocytes) between the epithelia; and

thickening of the epithelial basal membrane.

As nonspecific lesions of the cilia we recorded:

alterations of their membrane (megacilia, swollen and fusioned cilia, protrusions, decay and complete loss of the ciliary membrane) (Figs 5, 6, 7);

microtubular abnormities (abnormal arrangement of aconemata after ciliary fusion (Figs 6, 7); and

faded granulary drawing of microtubuli.

There were in addition

an increased number of goblet cells (Fig 8) with granula full of mucus;

many dense granulary structures of mucus caused probably by its increased viscosity;

frequent cell fragments and inflammatory cells in the mucus;

lesions of the apical cell membrane with loss of cilia and/or microvilli, leading to a flat surface; and

alteration of microvilli (swelling and loss) and disturbance of the fluid transport.

None of these findings were correlated to the degree of inflammation (bronchoscopic score), the bacteriological or biochemical findings of the secretions or the inflammatory signs in light microscopy.

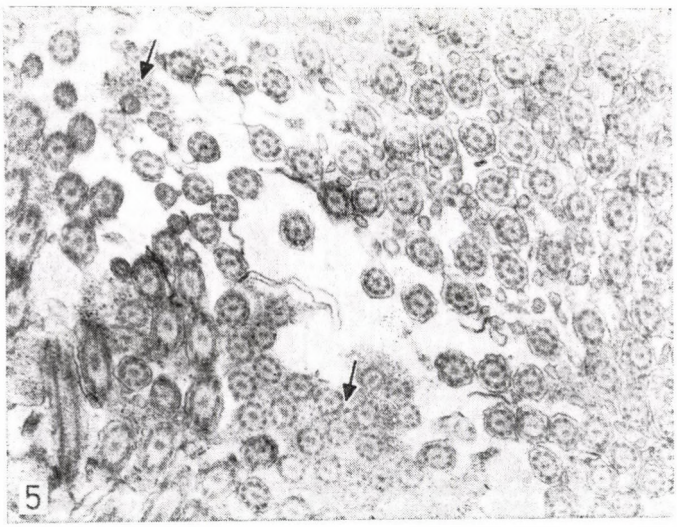
Discussion

It is well known that the severity and course of CF show a great variability among different patients. Although all the patients had a favourable course and only a mild form of CF (high Shwachman score and no Pseudomonas aeruginosa infection [14]), in most of them we could detect a pathologic endoscopic picture, pathologic findings of the bronchial secretions, chronic inflammatory changes in light microscopy and severe ultrastructural alterations.

There was only a slight correlation between the clinical severity and the bronchoscopic findings, between bronchoscopy and the content of DNA fibres and aMPS in the bronchial secretions, as well as between these semiquantitative biochemical parameters and the results of bacteriology.

Patients with staphylococcal infection had findings worse than those of the other children.

According to Chace et al [4] whose CF patients had a higher variability of the Shwachman score (from 5 to 80), there is a correlation between the content of hexose or sialic acid in bronchial expectorations and the severity of CF. It seemed that the light microscopic findings, i.e. signs of chronic infiltration with round cells, were in some agreement with the bronchoscopic picture of the mucosa. The alterations change intensely within weeks or months (see Fig. 1) due perhaps to intercurrent infections or exacerbations. Thus, bronchoscopic and histologic findings in CF are reflecting the degree of



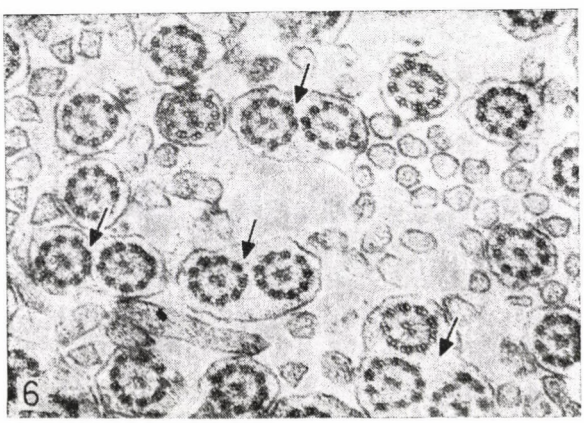
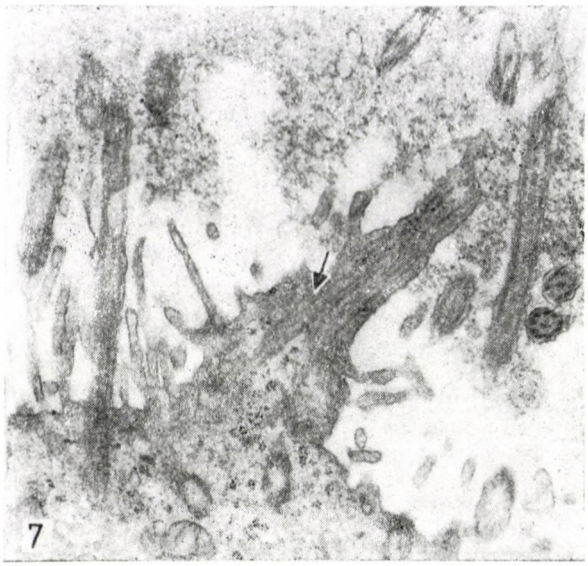


FIG. 5. Cross-sectional profile of a group of cilia. Some cilia show a destroyed outer membrane but maintained microtubuli (↓). Magnification, 1:30 000
FIG. 6. Fusioned cilia ↓ (cross section). Magnification, 1:54 000



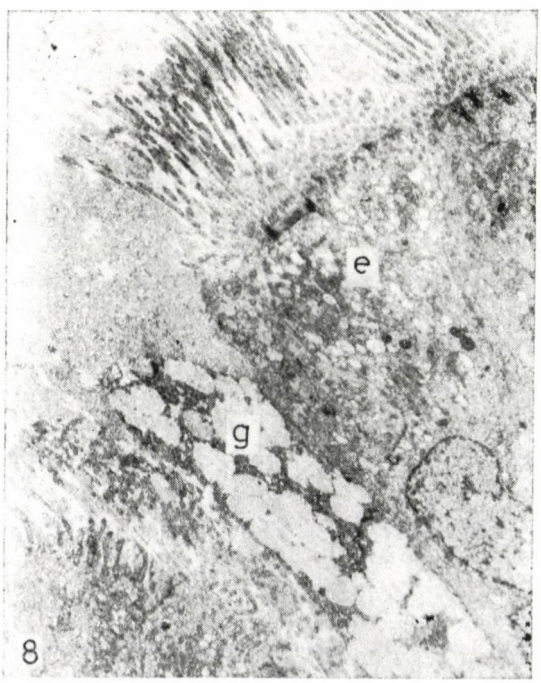


FIG. 7. Longitudinal section of a fusioned cilium (↓). Magnification, 1:28 000
FIG. 8. Goblet cell (g) among vacuolized ciliary epithelia (e). Magnification, 1:5200

chronic inflammation of the bronchial mucosa.

The ultrastructural findings seemed to be more constant. They were not correlated to the above-mentioned signs of inflammation such as the bronchoscopic findings of secretions and the light microscopic changes. These ultrastructural findings might be in correlation with the severity of the underlying disease, and so of prognostic value. This hypothesis should be examined in a group of patients with a variability of the Shwachman score higher than we had in our material. Since the detection of ultrastructural anomalies of cilia in Kartagener syndrome, there have been several investigations into the problem [1, 12, 16, 18, 19, etc.]. Differentiation between the primary ciliary insufficiency (mutated cilia, the immotile cilia syndrome or primary ciliary dyskinesis) and acquired ciliary defects is not always clear-cut. The main differences seem to be that the defects are specific in the first case, and restricted to a certain part of the ciliary apparatus (dynein arms, spokes and central sheath, central microtubules) in the second case. In the opposite acquired defects, the ciliated cells are non-specific and pleiotropic and they frequently involve swelling, fusing and shedding of cilia. Loss of the entire ciliated cell is even more common. An increased occurrence of internalized cilia or cilia with disorganized axononemes is also frequent [1]. Applying these criteria to our results, we are convinced that all ultrastructural changes in CF are of the secondary type, as stated also by others [12, 16, 19]. The acquired ciliary defects may be due to viral infections, infection by mycoplasma or bordetella, a cilio-toxic substance of Pseudomonas aeruginosa, proteolytic substances released from leukocytes in the bronchial secretion, inhalation of penicillin derivatives or mucolytic agents, and allergic reactions. In CF it is more likely that leukocytic enzymes, or drugs used for treatment may play a role in the aetiology of ciliary alterations. In our patients no certain differences could be seen between children who had been treated with mucolytic inhalations or by orally administered mucolytics before the examination.

Mucociliary clearance was normal or slightly impaired [12, 16], and no metaplasia of the bronchial epithelium[13] has been detected. The diagnostic and prognostic value of the present findings requires further investigations over a longer period.

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Variable expressivity of hypertelorism in three siblings with Greig syndrome

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Two sisters and one brother are reported with a complex of congenital malformations, hypertelorism, mental retardation, flattened nasal root, divergent strabism, mongoloid palpebral fissures, malformations of the ears, pathologic alterations of the eye-fundus in terms of optic nerve atrophy, all suggesting Greig syndrome. The major symptom of this syndrome, the hypertelorism, varied considerably in its expressivity in the three siblings. This fact is normally taken into consideration in the diagnosis of Greig syndrome, but we suggest that an alteration in skull formation should be the criterion for the syndrome rather than extreme hypertelorism.

The term "hypertelorism" was introduced by Greig [10] to indicate an extremely enlarged distance between the eyes. Presently the meaning of the term has been extended to include any pathologically enlarged distance between the inner corners of the eyes [3, 15]. Several authors have proposed that the term "euryopia" should be used for an interocular distance which exceeds the upper limit of the normal range but does not reach extreme values (Table I) [5, 11, 16, 18]. Enlarged interorbital distance may occur [13]

as a rare incident within the normal variability of the population. Mean interorbital distances were found to vary between human races [8];

as a symptom of a number of hereditary and non-hereditary pathological conditions associated with defects in skull development. This may not necessarily be present. Leiber and Olbrich [16] cite "hypertelorism" in more than 70 different syndromes,

as the major feature of the syndrome described by Greig [10] (Synonyms: Greig's hypertelorism, familial hypertelorism). Walker [20] differentiated this "primary" hypertelorism from "secondary" hypertelorism. The former is a result of other defects of skull development such as frontal encephalocele and meningocele, or medial skull cleft (De Meyer syndrome). It should be pointed out that Greig syndrome has not always been properly understood: as already indicated, conditions with hyperteloric symptomatology, as well as secondary defects in development, have sometimes been classified as Greig syndrome. Moreover, reports of cases vary according to the major field of interest of their author [9, 14]. Most of the older reports dealing with Greig syndrome concentrated on analysing

This work was carried out in the Department of Clinical Genetics, University Hospital, Bratislava, Czechoslovakia

TABLE I

Normal and pathological values of the medial canthal Index and circumference-interorbital Index (Günther, 1933). Stenopia is an abnormally small interorbital distance, metropia is a normal interorbital distance and euryopia is an intermediate condition between metropia and hypertelorism

	Stenopic	Metropic	Euryopic	Hypertelorism
Medial canthal Index	28	28–38	38–42	42
Circumference-interorbital Index	4.5	4.5–6.8	6.8–8.0	8.0

the dominant feature of the syndrome, the hypertelorism neglecting the remaining symptomatology. The repeated simultaneous occurrence of certain characteristics in the head (hypertelorism, flattened nose bridge, upward palpebral fissure, divergent strabism, ear shape, eye fundus abnormalities), together with mental retardation, slight abnormalities of the extremities and cryptorchism, cannot be accidental. It may be concluded that the complex of pathological findings described by Greig does not represent an isolated defect of interorbital distance but is a syndrome in the full meaning of the word [3, 15, 16]. Thus, Greig syndrome should be understood as a complex of abnormalities, the major one being a defect in the development of the chondral skull basis. In this paper we describe a family with three siblings affected by this syndrome.

CASE REPORTS

After having each of their children, the parents repeatedly requested the paediatrician to explain the reason for the illness. They also asked him to estimate the risk for another child they might have.

The proband, J.K., was the youngest daughter born in 1976. Overall psychomotor development was retarded. Mental retardation with marked facial dysmorphy (marked hypertelorism with no epicanti present, broad and flattened nose bridge continuing directly into the forehead, characteristic supraorbital arches parallel to the eyebrows), divergent strabism, the ears larger and set lower than usual and rotated backwards were observed (Fig 1). Diastema was present between the first upper incisors. The skull made a brachycephalic impression. Neurological examination revealed dyscrania with hypertelorism and non-progressive atrophy of the optic nerves, hypertonic syndrome with a characteristic walk. X-rays of the skull showed signs of hypertelorism with an interorbital distance of 12 cm, slight antimongoloid position of the orbits. The internasal bone was absent. Anthropometric examination of the skull is summarized in Table II. Dermatoglyphic examination revealed that the main lines tended to run longitudinally, pattern intensity on palms was low, carpal triradius was in

the t-position and transversal flexion creases were absent. Cytogenetic examination of peripheral blood lymphocytes showed a normal female karyotype (46,XX). Ophthalmologic examination was not possible because the patient would not cooperate.

A sibling of the proband, M.K., was born in 1972. She had begun walking at the age of 18 months, after which walking development stopped for one year. She had been able to control her body functions since the age of 3 years, started to

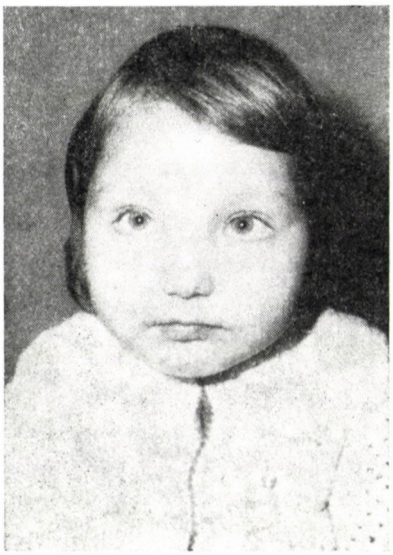

FIG. 1. Judita K. at 2 years of age

TABLE II

Anthropometric examination of the heads of the three siblings with Greig syndrome. (Results are expessed in mm; in parentheses, normal values for the corresponding age groups)

Parameters	Judita K. 1976	Monika K. 1972	Ladislav K. 1969
Head length (g-op)	145 (154)	142 (165.5)	164 (168)
Head breadth (en-en)	127 (133.6)	134 (142.4)	142 (146)
Forehead breadth (ft-ft)	100 (92.4)	100 (98.4)	110 (100)
Face width (zy-zy)	119 (106)	120 (116.4)	125 (118)
Bigonial width (go-go)	93 (81.9)	95 (88)	99 (90)
Physiognomic face height (tr-gn)	150	130	160
Morphological face height (n-gn)	90	100	117
Head circumference (g-op-g)	437 (474)	455 (500)	500 (510)
Medial canthal distance	38 (25.2)	35 (27.9)	31 (28)
Lateral canthal distance	94 (75.3)	92 (78.8)	98 (79)
Cephalic Index	87	94	86
Medial Canthal Index	40.4	38.04	31.6
Head-circumference-interorbital Index	8.4	7.7	6.2

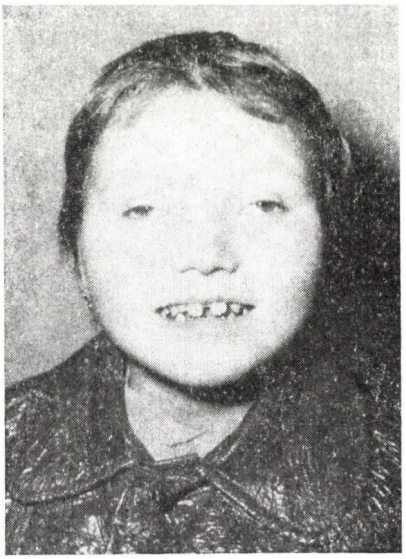

FIG. 2. Monika K. at 6 years of age

speak when she was 3 1/2 years old. At the age of 6 years her body weight was 16 kg, and length 105 cm. She was obviously mentally retarded with a characteristic facial dysmorphy, similar to that of her sister (enlarged interorbital distance without epicanthi, and a broad nose bridge, divergent strabism, large and lower set ears, Fig 2), diastema between the first upper incisors and flattened occiput. She had markedly larger and longer digits IV on her feet, nearly as large as digits II. Neurological examination gave similar results as those of her sister (mainly hypertonic syndrome and characteristic walk). X-ray of the skull showed that the distance between the outer orbital corners was 9 cm and the internasal bone was absent. Ophthalmological examination revealed that the otic disc was almost entirely discoloured, sited in level, the vascular gate was normal, arterioles and venules were all narrow, the peripheral retina showed no pathological changes, the macular reflex was absent, granular degeneration of the macular region was seen. Conclusion of the ophthalmological examination was: divergent strabism, macular degeneration, atrophia nervi optici in progression, nystagmus, hypertelorism. Anthropometric examination is summarized in Table II. Dermatoglyphic examination showed pattern frequencies in digits within the normal range. Total ridge count was normal (TRC = 135). Main lines tended to run longitudinally and pattern intensity on the palms was normal. The carpal triradius was in the t-position. Transversal flexion creases were absent. Chromatographic examination of the urine showed normal findings. Cyto-

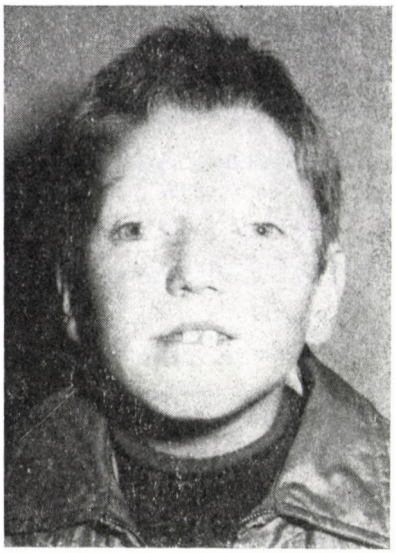

FIG. 3. Ladislav K. at 9 years of age

genetic examination of peripheral blood lymphocytes showed the normal female karyotype 46, XX.

The eldest brother L.K. of the proband was born in 1969 from the first pregnancy of a 19-year-old mother and a 27-year-old father. His overall psychomotor development was retarded. He had begun walking at the age of 2 years, controlling body functions at the age of 2 1/2 years and speaking at the age of 3 years. Physical examination of the 11-year-old boy showed mental retardation. He spoke in an unclear inarticulate manner. A marked facial dysmorphy was present (of the same type as that of his younger siblings) (Fig. 3) with slightly larger interorbital distance and broad nose bridge, divergent strabism, larger and lower-set ears, mouth often open, diastema between the first two incisors in the upper jaw. All the teeth were yellow and carious (he had often been treated with tetracycline), hypoplastic external genital organs with slack testes and clinodactylia on digits V of both hands. Neurological examination gave similar results as those of his younger siblings, mainly signs of hypertonic syndrome with a resulting characteristic walk. X-ray of the skull showed that the distance between the outer orbital corners was 10 cm. There were no other pathological signs, apart from an absent internasal bone. Ophthalmological examination disclosed discoloured papillae of the optic nerve in level, pigmented nasal conus, atypical vascular gate, slightly blunt and narrow branching of the vessels, pigmentation of the equatorial portion of the retina mainly on its periphery resembling pigment degeneration of the retina. Due to the disturbed

central fixation, both eyes had alternate divergence and there was total failure of focussing capacity. Anthropometric examination revealed a cephalic index of 86, i.e. hyperbrachycephaly. Neurocranium dimensions were below the normal range.

Discussion

Table III shows that the complete symptomatology of Greig syndrome was found in the youngest sibling. The canthal index did not reach the one agreed between euryopia and hypertelorism. However, as the child was only 3 years old at the time of examination, the head circumference/interorbital distance, which was clearly in the hyperteloric range, might be considered more reliable. Other symptoms of Greig syndrome included brachycephaly, serious mental retardation, divergent strabism, characteristic shape of the nose bridge and of the forehead, rotated low set ears with diasthema in the upper teeth, missing internasal bone and hypertonic syndrome. The eye fundus could not be examined as the patient did not cooperate.

The question then arose, how should the two elder siblings be classified ? They had all other symptoms including positive findings in the eye fundus that are characteristic of Greig syndrome, but they did not fulfil the criterion for hypertelorism. Should a diagnosis of Greig syndrome be made without the presence of extreme hypertelorism ? In fact, this has already been suggested by Günther [12] who stated that the constitutional abnormality of skull formation described by Greig does not always result in ex-

Table III

Symptomatology of the three siblings with Greig's syndrome

Symptomatology	Judita K. 1976	Monika K. 1972	Ladislav K. 1969
Mental retardation	++	++	++
Brachycephaly	++	+++	++
Microcephaly	+—	+	+—
Neuro-viscerocranium ratio	+	++	+—
Canthal Index	Euryopia	Euryopia	Normopia
Head-circumference-interorbital Index	Hypertelorism	Euryopia	Normopia
Absence of internasal bone	+	+	+
Diasthema in upper teeth	+	+	+
Atrophy of optic nerve	n.d.	+	+
Degenerative alterations of retina	n.d.	+	+
Hypertonic syndrome	+	+	+
Wide nasal bridge	+++	++	+
Enlarged set lower than usual	+	+	+
Others		Enlarged digit IV on feet	Clynodactily, genital hypoplasia, slightly open mouth

treme hypertelorism, and this leads to difficulties in diagnosis of certain cases. In familial occurrence with all other symptomatology suggesting constitutional skull abnormality there may be a decreased expressivity. This could be the case for the two eldest siblings: M.K. with an euryopic interorbital distance, and her brother L.K. with an interorbital distance within the normal range. The variability of findings of intercanthal distances in three siblings with identical, likely hereditary, developmental defects turns our attention to the importance of a defective skull formation rather than to extreme hypertelorism, the latter being purely a result of this prenatal defect.

It may be concluded from our observations that the diagnosis of the syndrome which has hypertelorism among its symptoms should be based on the establishment of a developmental defect of skull formation rather than on dimensions or indices; these should only be an aid to orientation. Aetiologically, the enlarged interorbital distance may be secondary to two kinds of defect [15]:

1. Defective development of the prechondral skull basis; here a number of neurological defects including Greig syndrome may be classified;

2. An early onset of pathological pressure inside the skull [11], as e.g. in Crouzon syndrome and Apert syndrome. Up till now, a condition for the Greig syndrome has been that hypertelorism should be "primary" and not secondary to another defect (e.g. to cleft defects). In our opinion, hypertelorism is secondary to a certain basic defect (depression or stimulation of growth of certain points of skull formation). Thus, the extent of hypertelorism as a result of such a defect may vary. This could explain the variable expressivity of hypertelorism and all the other symptoms in the three siblings. Basically, the entire complex process of postnatal skull formation is genetically determined. Several defects may, however, occur in this process caused by various external factors, leading to morphological alterations which when exceed the agreed limits are considered pathological. Günther [12], dealing with differential diagnostics of Greig syndrome and e.g. Crouzon syndrome, states as one feature the sporadic incidence of the former. Reports have, however, published on familial accumulation with possible autosomal dominant pathway over several generations [1, 6, 7, 19]. Families with suggested autosomal recessive heredity have also been reported [17].

Analysis of this family suggests an autosomal recessive heredity. The wide variability associated with Greig syndrome, the various modes of inheritance, and especially the mechanism of the pre- and postnatal development of these parts of the skull, suggest a heterogeneity in aetiology, pathogenesis and morphology of the observed clinical picture. Taking these facts into account, nosological entities associated with hypertelorism should be formed with extreme caution. For practical purposes of genetic counsel-

ling the genetic risk should primarily be based on analysis of the actual situation of the family, taking into account the possibility of decreased expressivity of individual symptoms as describied in this paper.

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Deletion 13q12.1 in a child with Coats disease

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Cytogenetic study of a child with the presumed clinical diagnosis of retinoblastoma of the right eye revealed del. 13q12.1. Histological examination of the removed eye showed changes which were characteristic of Coats disease. This finding is discussed.

The aetiology of Coats disease is unknown. It results from vascular anomalies in the retina (teleangiectasies, microaneurisms, etc.) and is fatal for the vision despite the benign character of the process. Other authors suggested the role of vascular degenerative lesions [2]. So far, genetic studies were not undertaken regardless of data showing the hereditary nature of the disease with predominant affliction of males in about 80% of all cases.

The aim of the present study was to explain the cytogenetic finding of del. 13q12.1 found in a child with Coats disease.

Report of a Case

The propositus (L.S.H.), a three years old male was referred to the Children's Eye Clinic because of squinting of the right eye and glistening of the pupil. The presumed clinical diagnosis was retinoblastoma of the right eye and the child was sent to us for cytogenetic examination.

Enucleation was performed. Histological examination of the nerve cells revealed total retinal detachment with subretinal exudate containing cholesterol crystals, pigment clusters and single foam cells.

The final diagnosis was Coats disease.

Chromosomal study of the patient and his parents was performed by means of peripheral blood leukocyte cultures. For high-resolution G-banding prometaphase analysis, chromosomes were obtained by a slight modification of the technique of Yunis [4, 5].

Results

At least 50 well-spread early metaphase and prometaphase plates were analysed.

Cytogenetic study revealed normal karyotypes of the parents. High resolution analysis of the patient's karyotype showed shortening of the white band 13q12 (Figs 1, 2). At the stage of 600 G-band chromosomal

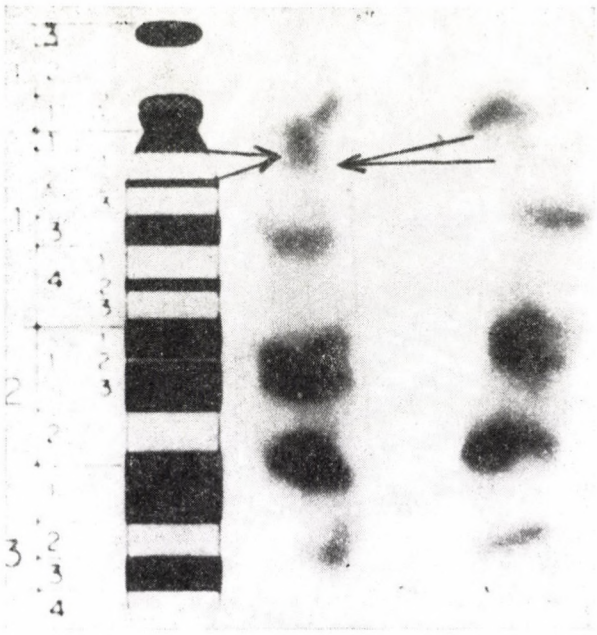

FIG. 1. The patient's chromosomes 13

FIG. 2. Chromosome 13 pair from patient with deleted chromosome 13 (del 13q12.1) on the left of the pair

differentiation it was identified as del. 13q12.1.

Discussion

To the best of our knowledge, del. 13q12.1 has never been reported in Coats disease. The present finding was casual and we have not studied other patients with Coats disease. For this reason it is difficult to comment upon the finding. Nevertheless, we suppose that the chromosomal abnormality 46,XY, del. 13q12.1 could be related to the child's disease. Chromosome 13 was namely found a major pathogenetic factor in blastomogenic processes: acute myelogenous leukaemia (del 13q12–14); myelofibrosis; Ph(—) chronic myelogenous leukaemia [3], and lymphoma and meningeoma [1], and deletion 13q14 has been accepted as a specific genetic marker for retinoblastoma.

We assume that the segment 13q12 might somehow occur in the eye's morphogenesis. New data over a large body of material and other kinds of eye tumours will help to establish the proper importance of chromosome polymorphism on which the clinical varieties of the ocular tumours are based.

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A recent aetiological study on facial clefting in Hungary

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A case-control epidemiological and family study was organized of 2024 index patients born between 1970 and 1976 affected by isolated cleft lip ± cleft palate, isolated posterior cleft palate and multiple congenital abnormalities including facial clefting and of their matched control cases. The specific rate of affected parents and sibs was 2.4% and 4.2% in the isolated cleft lip ± cleft palate sample, while 2.2% and 3.2% in the isolated posterior cleft palate group. The proportion of polygenic liability was about 77 ± 8% in isolated cleft lip±cleft palate cases. Among teratogens, the triggering impact of certain anticonvulsants was confirmed.

Previously we have published the data of epidemiological [2], genetic [3] and teratological [4, 5] studies on isolated cleft lip with or without cleft palate, and isolated posterior cleft palate of approximately all index patients born in Budapest, 1962–1967. These index patients were, however, analysed without matched control cases, therefore the environmental, maternal and obstetrical factors could not be evaluated adequately. This drawback, some new aetiological ideas and a concrete request have prompted us to conduct a new complex aetiological study (1977–1981) on Hungarian index patients with facial clefting born in 1970–1976. Some data of this second study were published elsewhere [8], here the aetiological factors will be discussed.

Materials and Methods

The material of the Hungarian Congenital Malformation Registry [5, 6] involved 2024 index patients with facial clefting born between 1970 and 1976. The completeness of notification was 92–98% in these types of congenital abnormalities (CAs). First, 8 groups of facial cleftings were separated as follows:

(i) Isolated cleft lip (CL) and cleft lip with cleft palate (CLP); the sum of these two subgroups CL(P) was N = 1086.

(ii) Specified CA-syndromes including CL(P) (N = 26).

(iii) Unspecified multiple CAs including CL(P) (N = 295).

(iv) Isolated posterior cleft palate (CP) (N = 365).

(v) Specified CA-syndromes including CP (N = 15).

(vi) Unspecified multiple CAs including CP (N = 143).

(vii) Robin sequence (N = 65).

(viii) Other facial cleftings (e.g., holoprosencephaly, median and oblique facial clefting) (N = 29).

The specified CA-syndromes and CA-entities with CL(P) and CP were trisomy 13 (Patau) 7, trisomy 18 (Edwards) 3, trisomy 21 (Down) 6, ADAM-sequence 6, Meckel-Gruber 3, fetal hydantoin 3, Apert 2, SCE 2, congenital rubella 1, OFD-I 1, thoracopagus conjoined twins 1 (2), Goldenhar 1, Marfan 1, diastrophic dysplasia 1, Larsen 1, popliteal web 1, Roberts 1. These CA-syndromes were not evaluated in this study. Owing to the low number of index patients with Robin sequence and the group entitled "Other facial cleftings" were also excluded. The distribution of CAs within unspecified multiple CAs including CL(P) and CP was published previously [8]. These two groups were combined under the abbreviation MCA. Eventually the aetiological factors were planned to be analysed in three groups of facial clefting: CL(P), CP and MCA involving 1,954 recorded cases.

A questionnaire was sent to the parents of all index patients studied. Lists of drugs and diseases were enclosed and parents were asked to have a look at them before filling in the questionnaire in order to standardize and refresh their memories. The prenatal logbook of pregnancies studied, furthermore photos and medical documentation of index patients and affected first degree relatives were requested from the parents.

A similar method was used in control cases matched with birth place, week of birth, sex and outcome (still- or livebirth, infant death) of index patients. Three matched control cases were ascertained in records of obstetrical institutions where index patients were born. As it appeared, 63 control cases also had CAs: liability for dislocation of the hip 21, congenital inguinal hernia 15, congenital cardiovascular malformations 8, eye anomalies 5, undescended testicles 4, pyloric stenosis 2, congenital clubfoot 1, polydactyly 1, omphalocele 1, dermoid cyst 1, anal atresia 1, choanal atresia 1, obstructive urological anomaly 1, auricular anomaly 1. The affected matched control cases represent a 5.2% total birth prevalence of CAs.

Response rates were significantly different within the groups of facial clefting (Table I). Only those cases were evaluated where all important questions were answered unequivocally. In order to prevent the further case loss, unpaired index patients were matched with the second or, if it was necessary, with the third control case. CA of first degree relatives was confirmed by the help of medical records or personal checks.

Biomathematical analysis was performed in two approaches: (i) index patients and their matched control cases were compared by the McNemar test, while (ii) all control cases were considered to be a total control group and this was compared with groups of index patients (χ^2 test). In general the latter is shown in the Tables.

TABLE I

Response and evaluated rates in different groups

Group	Registered cases	Index patients				Matched control			
		Respondent		Evaluated		Respondent		Evaluated	
		No.	%	No.	%	No.	%	No.	%
CL(P)	1086	727	66.9	630	58.0	504	46.4	471	43.4
CP	365	218	59.7	179	49.0	182	49.9	151	41.4
MCA	503	429	85.3	392	77.9	219	43.5	202	40.2
Total	1954	1374	70.3	1200	61.4	905	46.3	824	42.4

Results and Discussion

Genetics

The results of the *family study* are summarized in Table II. The unreliable data of 49,981 second and 44,748 third degree relatives were excluded and only the confirmed CAs of parents and sibs were included in the study. The specific familial cluster (K) was obvious in the relatives of index patients with CL(P), CP and MCA, respectively, except in the sibs of index girls with CP. The rates of affected parents and sibs showed some differences as compared to our previous study [3] conducted essentially in the same populations 10 years earlier by the same method.

Table II

Data of family study

Group	Index patient			Father				Mother			
	Sex	p	N	m	M	q	K	m	M	q	K
CL(P)	B	0.133	402	395	6	1.5	11	398	8	2.0	26
	G	0.077	228	223	8	3.6	27	225	4	1.8	23
	Σ	0.103	630	618	14	2.3	17	623	12	1.9	25
CP	B	0.036	80	80	1	1.3	36	80	2	2.5	52
	G	0.048	99	98	3	3.1	86	99	2	2.0	42
	Σ	0.042	179	178	4	2.2	52	179	4	2.2	46
MCA	B	0.039	181	170	1	0.6	15	176	3	1.7	33
	G	0.051	211	210	3	1.4	36	211	3	1.4	27
	Σ	0.045	392	380	4	1.1	28	387	6	1.6	31
Control	B	—	454	453	0	—	—	453	0	—	—
	G	—	368	365	0	—	—	368	0	—	—
	Σ	—	824	819	0	—	—	823	0	—	—

Group	Brother				Sister			
	m	M	q	K	m	M	q	K
CL(P)	216	9	4.2	32	232	5	2.2	28
	146	8	5.5	41	129	4	3.1	40
	362	17	4.7	35	361	9	2.5	32
CP	43	3	7.0	194	40	3	7.5	156
	50	0	—	—	52	0	—	—
	93	3	3.2	89	92	3	3.3	69
MCA	131	5	3.8	97	102	1	1.0	20
	152	5	3.3	85	132	5	3.8	75
	283	10	3.5	90	234	6	2.6	51
Control	269	0	—	—	217	1	0.5	—
	217	0	—	—	211	1	0.5	—
	486	0	—	—	428	2	0.5	—

p = birth prevalence (per cent)
N = number of index patients
B = boy (male)
G = girl (female)
Σ = boy + girl

m = number of relatives studied
q = per cent of affected relatives $\left(\frac{M}{m} \cdot 100\right)$
M = number of affected relatives
K = q/p

In the seventies, the affected rate of index patients' parents was 2.1% (1.9%), while a 3.6% (4.9%) sib-occurrence was found in the group of *CL(P)*. The percentage rates of affected relatives in the previous study are shown in brackets. Additionally, three further sisters and one brother were mentioned by the affection of facial clefting, but they died and the necropsy report or adequate medical documents were not available. With the inclusion of these cases, the sib-occurrence would be 4.2%. Taking into consideration the retrospective approach and questionnaire method (owing to the incompleteness of ascertainment), our figures may be minimal. The h^2 was estimated as 0.77 ± 0.08 based on data of the first degree relatives in this study (Table III).

The affected rate of *CP* was 0.6% and 2.2% in parents while the sib-occurrence was 2.5% and 3.2%, respectively, in the sixties and seventies. Carter et al [1] found a $1.3 \pm 0.6\%$ sib-occurrence and a $2.9 \pm 0.9\%$ affection rate in children of probands. The estimate of h^2 was 0.82 ± 0.16.

The *MCA* group may involve cases of heterogeneous origin. The affected rate of parents was 1.3% while a 3.1% sib-occurrence was found. Out of 392 index patients, 26 had affected first degree relatives. It is worth evaluating these familial clusters separately.

TABLE III

Data of GAMT computer-program in CL(P) group
(Explanation of abbreviations in text)

S	P	TYPE	D	S*	P*	m	M	2	H2	H2L	H2U	—ML	CHI2	DF
CL(P)														
B	1.33	PR	1	B	1.33	395	6	15.19	0.56	0.32	0.77	1.83		
B	1.33	PR	1	G	0.77	398	8	20.10	0.76	0.55	0.96	1.97		
G	0.77	PR	1	B	1.33	223	8	35.87	0.78	0.56	0.97	1.97		
G	0.77	PR	1	G	0.77	225	4	17.78	0.68	0.40	0.94	1.63		
		P TOTAL											2.45	3
B	1.33	SB	1	B	1.33	216	9	41.67	0.88	0.64	1.07	2.03		
B	1.33	SB	1	G	0.77	232	5	21.55	0.78	0.50	1.04	1.74		
G	0.77	SB	1	B	1.33	146	8	54.79	0.92	0.67	1.12	1.97		
G	0.77	SB	1	G	0.77	129	4	31.01	0.86	0.53	1.12	1.63		
		SB TOTAL							0.86	0.74	0.98	7.61	0.48	3
			1 TOTAL						0.77	0.69	0.84	18.05	3.62	1
					1 + 2 + 3 TOTAL				0.78	0.68	0.84	18.10	0.10	0
					PR + 2 + 3 TOTAL				0.78	0.55	0.83	9.71	2.17	0
					SB DIFFERENCE								1.55	1
						BB TOTAL			0.70	0.54	0.85	5.74	3.76	3
						BG TOTAL			0.77	0.60	0.93	3.72	0.02	3
						GB TOTAL			0.84	0.68	0.98	4.30	0.72	3
						GG TOTAL			0.76	0.55	0.94	3.57	0.61	3
						SEX DIFFERENCE							1.56	3

Data of second (2) and third (3) degree relatives were omittel.

The 2,556 first degree relatives of 824 matched control cases had 2 CL(P). Both CAs occurred in the sisters and these were CL and CLP. This 0.08% observed rate somewhat lower than the expected one (0.2%), i.e., the combined birth prevalence of isolated and multiple CL(P) and CP. The difference could, however, be explained by chance.

The most plausible hypothesis to explain the aetiology of CL(P) is the multifactorial-threshold model on the basis of a number of other studies (e.g. 1), including our previous survey. In this second study the family patterns of CL(P) were tested again by the *GAMT* (Gaussian-Additive-Multifactorial-Threshold) program [7] for the confirmation or exclusion of the role of the multifactorial threshold model.

The calculation is based on the sex (S), i.e., boy (B) and girl (G), specified birth prevalences (P), i.e., parents (PR) or sibs (SB) and the type (D) and sex (S^x) specified expected and observed rates of first degree relatives $\left(q = \frac{M}{m} \cdot 1000\right)$. The principle of the GAMT program is that the theoretically expected h^2 (H 2) values ($\pm$ confidence limit: H2L and H2U) are estimated by the maximum likelihood method (—ML) in the different segments of relatives (degree, type, sex). The comparison of estimated h^2 figures may be done by the use of appropriate statistics of an CHI2 type asymptotic distribution depending on the degree of freedom (DF). If there is no significant difference between the expected and observed h^2 figures, this proves that the familial pattern fits the GAMT program, i.e., the multifactorial threshold model.

Results of the GAMT program in the *CL(P)* group are shown in Table III. The familial pattern corresponded well to the GAMT program in the different sex-specified affected relative segments, too. The figures of h^2 seemed to indicate the multifactorial threshold model in the aetiology of CL(P) (Table III). However, according to our previous study the difference of h^2 values was significant in parents (0.68 ± 0.12) and sibs (0.95 ± 0.14). It was opposed to the classical multifactorial threshold model [10] and was explained by reduced fertility, i.e., selection in parents and the dominance variance. This recent study has confirmed the difference of h^2 values in parents and sibs, but the deviation did not reach the level of significance. Thus it is possible to state that the most plausible aetiological explanation for the origin of CL(P) is the multifactorial threshold model. Of course, when the observed figures fit the expected ones based on a model does not prove unequivocally the confirmation of the hypothesis unless alternative models can be excluded. Significant progress would be expected with a comparative analysis of different aetiological models [17, 22, 23, 24, 27] in the same materials.

In the case of *CP*, as a whole, the familial patterns corresponded to the GAMT-program. Some specific data were, however, opposed to the multifactorial threshold model (e.g., h^2 ex-

ceeded 1.0 twice in sibs, while h^2 was O in two other segments). The explanation may be the low number of relatives and the heterogeneous origin of the CP group.

The multifactorial threshold model was excluded in the MCA group; h^2 was obviously O in the matched control group.

The *main conclusions of the family study* were as follows:

(i) In the seventies the rates of affected relatives in CL(P) and CP groups were somewhat higher than in the sixties. [There was only one exception, the sib-occurrence of CL(P) cases.] A slight methodological progress may explain this.

(ii) As a rule, the sib-occurrence was higher than the rate of affected parents. It indicates the selection and/or the dominance variance.

(iii) Both the specific rates of affected parents (2.4% and 2.2%) and the sib-occurrences (4.2% and 3.2%) in the CL(P) and CP groups showed considerable similarities.

(iv) The origin of the CL(P) group is explained by the multifactorial threshold model (i.e., its polygenic liability is triggered or suppressed by environmental factors). The CP group showed a contraversial picture. The familial pattern, as a whole, fitted the GAMT-program, but some details were against it. This indicated a heterogeneous origin of the CP group. The familial cluster of MCAs did not fit the multifactorial threshold model; these groups involved different CA-entities of heterogeneous origin.

The occurrence of *non-specific CAs* was not higher in the first degree relatives of index patients than in those of the control cases (Table IV). The affected rate of relatives did not exceed the exceed the expected total prevalence of CAs (i.e., 6%), based on Hungarian experience. There was only one exception: the nearly 10% sister-occurrence in the CP group.

Particular stress was laid on the evaluation of other, so-called non-specific CA types in sibs of index patients with CL(P) and CP (Table V). (Data of parents were excluded owing to incompleteness caused by the selection and different levels of medical care.) The expected figures (E) were estimated on the basis of true birth prevalences (p) of CAs and the number of sibs (m). For evaluation of the comparison between the expected and observed figures (O) the χ^2 test was used. Only three significant differences were found in groups CL(P) and CP. Out of three, two were the *specific* familial cluster. The third one was a significantly lower figure in the group of congenital dislocation of the hip. The explanation may be an under-ascertainment. Five neural tube defects in sibs of CL(P) cases did not exceed the 0.05 level of significance in this study (Figs 1–5), however, their combination with some other materials of CL(P) indicated a higher sib-occurrence [14]. The relationship of these schisis-type CAs was published earlier [9]. According to the expectation, the other types of facial clefting did not occur more frequently in sibs of specified facial clefting groups, proving their independence

TABLE IV

Occurrence of non-specific (NS) congenital anomalies in the first degree relatives of index patients studied. (Facial cleftings and minor anomalies were excluded)

Group	Father			Mother			Brother			Sister		
	m	M_{NS}	q	m	M_{NS}	q	m	M_{NS}	q	m	M_{NS}	q
CL(P) (N = 630)	618	9*	1.5	623	8*	1.3	362	15*	4.1	361	16*	4.4
CP (N = 179)	178	2**	1.1	179	4**	2.2	93	4**	4.3	92	6**	6.5
MCA (N = 392)	380	3°	0.8	387	7°	1.8	283	10°	3.5	234	10°	4.3
Control (N = 824)	819	7°°	0.9	823	5°°	0.6	486	11°°	2.3	428	13°°	3.0

* dislocation of hip 1
cong. clubfoot 4
heart defect 1
cong. inguinal hernia 1
syndactyly 1
cong. myopia 1

** dislocation of hip 1
renal agenesis, unilat. 1

° polydactyly 2
spina bifida occulta 1

°° dislocation of hip 1
cong. clubfoot 1
syndactyly 1
limb reduction 1
cong. inguinal hernia 1
renal agenesis, unilat. 1
undescended testis, unilat. 1

* dislocation of hip 3
heart defect 2
polydactyly-syndactyly 1
spina bifida occulta 1
tongue defect 1

** dislocation of hip 2
cong. scoliosis 1
cong. myopia 1

° dislocation of hip 4
cong. clubfoot 1
heart defect 1
polydactyly 1

°° dislocation of hip 3
scoliosis 2

* dislocation of hip 1
cong. clubfoot 3
heart defect 1
pyloric stenosis 1
polydactyly 1
undescended testes 1
auricular CA 1
pectus excavatum 1
MCA (heart defect + renal agenesis, unilat.) 1
cong. inguinal hernia 4

** heart defect 1
pyloric stenosis 1
cong. clubfoot 1
cong. inguinal hernia 1

° heart defect 3
polydactyly 1
undescended testis 1
pyloric stenosis 1
spina bifida cystica 1
biliar atresia 1
multiple CA 2

°° dislocation of hip 1
cong. clubfoot 1
spina bifida cystica 2
cong. inguinal hernia 2
undescended testis 2
Down 1
heart defect 1
hypospadias 1

* dislocation of hip 4
cong. clubfoot 4
heart defect 3
anencephaly 3
spina bifida cystica 2

** dislocation of hip 3
heart defect 2
microphthalmia 1

° dislocation of hip 5
anencephaly + spina bifida 1
spina bifida cystica 1
haemangioma 3

°° dislocation of hip 3
scoliosis 1
heart defect 1
cong. inguinal hernia 3
pyloric stenosis 1
renal dysplasia 1
syndactyly 1
cong. clubfoot 2

TABLE V

Occurrence of non-specific type CAs in the sibs of index patients

Congenital abnormality (CA)	CL(P) (m = 723)		CP (m = 185)		Control (m = 914)	
	E	O	E	O	E	O
Anencephaly-spina bifida cystica (p = 2.6)	1.9	5	0.5	0°	2.4	2
Cleft lip ± palate (p = 1.0)	0.7	26□	1.2	1	0.9	2
Cleft palate (p = 0.4)	0.3	2	0.1	6□	0.3	0
Eye CAs (p = 0.5)	3.6	4	0.9	1	4.6	4
Ear CAs (p = 3.0)	2.2	4	0.6	0	2.7	1
Cardiovascular CAs (p = 10.7)	7.7	5°°	2.0	3	9.8	2*
Pyloric stenosis (p = 1.5)	1.1	1	0.3	1	1.4	1
Urogenital CAs (p = 3.0)	2.2	1°°	0.6	0	2.7	1
Hypospadias (p = 2.2)	1.6	0	0.4	0	2.0	1
Undescended testis (p = 7.8)	5.6	1	0.4	0	7.1	2
Cong. inguinal hernia (p = 11.4)	8.2	4	2.1	1	10.4	5
Cong. dislocation of hip (p = 28.0)	20.2	5x	5.2	3	25.6	4x
Clubfoot (p = 10.0)	7.2	7	1.9	1	9.1	3
Poly- and/or syndactyly (p = 0.5)	0.4	1	0.1	0	0.5	1
Other limb CAs (p = 0.6)	0.4	0	0.1	0	0.6	0
Vertebral and rib CAs (p = 0.5)	0.4	1	0.1	0	0.5	1
Down (p = 1.2)	0.9	0	0.2	0	1.1	0
Other CAs (~p = 10.0)	7.2	9*	1.9	4**	9.1	7***
Total	71.7	76	18.6	21	90.8	37*

□ = occurrence of specific type CA
x = $p < 0.05$
° = Anencephaly stated without medical record
°° = one component CA of multiple CA
* = haemangioma 5, hydrocele testis 3, micrognathia 1
** = haemangioma 4
*** = Down syndrome 1, tongue CA 1, haemangioma 3, torticollis 2

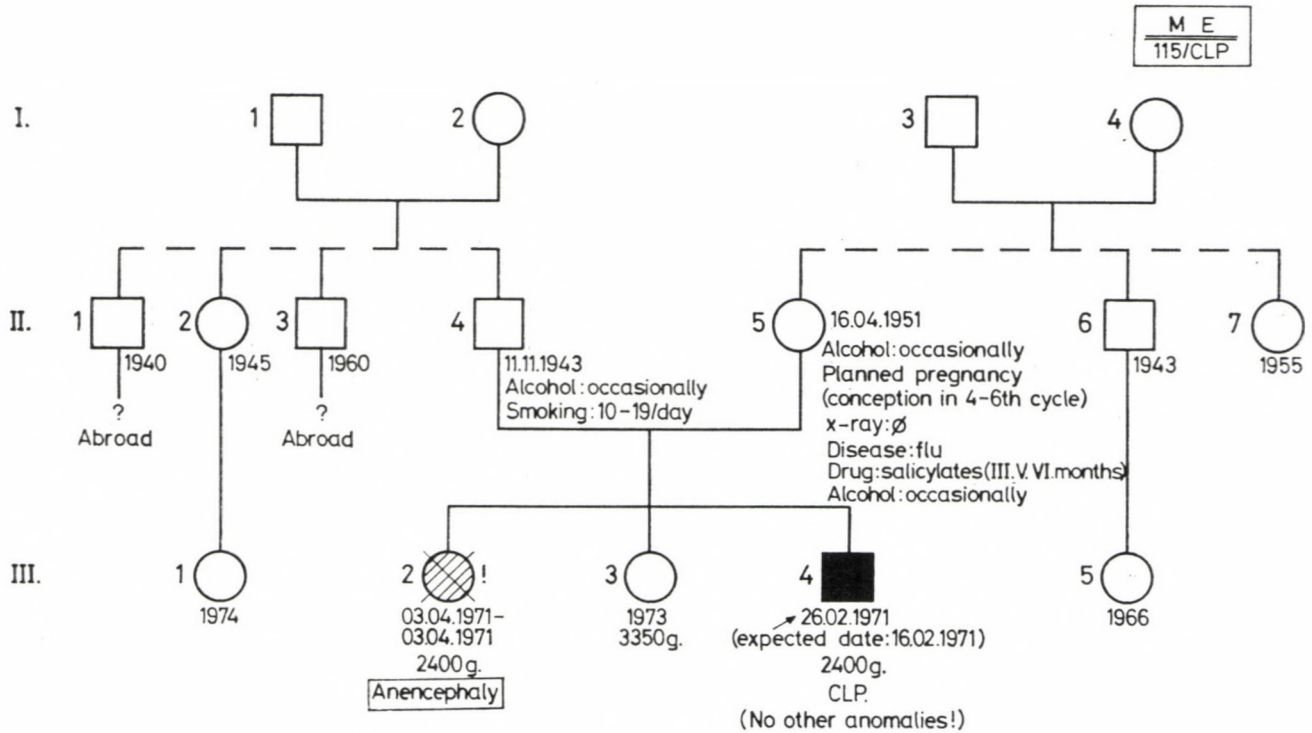

FIG. 1. Pedigree of Case 115

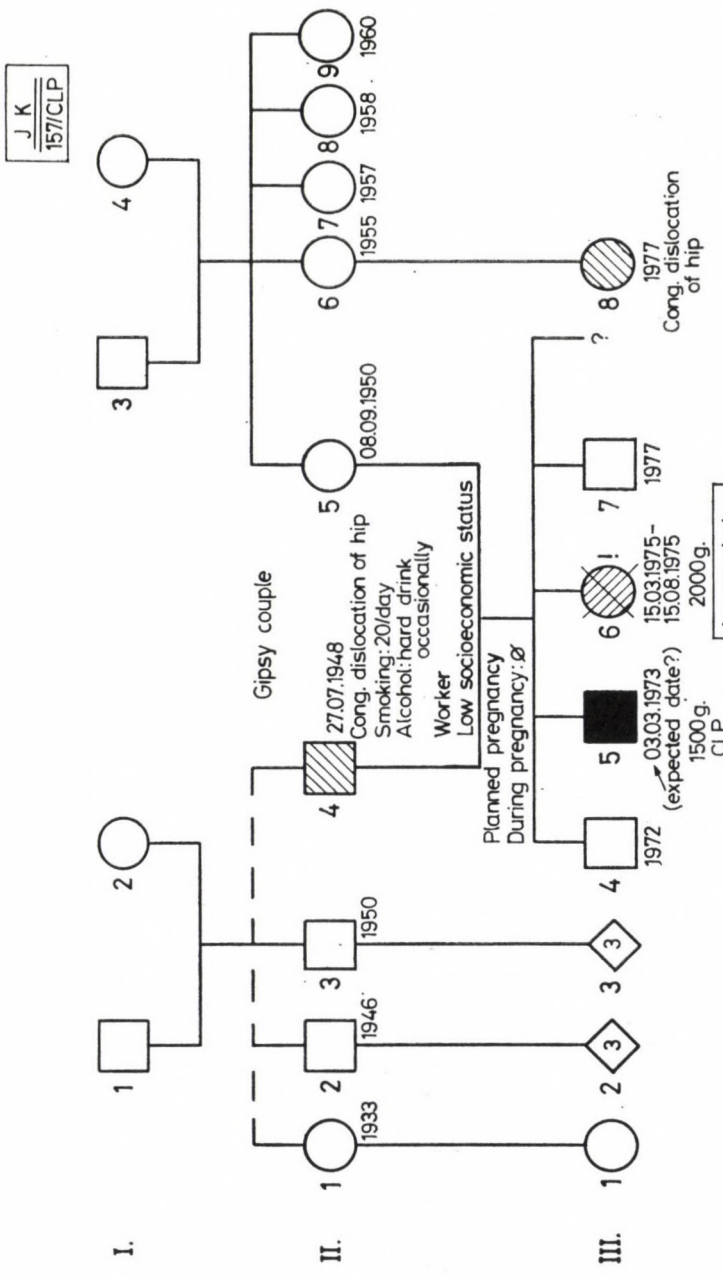

FIG. 2. Pedigree of Case 157

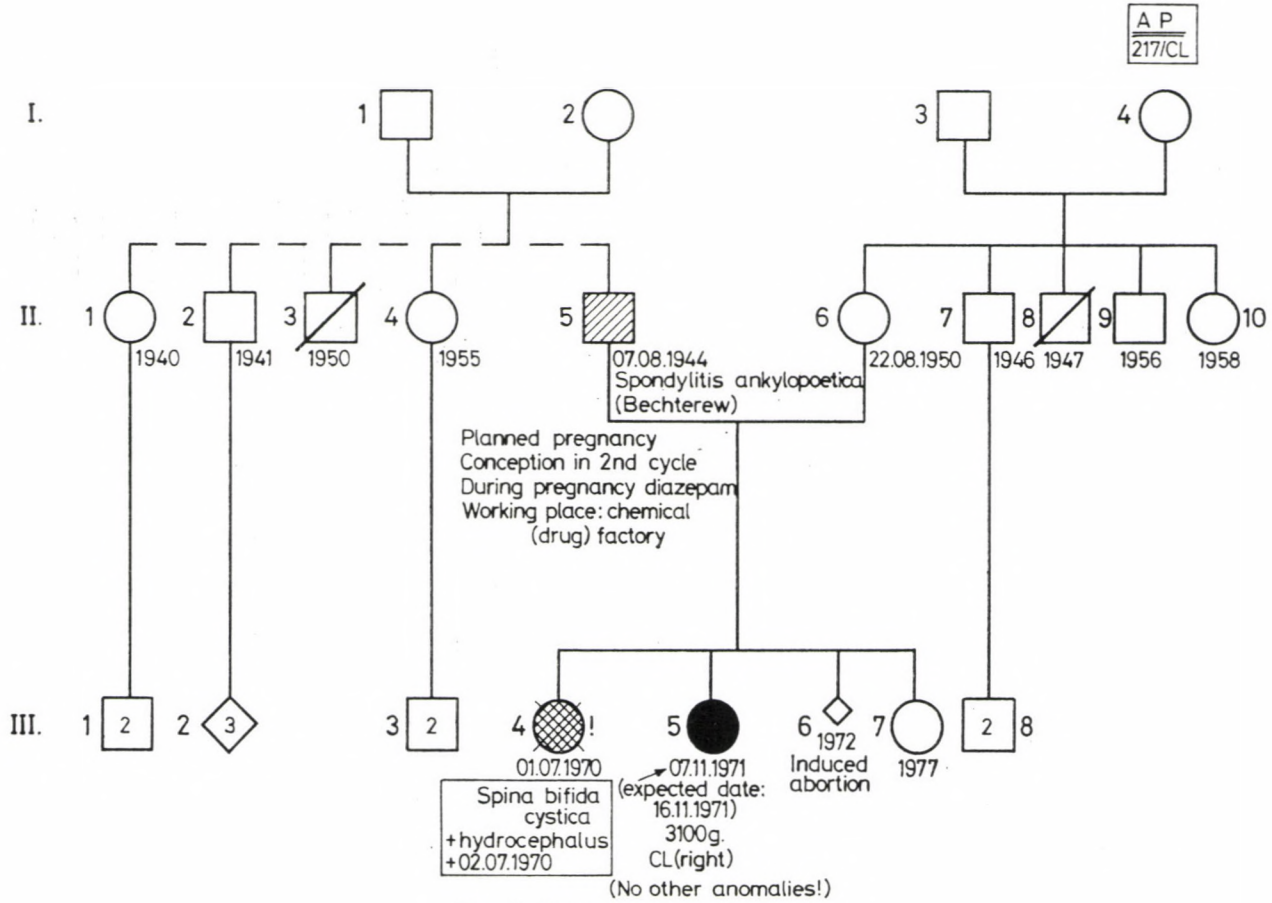

FIG. 3. Pedigree of Case 217

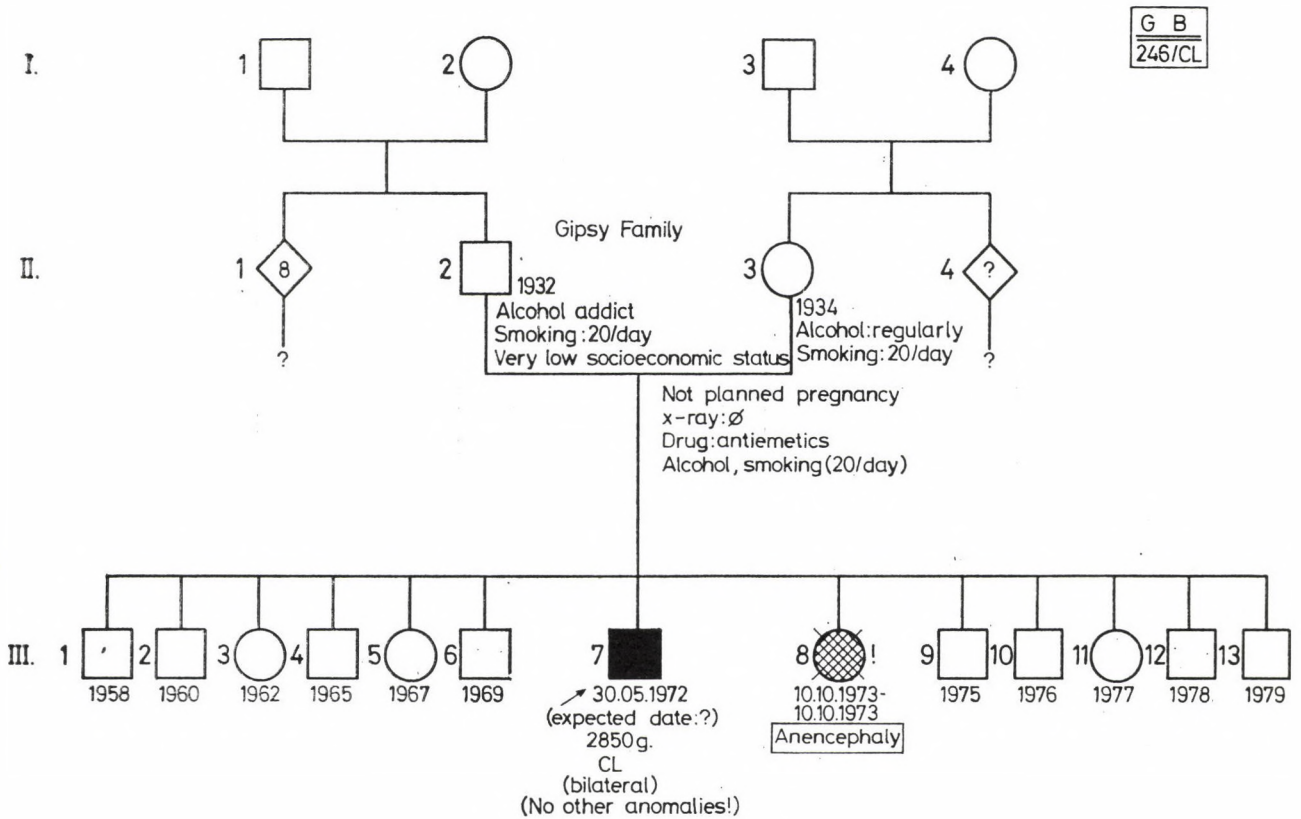

FIG. 4. Pedigree of Case 246

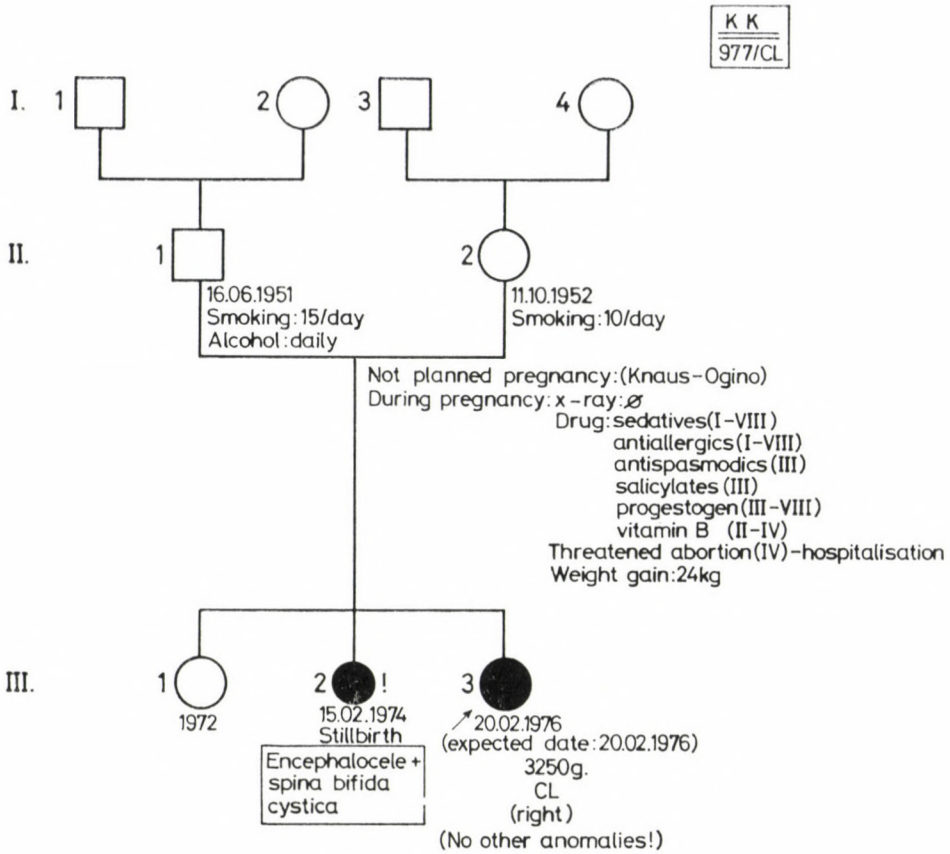

FIG. 5. Pedigree of Case 977

and different origin. In the control group the observed occurrence of liability for dislocation of the hip, congenital cardiovascular malformations and the total CAs were significantly lower, showing the ascertainment bias.

Prenatal selection may modify the specific and non-specific occurrence of CAs in sibs, therefore the outcome of previous and subsequent pregnancies of the mothers of index patients were also evaluated (Table VI). The rates of spontaneous abortion were significantly higher in the group of MCAs before ($\chi^2 = 6.87$; $p < 0.05$) and after ($\chi^2 = 12.28$; $p < 0.001$) the birth of index patients. The high rates of spontaneous abortion and the subsequent stillbirth did not exceed the significance level in the CP group owing to the low number of cases. Furthermore a lower per cent of subsequent induced abortions is worth mentioning in the MCA group ($\chi^2 = 17.96$; $p < 0.001$).

The data presented indicate the effect of prenatal selection for the

TABLE VI

Previous and subsequent pregnancy outcomes

Group	Previous pregnancies								Total pregnancy°	
	Induced abortion		Spontaneous abortion*		Stillbirth**		Livebirth°			
	No.	%	No.	%	No.	%	No.	$\bar{x}$	No.	$\bar{x}$
CL(P) (N = 630)	179	22.7	119 (1)	19.5	19	3.7	473	0.75	790	1.25
CP (N = 179)	45	20.4	40	22.9	5	3.7	130	0.73	220	1.23
MCA (N = 392)	146	25.9	87 (5)	20.9	11	3.3	319	0.81	563	1.44
Control (N = 824)	274	27.2	136 (2)	18.6	28	4.7	569	0.69	1007	1.22

* $\frac{\text{No. of spontaneous abortion}}{\text{No. of total birth} - \text{No. of induced abortion}} \cdot 100$

Ectopic pregnancies were included into spontaneous abortions, their absolute numbers are shown in brackets

sib-occurrence of MCAs. After the birth of index patients with MCA which is often lethal, the parents wanted more children or at least they terminated their pregnancies less often.

Summing up the results of genetic approach, the multifactorial threshold model seems to be the most plausible explanation of the origin of CL(P) with considerable polygenic liability (77%). On the other hand, CP and MCA groups may represent several entities of different origin.

Teratology

Owing to the high value of h^2 in the groups of CL(P) and CP, single and decisive environmental factors could not be expected. The triggering and suppressing external effects, i.e., in a narrow sense the teratogens and the possible maternal factors may, however, be important.

First, the *circumstances of conception* were studied, because these may be important from the teratological point of view. Only the MCA group had a significantly higher proportion of unplanned pregnancies than the control group (Table VII). Among unplanned pregnancies the failure of the calendar method (0.6–3.5 *vs* 2.4), coitus interruptus (7.0–10.8 *vs* 10.1), oral contraceptives (1.1–2.9 *vs* 1.3), condom-pessarium (0.3–1.1 *vs* 0.1), IUD (0.0–0.3 *vs* 0.0) did not show significant differences between groups of facial clefting and total matched controls. The time interval (number of female cycles) between the discontinuation of contraception or the beginning of sexual intercourse and the conception was studied in the cases of planned pregnancies (Table VIII). Owing to the high and different proportion of unknown figures,

of the mothers of index patients

Subsequent pregnancies								Total pregnancy°		Grand total°	
Induced abortion		Spontaneous abortion*		Stillbirth**		Livebirth°					
No.	%	No.	%	No.	%	No.	$\bar{x}$	No.	$\bar{x}$	No.	$\bar{x}$
115	28.8	30 (1)	10.5	5	2.0	250	0.40	400	0.63	1190	1.89
34	31.8	13	17.8	5	8.3	55	0.31	107	0.60	327	1.83
59	19.4	45 (2)	18.4	2	1.0	198	0.51	304	0.78	867	2.21
214	34.9	49 (3)	12.3	5	1.4	345	0.42	613	0.74	1620	1.97

** $\frac{\text{No. of stillbirth}}{\text{No. of total births}} \cdot 100$ ° $\frac{\text{No. of given pregnancy outcomes}}{\text{No. of index patients}} = \bar{x}$

it was difficult to evaluate this variable. The unknown percentage was significantly higher in the MCA group. The CP group had a considerably higher per cent of late conception (after the 10th month).

The duration of *working during pregnancy* and the possible dangerous occupational exposures (radiation, microbial, chemical, noise) were also studied, but no considerable differences were found between the study and control groups. Thus, we could not confirm the relation between facial clefting and organic solvent exposure during pregnancy [16].

Next, the so-called teratogens were analysed. In general, the occurrence of diagnostic *abdominal X-rays*, mechanical trauma and psychological

TABLE VII

Proportion of planned pregnancies which ended in birth of index patients and matched controls

Group	Planned		Unplanned		Unknown	
	No.	%	No.	%	No.	%
CL(P) (N = 630)	499	79.2	110	17.5	21	3.3
CP (N = 179)	146	81.6	25	14.0	8	4.4
MCA (N = 392)	297	75.8	82	20.9	13	3.3
Control (N = 824)	671	81.4	137	16.6	16	1.9

TABLE VIII

Time interval (number of female cycles) between beginning of reproductive activity and conception. (In brackets the percentage figures are shown)

Group \ Number of cycles	1	2	3	4–5	6–9	10–12	12–	Subtotal	Unknown
CL(P) (N = 630)	111 (28.3)	84 (21.4)	49 (12.5)	45 (11.5)	43 (11.0)	25 (6.4)	35 (8.9)	392	107 (21.4)
CP (N = 179)	32 (29.4)	18 (16.5)	11 (10.1)	13 (11.9)	13 (11.9)	8 (7.4)	14 (12.8)	109	37 (25.3)
MCA (N = 392)	62 (28.3)	39 (17.8)	32 (14.6)	28 (12.8)	29 (13.2)	10 (4.6)	19 (8.7)	219	173 (44.9)
Control (N = 824)	169 (30.0)	97 (17.2)	94 (16.7)	71 (12.6)	54 (9.6)	27 (4.8)	51 (9.1)	563	108 (16.1)

stress (Table IX) was higher in the critical period of CAs studied, i.e., roughly in the first trimester of gestation. This was the case in the next two trimesters of pregnancy as well, except for psychological stress. Thus the separation of true impacts from the recall bias was difficult. The role of psychological stress was discussed several times in the aetiology of facial clefting [12] but this has not been confirmed in human beings.

Maternal disorders including microbial infections were evaluated independently for the duration of pregnancy (Table X). Influenza or influenza-like diseases (so-called "flu") during pregnancy were mentioned more frequently by the mothers of index patients in all groups. There are, however, two important arguments against the role of flu in the aetiology of facial cleftings. First, the flu occurred after the critical period

TABLE IX

Occurrence of so-called physical teratogens and psychological stress

Group	Diagnostic abdominal X-ray			Mechanical trauma			Psychological stress		
	Month		Total	Month		Total	Month		Total
	1–3	4–9		1–3	4–9		1–3	4–9	
CL(P) (N = 630)	1 (0.2)	9 1.4	10 1.6	18 2.9	27 4.3	45 7.1	51 8.1	30 4.8	81 12.9
CP (N = 179)	0 —	1 (0.6)	1 (0.6)	0 —	11 6.1	11 6.1	10 5.6	13 7.3	23 12.8
MCA (N = 392)	1 (0.3)	4 (1.0)	5 1.3	5 1.3	10 2.6	15 3.8	24 6.1	19 4.8	43 11.0
Control (N = 824)	0 —	2 (0.2)	2 0.2	5 0.6	6 0.7	11 1.3	29 3.5	42 5.1	71 8.6

TABLE X

Maternal disorders during pregnancy (If there were several diseases, only the most serious one was considered)

Group	No occurrence	Rubella	Flu	Urinary infection or disease	Respiratory infection or disease	Unindentified fever	Liver disease	Mumps	Hypertension	Epilepsy	Anaemia	Others	Total
CL(P)	359	1	175	39	11	7	6	5	7	5	10	5	271
(N = 630)	57.0	0.2	27.8	6.2	1.7	1.1	1.0	0.8	1.1	0.8	1.6	0.8	43.0
CP	99	1	56	7	2	0	1	0	3	1	8	1	80
(N = 179)	55.3	0.6	31.3	3.9	1.1	—	0.6	—	1.7	0.6	4.5	0.6	44.7
MCA	222	6	119	17	9	3	1	1	6	1	5	2	170
(N = 392)	56.6	1.5	30.4	4.3	2.3	0.8	0.3	0.3	1.5	0.3	1.3	0.5	43.4
Control	648	1	92	25	14	4	8	3	13	2	9	5	176
(N = 824)	78.6	0.1	11.2	3.0	1.7	0.5	1.0	0.4	1.6	0.2	1.1	0.6	21.4

of facial cleftings in the majority of cases. Furthermore, the detailed analyses of the correlation between the time of influenza epidemics in Hungary and the monthly distribution of CL(P) and CP did not give a positive result (Fig 6), i.e., birth prevalences of facial clefting did not increase after influenza-epidemics. Previously, Leck [19] and Leck et al [20] found a high rate of facial clefting in index patients born 6–9 months after influenza epidemics, although not in any for whom the related epidemic was the initial outbreak of A2 influenza. However, later Leck [21] wrote: "it is difficult to believe that maternal exposure to infection caused the defects in these children, since the epidemics apparently happened when many of those concerned had already passed the stage at which facial clefting is laid down".

Furthermore, 6 rubella infections and/or diseases were noteworthy in the group of MCAs [13]. Out of 9 mumps infections 2, 3, 2 and 2 occurred in the 2nd, 3rd, 4th and 6th month of gestation, respectively. Finally, within the CL(P) group 5 mothers had epilepsy and were treated with anticonvulsants during pregnancy. It is well known that facial clefting is a principal component CA in both the fetal hydantoin and the fetal trimethadione syndromes.

Drug ingestion during pregnancy was also analysed (Table XI). The per cent of no drug use was significantly lower in the MCA group ($\chi^2 = 4.12$; $p < 0.05$). The recorded occurrence of drugs usual in prenatal care, i.e. different vitamins, iron and calcium preparations did not show any significant difference among the groups, and this may be an argument against the recall bias. Anticonvulsants were used by the mother of 11 index patients with CL(P) and caused a significant increase ($\chi^2 = 9.11$; $p < 0.01$). (It is disturbing that only 5 epilepsies were mentioned among the

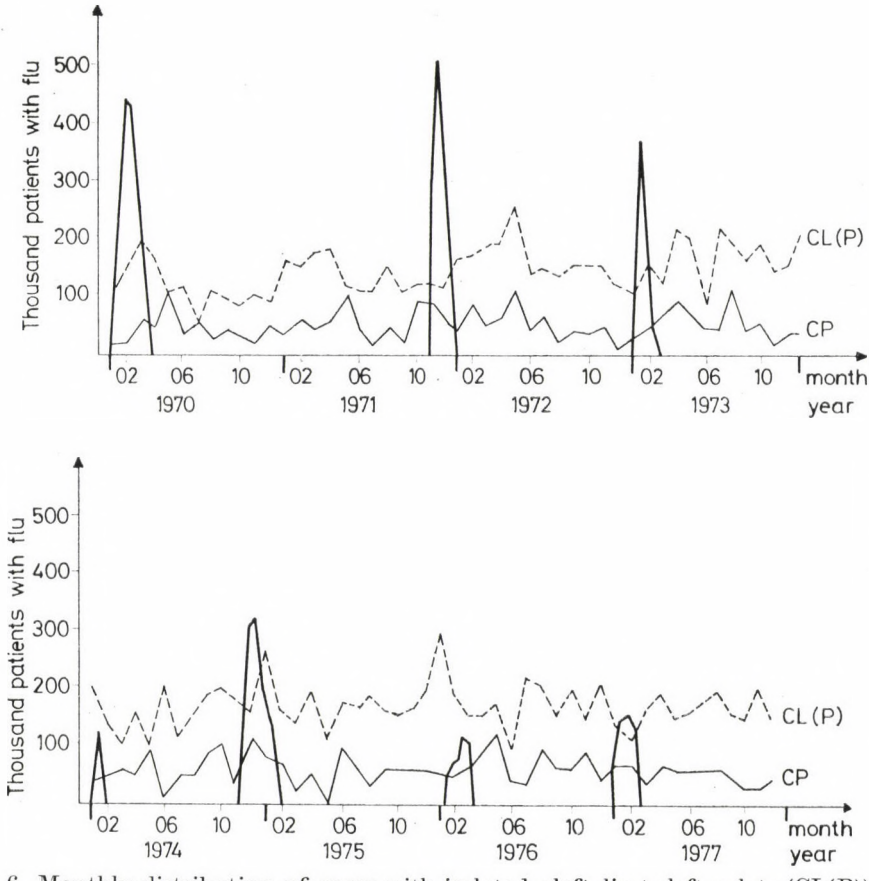

FIG. 6. Monthly distribution of cases with isolated cleft lip±cleft palate (CL(P)) and cleft palate (CP) and the time of influenza epidemics in Hungary, 1970-1976

maternal disorders.) All anticonvulsants had been taken in the first month of pregnancy as well. As it was mentioned previously, three fetal hydantoin syndromes were excluded. The higher frequency of isolated CL(P) after the ingestion of hydantoin (4), trimethadione (3) and primidone (2) indicated that in general practice mainly CL(P) is diagnosed after anticonvulsant treatment.

The use of antibiotics ($\chi^2 = 20.65$; $p < 0.001$), chemotherapeutics ($\chi^2 = 10.84$; $p < 0.01$), antipyretics ($\chi^2 = 40.20$; $p < 0.001$), analgesics-antispasmodics ($\chi^2 = 28.59$; $p < 0.001$) and sedatives ($\chi^2 = 9.41$; $p < 0.001$) was also more frequent in the mothers of CL(P) index patients than those of the control group.

The use of antiemetics was significantly lower in the CL(P) group, though this type of drug was supposed to play a role in the aetiology of facial clefting [15, 26]. In the CP group antibiotics ($\chi^2 = 6.43$; $p < 0.05$)

Table XI

Drug ingestion during pregnancy

Group	No drug use	Drug use during pregnancy	Hormones						Antibiotics						Chemotherapeutics			
			Oral contraceptives	Oestrogens	Progesterone	Methandrostenolone	Prednisolone	Insulin	Penicillins	Streptomycin Isoniazid	Chloramphenicol	Oxytetracycline	Moroxydine	Nalidixic acid	Sulfonamides	Ninofurantoin	Metronidazole	Natamycin
CL(P)	196	434	25	13	73	1	0	0	55	2	4	11	0	1	15	15	3	0
N = 630	31.1		*5.8*	*3.0*	16.8	0.2	—	—	*12.7*	0.5	0.9	*2.5*	—	0.2	*3.5*	*3.5*	0.7	—
CP	58	121	1	2	30	0	0	1	17	0	1	0	0	0	1	3	0	0
N = 179	32.4		0.8	1.7	24.8	—	—	0.8	*14.0*	—	0.8	—	—	—	0.8	*2.5*	—	—
MCA	96	296	17	10	74	0	1	0	45	1	1	9	0	0	6	5	1	2
N = 392	24.5		*5.7*	*3.4*	25.0	—	0.3	—	*15.2*	0.3	0.3	*3.0*	—	—	2.0	1.7	0.3	0.7
Control	248	576	15	6	117	0	1	0	31	1	4	5	0	1	10	5	2	0
N = 824	30.1		2.6	1.0	20.3	—	0.2	—	5.4	0.2	0.7	0.9	—	0.2	1.7	0.9	0.3	—

Group	Antipyretic and Anti-inflammatory Agents			Analgesics				Antiemetics		Antihypertensive Agents	
	Salicylates	Aminophenazone	Oxyquinoline sulphonic acid	Codeine and its salts	Amobarbital	Combinations	Spasmolytics	Dimenhydrinate	Thioethylperazine	Reserpine	Reserpine + hydrochlorothiazide
CL(P)	66	24	6	6	1	35	28	75	12	2	0
N = 630	*15.2*	*5.5*	*1.4*	1.4	0.2	8.1	6.5	17.3	2.8	0.5	—
CP	13	3	1	0	0	5	7	24	8	2	0
N = 179	*10.7*	2.5	0.8	—	—	4.1	5.8	19.8	6.6	1.7	—
MCA	47	12	4	2	0	14	23	52	9	1	1
N = 392	*15.9*	*4.1*	1.4	0.7	—	4.7	2.3	17.6	3.0	0.3	0.3
Control	36	8	0	3	0	12	17	119	24	6	0
N = 824	6.3	1.4	—	0.5	—	2.1	3.0	20.7	42	1.0	—

Group	Sedatives–Tranquillizers													Anticonvulsants										
	Barbital	Promethazine	Metofenazin	Chlorpromazine	Chlordiazepoxide	Amitriptyline	Nitrazepam	Levopromazine	Glutethimid	Diazepam	Methylpentynol	Meprobamate	Phenobarbital	Phenytoin	Phenacemide	Pirimidone	Mephenytoin	Morpholep	Sultiame	Trimethadione	Together	Vitamins	Iron and iron salts	Calcium
CL(P)	5	48	4	2	4	0	1	0	1	16	1	12	5	2	0	2	2	1	1	3	11	36	25	0
N = 630	*1.2*	11.1	0.9	0.5	0.9	—	0.2	—	0.2	3.7	0.2	2.8	1.2	0.5	—	0.5	0.5	0.2	0.2	0.7	*2.5*	8.3	5.8	—
CP	1	16	1	0	2	0	0	0	0	3	0	2	0	0	0	0	0	0	0	0	0	7	3	0
N = 179	0.8	13.2	0.8	—	1.7	—	—	—	—	2.5	—	1.7	—	—	—	—	—	—	—	—	—	5.8	2.5	—
MCA	5	33	0	2	5	1	2	1	0	13	1	5	8	0	1	0	0	0	0	1	2	21	10	2
N = 392	*1.7*	11.1	—	0.7	1.7	0.3	0.7	0.3	—	*4.4*	0.3	1.7	2.7	—	0.3	—	—	—	—	0.3	0.7	7.1	3.4	0.7
Control	1	57	0	2	3	0	1	0	0	16	0	3	5	1	0	1	0	0	0	0	2	41	36	3
N = 824	0.2	9.9	—	0.3	0.5	—	0.2	—	—	2.8	—	0.5	0.9	0.2	—	0.2	—	—	—	—	0.3	7.1	6.3	0.5

Significant increases are indicated by italics

and antipyretics ($\chi^2 = 4.45$; $p < 0.05$), in the group of MCAs antipyretics ($\chi^2 = 38.12$; $p < 0.001$), some antibiotics ($\chi^2 = 17.78$; $p < 0.01$), sedatives ($\chi^2 = 19.03$; $p < 0.001$), some hormones ($\chi^2 = 14.00$; $p < 0.001$) (mainly hormonal supportive therapy) were reported more often.

These differences of several types of drug use during pregnancy may be embarrassing for experts because they indicate mainly the effect of recall bias. The analysis of time-distribution of the above-mentioned drugs with a significant increase during pregnancy showed that the majority had been taken *after* the critical period of facial clefting.

Alcohol consumption during pregnancy did not show any significant difference among the groups (Table XII). Only the per cent of hard drinkers was higher in the CP group ($\chi^2 = 9.41$; $p < 0.01$).

The possible aetiological role of *smoking* was also raised in the literature [11, 26]. The per cent of non-smoker mothers was significantly lower in the CL(P) group ($\chi^2 = 6.31$; $p < 0.05$) (Table XIII). Accordingly,

TABLE XII

Maternal alcohol consumption

Group	Alcohol consumption							
	Total abstinence	Only before conception	Only 0–3 months		Whole pregnancy		Hard drink	
			occasionally	habitually	occasionally	habitually	No.	%
CL(P)	442	28	12	0	144	4	70	11.1
(N = 630)	70.2	4.4	1.9	—	22.9	0.6		
CP	99	23	7	0	48	2	31	17.3
(N = 179)	55.3	12.8	3.9	—	26.8	1.1		
MCA	272	14	6	1	101	3	46	11.7
(N = 392)	69.4	3.6	1.5	0.3	25.8	0.8		
Control	555	6	6	0	251	6	87	10.6
(N = 824)	67.4	0.7	0.7	—	30.5	0.7		

TABLE XIII

Maternal smoking

Group	No smoking	Only before conception	Only 0–3 months			Whole pregnancy			Total
			0–10	11–20	21–	0–10	11–20	21–	
CL(P)	418	30	30	4	0	100	25	23	148
(N = 630)	66.3	4.8	4.8	0.6	0.0	15.9	4.0	3.7	23.5
CP	130	16	9	0	0	12	10	2	24
(N = 179)	72.6	8.9	5.0	0.0	0.0	6.7	5.6	1.1	13.4
MCA	302	7	14	3	0	35	16	15	66
(N = 392)	77.0	1.8	3.6	0.8	0.0	8.9	4.1	3.8	16.8
Control	597	30	32	4	6	89	43	23	155
(N = 824)	72.5	3.6	3.9	0.5	0.7	10.8	5.2	2.8	18.8

TABLE XIV

Symptoms of early toxaemia

Group	Pronounced nausea				Total	Continuous and strong vomitus				Total	Weight*			Together
	No occurrence	Months				No occurrence	Months				Gain 7 kg or more	0–6 kg	Loss	
		1–3	4–6	Whole			1–3	4–6	Whole					
CL(P)	344	148	90	48	286	533	54	26	17	97	561	63	6	69
(N = 630)	54.6	23.5	14.3	7.6	45.4	84.6	8.6	4.1	2.7	15.4	89.1	10.0	1.0	10.9
CP	97	52	13	17	82	145	19	6	9	34	158	19	2	21
(N = 179)	54.2	29.0	7.3	9.5	45.8	81.0	10.6	3.4	5.9	19.0	88.3	10.6	1.1	11.7
MCA	209	96	57	30	183	320	35	26	11	72	324	60	8	68
(N = 392)	53.3	24.4	14.5	7.7	46.7	81.6	8.9	6.6	2.8	18.4	82.7	15.3	2.0	17.3
Control	428	206	131	59	396	682	76	38	28	142	730	91	3	94
(N = 824)	51.9	25.0	15.9	7.2	48.1	82.8	9.2	4.6	3.4	17.2	88.6	11.0	0.4	11.4

* During the first six months of gestation

TABLE XV

Symptoms of late toxaemia

Group	Symptoms								Total	Weight gain		Together
	No occurrence	H	P	O	H + P	H + O	P + O	H + P + O		16–19 kg	20 kg or more	
CL(P)	389	26	33	142	0	18	15	7	241	61	54	115
(N = 630)	61.7	4.1	5.2	22.5	0.0	2.9	2.4	1.1	38.3	9.7	8.6	18.3
CP	111	7	5	39	3	4	8	2	68	16	12	28
(N = 179)	62.0	3.9	2.8	21.8	1.7	2.2	4.5	1.1	38.0	8.9	6.7	15.6
MCA	223	29	7	97	4	14	10	8	169	24	28	52
(N = 392)	56.9	7.4	1.8	24.7	1.0	3.6	2.6	2.0	43.1	6.1	7.1	13.3
Control	454	76	28	198	7	38	10	13	370	55	54	109
(N = 824)	55.1	9.2	3.4	24.0	0.8	4.6	1.2	1.6	44.9	6.8	6.6	13.2

Abbreviation:
H = Hypertension (>150 mm Hg)
P = Proteinuria
O = Oedema in leg

TABLE XVI

Occurrence of threatened abortion

Group	No occurrence	1–3 months				Total	4–9 months				Total
		V	U	V + U	H		V	U	V + U	H	
CL(P)	409	32	17	5	41	95	10	25	3	88	126
(N = 630)	64.9	5.1	2.7	0.8	6.5	15.1	1.6	4.0	0.5	14.0	20.0
CP	122	11	6	2	12	31	2	10	0	14	26
(N = 179)	68.2	6.1	3.3	1.1	6.7	17.3	1.1	5.6	—	7.8	14.5
MCA	220	14	16	7	29	66	7	33	3	63	106
(N = 392)	56.1	3.6	4.1	1.8	7.4	16.8	1.8	8.4	0.8	16.1	27.0
Control	577	29	8	4	51	92	13	31	0	111	155
(N = 824)	70.0	3.5	1.0	0.5	6.2	11.2	1.6	3.8	—	13.5	18.8

V = vaginal bleeding
U = uterine contraction
H = hospitalization

the rate of smokers during the first three months of gestation and the whole pregnancy was somewhat higher in the CL(P) group than in the total control group.

Finally, some categories of *pregnancy complication* were evaluated. The symptoms of early toxaemia, i.e. pronounced nausea as well as continuous and strong vomitus did not occur more frequently in the pregnancies of the mothers of index patients (Table XIV). The reported occurrence of single, pair and triplet symptoms of late toxaemia was not higher in the group of index patients (Table XV). There was only one exception: an extreme weight gain occurred more frequently in the CL(P) group.

The occurrence of *threatened abortion* was analysed on the basis of vaginal bleedings, uterine contractions, their combination, and hospitalization (Table XVI). While the mothers of index patients reported a higher rate of vaginal bleeding and uterine contraction in the first trimester, the combination of these symptoms, and mainly the hospitalization, had a similar occurrence in the study and the control groups. There was a higher rate of symptoms in the second and third trimesters in the MCA group ($\chi^2 = 22.30$; $p < 0.001$).

Consequently, a number of possible teratogens and maternal factors were found in this study, but our conclusions have to be limited, owing to the well-known difficulties of a retrospective epidemiological approach (ascertainment and recall bias). Nevertheless, the triggering impact of certain types of anticonvulsant seemed to be well-founded.

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Book reviews

H Ewerbeck: *Differentialdiagnose von Krankheiten im Kindesalter.* 2., vollständig überarbeitete Auflage. XIV + 318 Seiten mit 23 Tabellen. Springer-Verlag, Berlin–Heidelberg–New York–Tokyo 1984. Preis DM 58,—

Den berechtigten Anspruch auf eine Neuauflage dieses ausgezeichneten Werkes haben das weltweite Interesse und die zahlreichen Übersetzungen bewiesen. In dem vorliegenden Band bot sich die Gelegenheit, neue Ergebnisse, weitere Krankheitsbilder und bei schon früher geschilderten Krankheiten neue pathogenetische Gesichtspunkte zu berücksichtigen. Das ursprüngliche Konzept — den Leser in unklaren Fällen von den auffälligsten Symptomen ohne Irrwege und unnötige Untersuchungen zur richtigen Diagnose zu verhelfen — wurde beibehalten. Beibehalten wurde auch die ursprüngliche Einteilung. In den einzelnen Kapiteln findet man jedoch viele neue Krankheitsbilder, solche, deren Bedeutung in letzter Zeit zugenommen hat oder aber erst jetzt erkannt wurde (z.B. Yersiniose); Obstipation wurde in der ersten Auflage unter den »kleinen Leiden und Anfälligkeiten« besprochen, des häufigen Vorkommens zufolge ist diesem Thema jetzt ein eigenes Kapitel gewidmet. Es ist nicht leicht, all die geistreichen, logischen und praktischen Umgruppierungen einzeln hervorzuheben, die durch Zeit, Praxis und neue Erkenntnisse geformt wurden, jedenfalls tragen auch diese dazu bei, den Arzt zur richtigen Diagnose zu leiten. Dasselbe bezieht sich auch auf die informativen Tabellen und das einleitende, ausführliche Inhaltsverzeichnis in jedem Kapitel. Um den kompakten Umfang des Buches zu sichern, wurde auf eine Angabe der umfangreichen Einzelliteratur verzichtet, das Verzeichnis der differentialdiagnostisch weiterführenden Übersichtsarbeiten wurde jedoch wesentlich erweitert.

Zusammenfassend: der sich seit einem Jahrzehnt so nützlich erwiesene differentialdiagnostische Wegweiser wird in der auf den aktuellen Stand gebrachten neu verfaßten Form von den in der Kinderheilkunde tätigen Ärzten mit Freude begrüßt.

K Schmidt

A. J. Ayres: *Bausteine der kindlichen Entwicklung.* XI + 274 Seiten mit 4 Abbildungen. Springer-Verlag, Berlin–Heidelberg–New York–Tokyo 1984. Preis DM 38,—

Die aus dem Amerikanischen übersetzte Monographie befaßt sich mit der Bedeutung der Integration der Sinne für die Entwicklung des Kindes. Die weltweit bekannte Autorin hat sich mit dem sensorischen integrationsdiagnostischen Test und dem 1973 veröffentlichten und an Fachläute gerichteten Buch »Lernstörungen, sensorisch-integrative Dysfunktionen« ihre internationale Reputation erworben. Das vorliegende Buch wendet sich in erster Linie an die Eltern und Lehrer von Kindern mit Entwicklungsstörungen, um ihnen die Erkennung einer sensorischen Integra-

tionsstörung zu erörtern und das Verstehen der Behandlungsmöglichkeiten zu erleichtern.

Im ersten Teil werden in 3 Kapiteln das Gehirn, die Struktur und Funktion des Nervensystems, die Entwicklung der Wahrnehmungsintegration erläutert.

Den Störungen der sensorischen Integration sind 6 Kapitel gewidmet, in denen die Störungen des Gleichgewichtssystems, der visuellen Wahrnehmung, des Hörens und der Sprache, ferner die Bewegungsplanung und schließlich die übertriebene Reaktion auf Sinneseinwirkungen und der Autismus besprochen werden.

Im dritten Teil werden in 3 Kapiteln die therapeutischen Möglichkeiten mittels entsprechende Umweltbedingungen, verschiedene Aktivitäten, Reizwirkungen und einem Wahrnehmungstraining behandelt. Der Laie findet anschließend die Erläuterungen einiger Begriffe, die im Buch verwendet werden.

Das Verdienst der Arbeit besteht darin, daß es als Beispiel dient, wie ein wissenschaftliches Thema allgemeinverständlich bearbeitet werden kann. Deshalb soll das Buch auch kinderneurologisch tätigen Ärzten und Therapeuten empfohlen werden, die die Behandlung von lernbehinderten oder hirngeschädigten Kindern durchführen wie auch deren Familienangehörigen und Betreuern.

T Kolos

Pädiatrische Pneumologie. Herausgegeben von A Fenner, H von der Hardt. XX + 708 Seiten mit 198 Abbildungen und 114 Tabellen. Springer-Verlag, Berlin–Heidelberg–New York–Tokyo 1985. Preis DM 198,—

In dem letzten Jahrzehnt hat sich die Tendenz verstärkt, die pädiatrische Pneumologie als eine selbständige Disziplin zu betrachten. In diesen Zeitraum fällt auch die Gründung der Gesellschaft für Pädiatrische Pulmonologie und einer Fachzeitschrift mit diesem Profil. Dieses Buch hätte schon vor Jahren erscheinen sollen, die Redaktion wurde damals von J. Wenner und A. Fenner übernommen. Durch Prof. Wenners frühen Tod konnte aber diese Aufgabe nicht vollendet werden. Unter diesen Umständen mußte das Werk mit Prof. Hardt neu konzipiert und bearbeitet werden, wobei jedoch die ursprünglichen Vorstellungen weitgehend berücksichtigt wurden. Die einzelnen Kapitel oder Abschnitte wurden ferner von mehreren deutschen Fachautoritäten verfaßt.

Die Verfassung des Buches entspricht jener eines Lehrbuches, indem es sich auf sämtliche mit dem Thema zusammenhängende Fragen erstreckt.

Im Allgemeinen Teil soll als besonders wertvoll der alle wesentliche Kenntnisse zusammenfassende Abschnitt über die Funktionsdiagnostik (Hardt) hervorgehoben werden. Die Nomogramme sind äußerst übersichtlich und enthalten teilweise auch die Gleichung der Kurve, wodurch diese direkt verwendbar werden; wo diese fehlt, können nur approximative Werte abgelesen werden.

In den Kapiteln über Fehlbildungen und Neubildungen sind auch seltenere Erkrankungen zu finden. Bei der Besprechung der entzündlichen Erkrankungen der extrathorakalen Atemwege wird auch das Problem der Tonsillektomie behandelt; der Indikationskreis für eine Tonsillektomie wird von den Fachbüchern verschiedenartig beurteilt, hier handelt es sich um eine zeitgemäß-konservative Stellungnahme, ein begrenztes Indikationsgebiet: sich wiederholende Tonsillitis soll als relative Indikation betrachtet werden und — ähnlich wie die amerikanische Meinung — soll eine jährlich siebenmal auftretende beta-hämolysierende Streptokokkus Tonsillitis, oder 5 Episoden 2 Jahre lang oder aber 3 Episoden 3 Jahre lang die Indikation einer Tonsillektomie rechtfertigen.

Das Wesen und Bestehen einer chronischen Bronchitis ist stets Gegenstand von Diskussionen. Die WHO-Empfehlung für Erwachsene wird für das Kindesalter nicht akzeptiert, sondern die Definition von

Weingärtner u. Dietzsch, laut der sie innerhalb des letzten Jahres in 3 Schüben von mindestens 2wöchiger Dauer, mit Husten und Auskultationsbefund bestehen sollte. Die chronische Schleimhautentzündung besteht oft ohne Symptome. Hinsichtlich der obstruktiven Bronchitis vertreten die Autoren die Meinung, daß diese Erkrankung einer milden Form des Asthma-Syndroms entspricht und daß es sich bei wiederholtem Vorkommen empfiehlt, eher von einem Asthma bronchiale zu sprechen.

Weitere Kapitel befassen sich mit der zystischen Fibrose (Mukoviszidose), mit den Pneumonien, der Tuberkulose, Sarkoidose, ferner den Mykobakteriosen, Systemerkrankungen mit pulmonaler Beteiligung, Ventilationsstörungen, traumatischen Erkrankungen, Intoxikationen, mit der respiratorischen Insuffizienz, schließlich mit den pulmonalen Manifestationen der autoimmunen und systemischen Erkrankungen, um nur einige der Themen zu erwähnen. Das Kapitel über die Differentialdiagnostik des Atemdurstes soll auch hervorgehoben werden.

Das Buch ist sowohl ein praktischer Leitfaden für Diagnose und Therapie wie auch ein Nachschlagwerk über seltenere Erkrankungen und spezielle Techniken und dürfte sich in der Praxis und zur Fortbildung von Kindernärzten sehr nützlich erweisen.

E Cserháti

D. Kleinmann: *Sport als Medizin.* 190 Seiten mit 27 Abbildungen und 11 Tabellen. Hippokrates Verlag, Stuttgart 1985. Preis DM 26,—

Als Internist und Sportmediziner verfügt der auch selbst vielseitig sportlich aktive Autor über reichliche eigene Erfahrungen, was dem vorliegenden Buch gleich anzumerken ist. Es besteht aus 2 Teilen, von denen im ersten allgemeinen Teil diverse Gesichtspunkte wie Training, Einflüsse auf die Leistungsfähigkeit, Sport unter besonderen Bedingungen (Kälte, Wärme, Höhe, Tiefe), Belastbarkeit bei Kindern, Sport im Alter oder bei Frauen usw., ferner ein Trainingprogramm erörtert werden. Im zweiten Teil findet man die Besprechung von Sport bei Krankheiten. Hier wird auch die Rolle des Sportes in Prävention und Rehabilitation ausführlich behandelt und auf die Risikofaktoren hingewiesen.

Die Literaturdaten sind lediglich eine Ergänzung zu jenem umfangreichen Literaturverzeichnis, das in dem von demselben Verfasser 1980 erschienenen Werk »Sportmedizin für die Praxis« enthalten ist.

Das Buch wendet sich an Sport- und Schulmediziner und bietet mit zahlreichen nützlichen Ratschlägen Hilfe zum besseren Verständnis des Sportes beim gesunden und kranken Mensch und Kind.

J Kelemen

R. Becker: *Die Lese—Rechtschreib—Schwäche aus logopädischer Sicht.* 5. überarbeitete Auflage. 312 Seiten mit 27 Abbildungen und 38 Tabellen. Verlag Volk und Gesundheit, Berlin 1985. Preis DM 29,—

Lesen und Schreiben sind hochentwickelte menschliche Fähigkeiten, die mit anderen Leistungen in Wechselwirkung und gegenseitiger Abhängigkeit stehen. Die Schwäche dieser Funktionen kann bei den heutigen erhöhten Bildungsanforderungen auch auf die kindliche Psyche und Persönlichkeitsentwicklung ausschlaggebend negative Auswirkung haben. Pädagogen, Psychologen und Logopäden haben in letzter Zeit besonderes Interesse für dieses Problem gezeigt, und so ist dieses Buch als eine lückenfüllende Arbeit zu begrüßen.

Das Buch gliedert sich in 5 Teile. Einleitend werden Begriffe und Benennungen (Wortblindheit, Dyslexie, Legasthenie usw.) geklärt; in der DDR hat sich die Bezeichnung Lese-Rechtschreib-Schwäche (LRS) eingebürgert, wobei es sich um eine LRS bei normalen oder nahezu normalen Kindern handelt, die in dieser Funktion län-

gere Zeit hindurch behindert waren. Hiernach werden die psychologischen, pädagogischen, logopädischen und neurologischen Gesichtspunkte besprochen und die biologischen, psychischen und sozialen Faktoren in der Entwicklung des Syndroms zusammengefaßt.

Der zweite Teil befaßt sich mit der Diagnose. Es wird eine leicht bewertbare Methode zur Untersuchung der LRS empfohlen und die damit erzielten Ergebnisse angeführt. Im dritten Teil findet man Angaben über Vorkommen und Häufigkeit der LRS unter der behinderten und normalen Population. Der vierte Teil ist der rehabilitativen Bildung und Erziehung von Kindern mit LRS gewidmet. Neben der Diagnostik bietet dieser Teil die meisten nützlichen Informationen und Ratschläge für die Praxis. Das letzte Kapitel faßt die Schlußfolgerungen und Perspektiven zusammen.

Das Buch soll Logopäden, Foniatern, Psychologen und auch Pädagogen und Erzieher und Studenten der Heilpädagogik empfohlen werden.

Erzsébet S Nagy

Pädiatrie. Band II. Herausgegeben von P Grossmann, W Plenert mit Beiträgen von 10 Fachwissenschaftlern 427 Seiten mit 166 Abbildungen und 78 Tabellen. Georg Thieme, Leipzig 1984. Preis DM 140,—

Der vorliegende zweite Band des auf 3 Bände geplanten Gesamtwerkes befolgt die Zielsetzung, den Zusammenhalt der Pädiatrie vor Augen zu halten. In dem Buch finden wir die traditionellen Kapitel über Organ- und Systemerkrankungen und der Grenzgebiete. Hämatologie, Gastroenterologie, Endokrinologie, Osteologie, Neurologie und Onkologie sind die Themen der 6 größeren Kapitel. Jedes Kapitel wird von einem physiologischen Überblick eingeleitet, in dem die pädiatrischen Beziehungen hervorgehoben werden. Bei der Besprechung der einzelnen Krankheitsbilder werden Häufigkeit, Bedeutung und praktische Maßnahmen berücksichtigt. Es wurden lediglich wissenschaftlich begründete und in ein Lehrbuch gehörende Kenntnisse angeführt, weshalb das die Kapitel abschließende Schrifttum jedoch nicht immer up-to-date erscheint.

Der Aufbau und die Einteilung der Kapitel dienen der guten Orientierung, die auch durch übersichtliche Tabellen und gute Abbildungen gefördert wird. Demselben Ziel dient auch ein ganz ausführliches Inhaltsverzeichnis am Amfang jedes Kapitels. Man vermißt jedoch das übliche Sachwortverzeichnis, welches erst für den in der Zukunft erscheinenden dritten Band vorgesehen ist.

Das Buch ist ein Lehrbuch und Nachschlagwerk und dürfte sich deshalb sowohl zur Facharztbildung wie als Informationsquelle für den in Klinik und Ambulanz tätigen Kinderarzt eignen.

K Schmidt

Kozlowski, P Beighton: *Gamut Index of skeletal dysplasias. An aid to radiodiagnosis.* XV + 196 pages. Springer-Verlag, Berlin–Heidelberg–New York–Tokyo 1984. Price DM 40.—

Although we are living in a computerized era, books are easy to handle and give as much help as the thinking machines. Books have a further advantage: they are cheaper and can be used by those who do not have a computer. This aim has certainly been achieved by the book at issue.

The subtitle "An aid to radiodiagnosis" expresses what this book can give to the reader. X-ray reproductions are missing, therefore the first and essential step is an extremely accurate evaluation of the radiological changes. On the basis of the findings, by the help of the index system we can look after the clinical entities for which the given X-ray alteration may be characteristic.

The book is divided into three main sections. In the first, the general skeletal

abnormalities are systematized in 12 chapters. In the second section regional skeletal abnormalities are dealt with in 6 chapters. If the radiological alteration fits an appropriate category of the first or second sections, the reader consults the third section. This section contains short, well summarized descriptions of more than 170 malformation syndromes in which skeletal changes are the main features or their presence has a diagnostic value.

In the Appendix, after a short list of textbooks, the International Nomenclature of Constitutional Skeletal Diseases of Bone, as well as an extended subject index helps the reader in further orientation.

Skeletal changes represent cardinal patterns of many dysmorphic syndromes and often they are important and characteristic features of the phenotype. These signs and symptoms are easy to find in possession of the X-ray diagnosis. The examination can be performed without much burden to the patient in any age, even in early infancy. Therefore in certain syndromes X-ray imaging of skeletal abnormalities is the starting point to establish the correct and accurate diagnosis. This book offers help for radiologists, paediatricians, geneticists and especially for those who are engaged in the field of syndromatology.

The Gamut Index has many advantages, but the drawbacks of this first edition should also be mentioned. First of all, some names are misspelt, e.g. De Barsey instead of De Barsy, or Reinardt or Rheinhardt instead of Reinhardt. Then in the third section a number of syndromes are drawn together although nowadays they are considered separate clinical and genetic entities. Among others, the tricho-rhino-phalangeal (TRP) syndrome should be divided into two different forms, namely the TRP I and TRP II or Langer–Giedion syndrome. The latter is incorrectly mentioned as Giedion–Langer syndrome. The Coffin–Lowry and Coffin–Siris syndromes should also be separated, as well as the Murcs and Vater associations. The cerebro-oculo-facio-skeletal (COFS) syndrome must be distinguished from the Pena–Shokeir I syndrome since the former one is the Pena–Shokeir II syndrome. The differences are important, since the clinical picture and heredity in the mentioned syndromes are not identical and therefore at genetic counselling the consequences and the therapeutic and preventive measures should also be different. Many syndromes are missing in which skeletal abnormalities are characteristic, for instance the Aase, Greig, Pfeiffer, Proteus, Saethre-Chotzen and sclerosteosis syndromes. On the other hand, in the third section, a series of very rare conditions are mentioned of which only one or two cases have been published.

The list of skeletal dysplasia syndromes should be complete as far as possible, otherwise the reader has to consult other, more comprehensive, textbooks to be sure that the diagnosis established by the help of this book is really correct.

Since the idea of this type of Gamut Index is promising, we must hope that the next edition will fulfil the aim to be a real aid to radiodiagnosis.

P Kiss

PRINTED IN HUNGARY
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Golden Jubilee Conference and Northern European Epilepsy Meeting

University of York (UK)
13—16 September 1986

MAIN TOPICS:

Liaison Psychiatry and Epilepsy
Fifty Years of the EEG in Europe
Fifty Years' Experience of Phenytoin
The Anatomy of Epileptogenesis
The Development of New Anticonvulsants
Epilepsy in Children and Adolescents
Fifty Years Epilepsy Centres
Non-drug Management of Epilepsy
Invited Topics
European Audio-Visual Festival

ABSTRACTS:

The deadline for submission of abstracts for all sessions (oral and poster) is 1 June 1986. Further details can be obtained from the Nothern European Epilepsy Meeting Secretariat
Sunderland House, Sunderland Street,
Macclesfield, Cheshire, SK 11 6JF, UK

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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XXIIIrd Congress of the

EDTA — EUROPEAN RENAL ASSOCIATION

June 29—July 3

XVth Annual Conference of the

EUROPEAN DIALYSIS AND
TRANSPLANT NURSES ASSOCIATION

July 1—4

President István Taraba

The XXIIIrd Congress of the European Dialysis and Transplant association — European Renal Association (EDTA—ERA) will be held between June 29—July 3, 1986, and the XVth Annual Conference of the European Dialysis and Transplant Nurses Association — European Renal Care Association (EDNA—ERCA) between July 1—4, 1986, in Budapest, Hungary

The EDTA—ERA Congress will focus on general nephrology, dialysis and transplantation. Main topics selected are

1) dialysis
2) transplantation
3) computing in clinical nephrology
4) hormonal changes in end stage renal diseases
5) drugs and kidney diseases
6) role of vasoactive mediators in renal functions.

Deadline for submission of abstracts will be the 1st of February 1986.

For further information and abstract forms write to Congress Bureau MOTESZ: P. O. B. 32, H-1361 Budapest, Hungary.
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Abbreviations should be spelled out when first used in the text. *Drugs* should be referred to by their WHO code designation (Recommended International Nonproprietary Name); the use of proprietary names is unacceptable. The *International System of Units* (SI) should be used for all measurements except blood pressure.

References should be numbered in alphabetical order and only the numbers should appear in the text (in parentheses). The list of references should contain the name and initials of all authors (the use of et al instead of authors' names is not accepted). For journal articles the title of the paper, title of the journal abbreviated according to the style used in Index Medicus, volume number, first page number and year of publication should be given; for books, the title followed by the publishers and place and year of publication.

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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Figures should be identified by number and author's name. The top should be indicated on the back, and the approximate place of the figure in the text. Captions should be typed on a separate page.

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Acta Paediatrica Hungarica, 27 (3), pp. 173—187 (1986)

Cranial nerve damage after paediatric head trauma: a long-term follow-up study of 741cases

G. Jacobi, Annegret Ritz, Rita Emrich

Department of Paediatric Neurology, University Hospital Frankfurt/Main, GFR

A report is given on transient and permanent (6 months) impairment of cranial nerves after paediatric head injuries (N: 741). There is a link between severity of the injury, fractures on the base of the skull, its foramina and channels, and the frequency of cranial nerve involvement. One should try to establish whether a posttraumatic dysfunction of the cranial nerves is primary or secondary in nature, i.e. due to raised intracranial pressure or haemorrhage. In children after head injuries often the cranial nerves of the oculomotor system are affected (20.2%/7.0%) — transient (permanent), followed by optic atrophy (4.88%), lesion of the trigeminal nerve (4.2%/2.2%), and the facial nerve lower motor type (4.1%/1.7%). Loss of hearing (3.3%/1.2%) and of smell (3.2%/1.2%) are less frequent in children than in adults.

There are few reports on cranial nerve (CN) damage after paediatric head injuries (HI) [19, 20, 25, 26, 28, 36, 37, 46, 53]. Most comprehensive reports are mixed series of a few children and mainly adults [1, 24, 31, 32, 35, 41, 51, 64]. CN impairment might indicate raised intracranial pressure (ICP) or expanding haematoma and is a secondary lesion then. On the other hand, all CN are close to the base of the skull or are even harboured within by bony channels or passing through various foramina. So, if there is a fracture on the base of the skull one has to look carefully for every kind of CN impairment. On the other hand, their course is beneath "silent" areas of the brain: the fronto-orbital and basal temporal lobes. If there is an additional contusion of those brain structures, the child later might present with mental retardation or regression or with perl sonality, language or memory problems; or there may be a permanent brain-organic syndrome, in particular if the damage was bilateral and/or symmetrical. Therefore it seems important to look attentively for CN impairment in every child with HI.

Patients

From January, 1970, to July, 1984, we have seen and treated 741 children who had suffered HI. They were grouped according to the duration of post-traumatic amnesia (PTA) (Table I). The ratio boys/girls was 2/1, (471/270).

As to aetiology and age, (Table II), 45.2% were involved in traffic accidents. Mortality was 4.9% in the whole material, but 8.0% in those after traffic accident. Mortality was much influenced by poly-

TABLE I

Duration of post-traumatic amnesia (PTA) in 741 children

I: no PTA, concussional symptoms present	235
II: PTA < 1 hour	168
III: PTA 24 hours	97
IV: PTA < 1 week	93
V: PTA > 1 week	111
VI: died within the first 4 weeks after trauma	37
Total:	741

NB: of the 204 patients in IV and V 183 were controlled after 6 months, some of them were followed-up more than 10 years.

trauma: 9.3% (N:289) versus 2.2% in those with only HI anaemia and hypovolaemia (N:111); severe hypoxia/cardiorespiratory arrest (N:11) occurred more often after polytrauma than after cranial monotrauma. Many of the children who were hypovolaemic or developed shock later had to be classified into groups IV—VI with the worst prognosis [14, 42, 43]. Of the 35 children who had been battered; 30 were younger than 2 years; 3 of them, died. In these physically abused children one has to accept that head injury is a recurrent event and the addition of minor and major trau'sвш even in subconcussional doses, finally might end in profound mental retardation or severe brain (and ocular) damage [17, 18, 27, 36, 37].

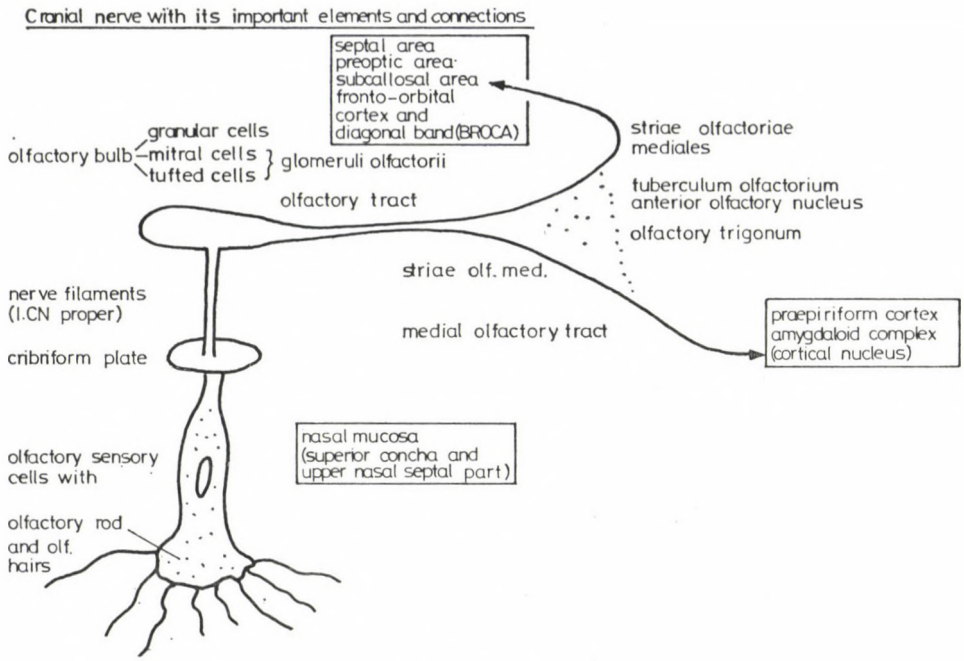
CN impairment, general considerations

Primary lesions are due to stretch, shearing, intruding bone fragments and ischaemic necrosis [1, 5, 31, 32, 44, 64]. Some damage may depend on shearing of the nerves' vessels (vasa vasorum). Basically, every accident may bring direct concussional or rotational forces into action on the human skull. Apart from its direction, the impact might be of crushing or high velocity type with acceleration-deceleration of the brain as a soft tissue content of the skull. CN passing through bony channels (II, V, VII, VIII) are more exposed to crushing forces, whereas those running through the subarachnoidal space are more exposed to shearing (rotational) forces (Graph 1) if, additionally, they are neighboured by sharp edges, wings or ligaments (I, III, IV, VI).

Secondary lesions are mainly prompted by raised ICP and intracranial haemorrhage, including intradural and interstitial bleeding of the nerve itself [5, 39, 64]. Rare causes of secondary CN damage are bacterial meningitis, adhesive arachnoiditis, aerocele, and callus formation [39, 64]. Most important is a secondary damage to the midbrain which is prompted by herniation, vasospasm and circulatory failure; there is a predilection for these in the periaqueductal grey matter and the basis pontis/mesencephali [29, 64].

Site of damage. There are four main areas (Graph 1):

1. within the brainstem in the brainstem nuclei, internuclear network (medial longitudinal bundle) and roots of exit;



GRAPH 1

2. in their intracisternal course;

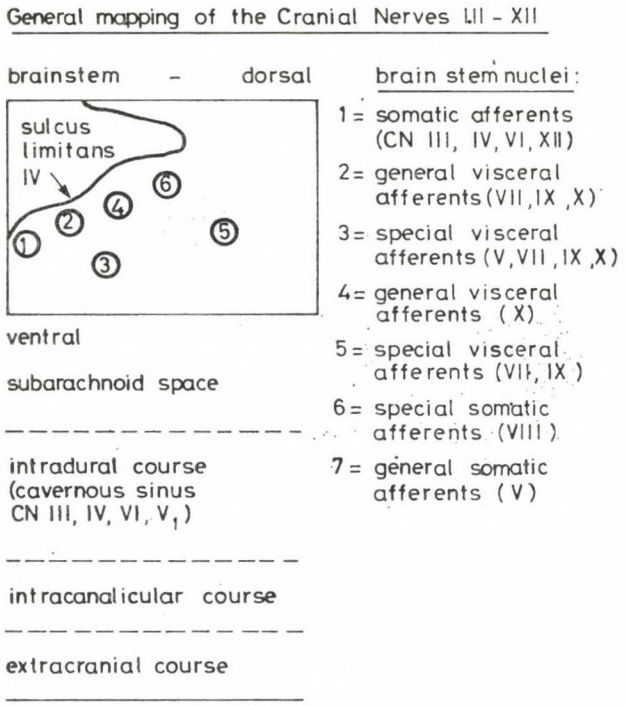
3. in their intradural (intracanalicular, intraforaminal) course;

4. in its most peripheral course the CN itself or its branches might be severed.

CN impairment, particular considerations

CN I, olfactory nerve

Disturbance of smell after HI which has an overall incidence of 3 to 20% [1, 31, 32, 35, 40, 41, 51, 60, 61, 62, 64] is age-related and not dependent on the severity of trauma. Older people (50 years and more) suffer in 50% from loss of smell after HI, even when there was not PTA at all or one of less than one hour [60, 61]. In most cases the blow was frontal or fronto-temporolateral in direction, but in 1/3 it was occipital [41, 64] and may be regarded as a contre coup effect. If recovery takes place, this occurs within 3 months in most cases [31, 32, 40, 61], but recovery has been observed even 5 years after the trauma [61]. Parosmia or olfactory scotomas arise from tearing off the olfactory filaments when they are leaving the dura (Graph 2), but it is thought to be a cortical phenomenon [1, 31, 41, 61, 62, 64]. Parosmia nearly always presents as kakosmia [40, 60, 61]. It is not always due to the violence of the accident itself but to general anoxia comparable to transient loss of smell at high altitude (>7 500 m) too [61]. Bilateral loss of taste and smell is encountered after severe HI and may be attributed to



Graph 2

damage of the ventromedial thalamic nuclei or, if there is additional nystagmus and/or IIIrd nerve palsy, of the tegmental part of the brainstem [8]. In contrast to adults, posttraumatic anosmia in children is rare, 1.4% [14, 28]. In the present series (Table III) it was transient in 2%, and permanent in 1.2%. All these children had suffered from frontobasal injuries with fractures of the anterior fossa and most of them had nasal CSF discharge and needed surgical repair of dura tears and lacerations. Loss of smell was found bilaterally only in 1 child; there was some predilection of groups IV and V with longstanding PTA, and in some very young children and some with very severe brain damage, testing of smell could not be done adequately. Parosmia or olfactory scotomas were never seen in our paediatric patients.

CN II, optic nerve and visual pathway

Optic atrophy (OA) was found in 34 children (4.8%), 33 of them were in groups IV and V (long PTA), and in 20 it was bilateral. 19 of these children were blind on one or two eyes. The worst findings were in the battered children who had had retinal haemorrhages, some of them reaching into the vitrous body, some with partial retinal detachment. All these abused children had a raised ICP, too.

TABLE II

Aetiology, length of PTA and age in 741 children with head injury

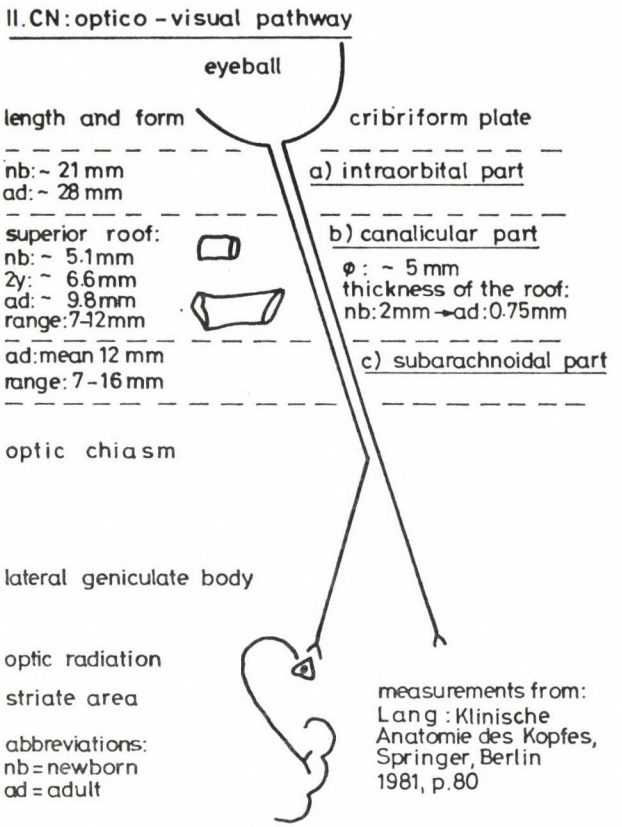
Aetiology PTA:	I	II	III	IV	V	VI	N	in per cent	1—24 mo	< 6y	> 6y
at home	122	39	12	9	2	—	184	24.8	94	58	32
at play and sport	46	49	12	6	3	4	120	16.2	1	40	79
falls (> 3m)	11	12	9	16	6	3	57	7.7	9	29	19
parental assault	11	2	4	3	12	3	35	4.7	30	4	1
other: shoot/stab	1	3	1	—	2	—	7	1.0	—	2	5
all traffic accidents	44	63	59	59	86	27	338	45.3	17	105	216
as seat passenger	4	6	10	11	14	6	51	6.9	15	15	21
as pedestrian	22	36	29	34	62	17	200	27.0	2	81	117
as (motor)cyclist	18	21	20	14	10	4	87	11.7	—	9	78
within each group:	235	168	97	93	111	37	741		151	238	352

NB: lethality figures: the whole group: 4.9% for all traffic accidents: 8.0%; for the rest: 2.5%

These findings were in accordance with data of other authors [16, 17, 27, 36, 37]. Raised ICP was the only cause for OA in 13 patients. The sites of damage to the optic nerve were besides the retina as follows (Graph 3).

During its intraorbital course, direct lesions of the II CN may occur [6, 13, 22, 24] prompted by penetrating objects. Anterior marginal tearing and ischaemic damage may lead to field defects [6, 24]. Of practical importance is a retrobulbar haematoma; clinically it should be suspected if there was an orbital trauma and unilateral amaurotic pupil. Diagnosis to-day is made by high resolution orbital computerized tomography, and after its imaging the haematoma should be drained immediately by needle aspiration. We had one such case in our series.

Lesions of the optic nerve in the optic canal: the amaurotic pupil has no direct light reaction but the consensual one is well preserved or even overshooting. The amaurotic pupil fluctuates in its width according to the light in the room. In every child with an amaurotic pupil a canal or a chiasmal lesion should be suspected and excluded [41]. We had 4 cases of tearing of the optic nerve in our patients, two of them had minor trauma [1]. One has to consider that the roof of the optic canal is thicker in young children than in adults [38]. Most lesions occur after direct frontal or frontolateral trauma; it was shown that the isochromatic lines after experimental frontal or frontolateral concussion converge to the roof of the optic canal and ipsilaterally to the suprasellar region [21]. Therefore, some authors stress the importance of canal fractures in optic nerve damage [7, 11, 13, 21] while others do not [1, 6, 22, 24, 31, 32, 59, 62]. If there is some recovery of the direct light reflex within 48 hours after the trauma, some or much restitution of vis-



GRAPH 3

ual acuity may be expected [41, 64]. As far as operative procedures are concerned, an inferior-medial approach through the middle and posterior ethmoid cells without opening of the dural sheath of the optic nerve is indicated [7, 11, 65]. Fukado is for optic nerve decompression in every case, and reviewed 353 own cases [11]. According to this author, some improvement can be achieved by this operative procedure even weeks after the trauma. All other authors indicate operative decompression only if there is progressive visual loss, short interval trauma/operation (< 48 hours) and the patient is not comatose [13, 22, 24, 62, 64]. Again, some other authors disfavour operative treatment completely [1, 31, 32]. According to Fukado [11], the symptoms of optic nerve damage in its intracanicular course are a loss of direct light reflex (100%), lesion of the lateral eyebrow (97%) and blood and/or CSF discharge from the homolateral nostril (80%). If the optic nerve is severed at the orbital apex, some other CN are involved: II, IV, VI and the first division of the Vth [13, 22, 52]. OA develops 12 days to 6 weeks after the trauma [1, 6, 20, 22, 24, 31, 32,

41, 59, 62]. The outcome is always doubtful. All of our four patients became blind permanently, as were more than 50% in larger series. In some patients light/dark discrimination or finger counting may be possible, in a few a tolerable visual acuity is restored [6, 20, 22, 24, 31, 41, 59, 62]. If there is an altitudinal or inferior quadrant field defect, this finding is in favour of an ischaemic or vascular lesion of the optic nerve [6, 22, 41, 62].

Chiasmal lesions should be suspected clinically if there is a bilateral loss of direct light reflex initially with recovery on one side during the next days/weeks, hemianopic field defects with macular sparing or splitting [1, 6, 13, 22, 31, 47] in long-term observation, and hypothalamic symptoms like transient diabetes insipidus, polyphagia with obesity [1, 13, 39, 47, 48] and, very rarely, sexual delinquency or narcoleptic attacks [48]. Further symptoms found in chiasmal lesions are fractures of the tuberculum sellae [1, 13, 41], CSF-discharge from one nostril or one of the ears [1, 13, 24, 39], see-saw nystagmus [39, 41], anosmia, or involvement of the CN III, VII and VIII. Lesions of the optic chiasm may be prompted by longitudinal shearing up to 3.5 cm [13], ischaemic necrosis and interstitial haemorrhages [5, 31]. The hypothalamic symptoms are attributed to damage of the hypothalamic-hypophyseal tract and the supraoptic/paraventricular nuclei of the hypothalamus [1, 13, 39, 41, 47, 48]. Longstanding PTA has been observed [13, 24, 31, 39, 47, 48] as it was in our 5 patients; they all belonged to group V with a PTA longer than 1 week. Posttraumatic diabetes insipidus and chiasmal lesions are not linked invariably: there are chiasma lesions without diabetes insipidus in 50% [39] and vica versa in 5/18 [48]. OA develops within months in patients with chiasmal lesions [6, 22, 31] and the disk might present with a more yellow colour [39] after peri-parasellar bleedings: yellow OA.

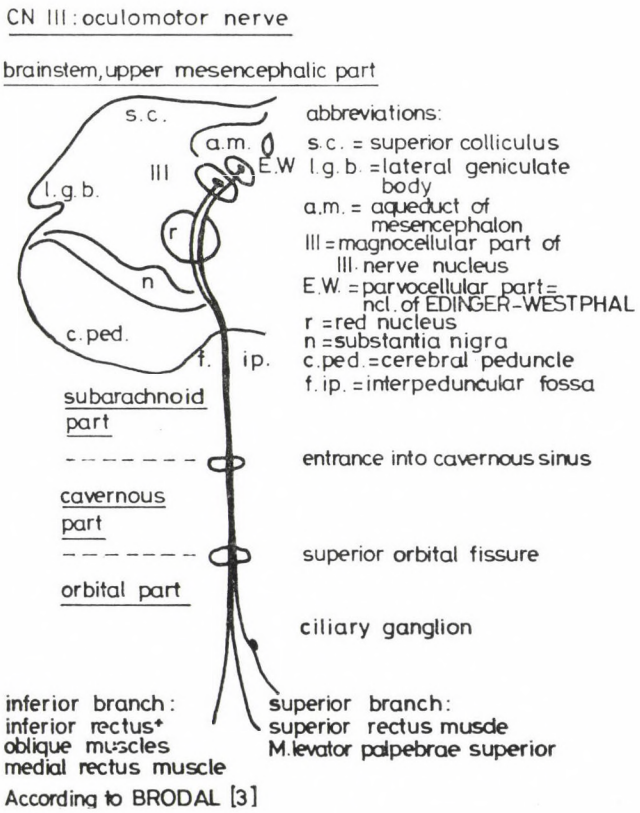
Traumatic damage to the optic tract and lateral geniculate body is exceptional; it is found mostly after penetrating injuries [24, one own case] and also after blunt trauma [5, 6]. It may be followed by OA after months and even years [13]; clinically it presents with homonymous quadrant- or hemianopsia with hemianopic loss of the light reflex and macular splitting [6, 13, 24].

Cortical blindness and damage to the optic radiation occurred in 18 (2.6%) of our patients, but it may have been missed in a few who survived in a vegetative state. Cortical blindness was observed with a short PTA as well as after long PTA at a ratio of 8/10 in our patients; the difference is in the duration of this clinical symptom, which is a few hours or days in the "commotional group" and long, weeks and even months, in the „concussion group". Most patients with posttraumatic cortical blindness have a good prognosis [6, 13, 31, 32] with the exception of penetrating occipital injuries [41]. Cortical blindness is a secondary phenomenon in

most patients, due to circulatory failure in the territory of the posterior cerebral and the anterior choroid artery, for instance during uncal herniation. Cortical blindness in children after trivial trauma with or without PTA might be related to complicated migraine in some cases [13, and some own observations].

CN III, IV and VI: oculomotor, trochlear and abducens nerves

Oculomotor nerve impairment was found most often in our series; CN 15.9% in the acute state, and 5.6% in the chronic course. This finding split up into total ophthalmoplegia in 3.9% (permanent 0.9%), and external ophthalmoplegia in 2.4% (2.5%) which means that some of the total ophthalmoplegias had resolved to partial external ones and internal ophthalmoplegia in 11.6% (2.2% permanently impaired). The III CN might be damaged in its course through the subarachnoid space (Graph 4) by mass lesions or raised ICP lying between the posterior cerebral and the superior cerebellar artery and the tentorial notch; or at its entrance into the cavernous sinus when passing the



GRAPH 4

ridge of the lesser sphenoid wing [1, 44]. When there is a raised ICP to which are due 75% of all IIIrd nerve traumatic lesion [24, 31, 32], the most vulnerable part of the nerve are its parasympathetic fibres lying superior-medially in its cisternal course [52]. If there is a III and VI CN damage without orbital fracture or sinus cavernosus fistula [1] we have to think of raised ICP in every case very carefully [20, 24, 25, 53]. In follow-up, there are some differences in the width of the pupils after uncal herniation in most cases [31, 32]. If loss of the round shape of the iris is observed, nuclear damage within the upper brainstem has most likely occurred [6, 44]. After orbital trauma, branches of the III CN may be impaired: if the medial orbital wall is broken out, there are more oculomotor nerve branches affected while after lateral wall fracture the VI, and after inferior wall fracture, the 2nd division of the V may be impaired [6, 22, 24, 31, 59, 63]. If there is enophthalmus (4 of our patients), it may be progressive at follow-up [59]. Exophthalmus, on the other hand, means a clot formation behind the globe (blood, pus), foreign body in the orbit or, if it is pulsating, encephalocele after blow-out fracture of the lesser sphenoidal wing. If there is a pulsating exophthalmus with bruit, this means a cavernous sinus a.-v. fistula, or in a child with Recklinghausen disease and no or only a faint bruit, a premorbid condition [22, 59, 63]. Recovery after the trauma can be expected in about 75% as far as all eye muscles are concerned [1, 20, 22, 24, 25, 31, 35, 59, 63, 64]; it often occurs within the first weeks and then is most likely be due to raised ICP. When fibre interruption had occurred. misdirectional phenomena are encountered, such as globe retraction on vertical gaze, horizontal gaze lid dyskinesis, adduction of the homolateral eye during upvergence, monocular vertical optokinetic nystagmus and pseudo-Graefe's sign or pseudo-Argyll Robertson pupil phenomenon [1, 31, 52, 63].

Trochlear nerve palsies most often are due to trauma [55, 56, 59]. Of our 5 cases, one was due to penetrating trauma [24], 2 to raised ICP [41] and 2 to orbital fractures [6, 31, 32, 52]. Although the IV CN has the longest course through the subarachnoid space: 75 mm, as compared to the III CN: 20 mm, and the VI CN: 15 mm [34, 38, 52], there is no preponderance of pressure damage. Apart from the 3 causes already mentioned, it might be damaged in fractures of the ala parva of the sphenoid, traumatic a.-v. aneurysms of the intracavernous part of the carotid artery, and in the superior orbital fissure syndrome [1, 31, 32, 41, 59].

Abducens nerve palsies may be uni- or bilateral. Most of these palsies are due to raised ICP and then may disappear within a few days, even when present bilaterally [own observation]. A posttraumatic abducens nerve paresis is not likely to be due to brainstem damage [63, 64]; in the young adult, preexisting disseminated sclerosis or diabetes mellitus [31, 32,

TABLE III

CN-involvement in children after head injury: a) (sub)acute = < 6 months (N: 704)
b) chronic = > 6 months (N: 683)

CN-function concerned	PTA: < 6 mo						> 6 mo					
	I—III	in %	IV+V	in%	N	in %	I—III	in %	IV—V	in %	N	in %
I:olfactory nerve	8	1.6	15	7.3	23	3.2	2	0.4	6	3.4	8	1.2
II: optic nerve: optic atrophy	1	0.2	33*	16.2	34	4.8	1	0.2	33*	16.2	34	4.8
optic nerve: amaurosis	1	0.2	18*	8.8	19	2.7	1	0.2	18*	8.8	19	2.7
cortical blindness	8	1.6	10	4.8	18	2.5	—	—	2	1.1	2	0.4
III: oculomotor nerve	28	5.6	81	39.7	109	15.9	3	0.6	35	19.1	38	5.6
IV: trochlear nerve	—	—	5	2.4	5	0.7	—	—	4	2.2	4	0.6
VI: abducens nerve	9*	1.8	19*	9.3	28*	4t0	2	0.4	5	2.7	7	1.0
III/IV/VI	37	7.4	105	51.5	142	20.2	5	1.0	44	24.0	49	7.0
all oculomotor brainstem signs	1	0.2	50	24.5	51	7.2	—	—	23	12.6	23	3.3
V: trigeminal nerve	1	0.2	29	14.2	30	4.2	1	0.2	14	7.7	15	2.2
VII: facial nerve, lower motor neurone	5	1.0	24	11.8	29	4.1	1	0.2	11	0.6	12	1.7
facial nerve, upper motor neurone	11	2.2	40	19.6	51	7.2	1	0.2	27	14.2	27	3.8
VIII: stato-acoustic nerve	13	2.6	14	6.8	27	3.8	1	0.2	7	3.8	8	1.2
IX—XII: basal CN group	2	0.4	1	0.5	3	0.4	1	0.2	1	0.5	2	0.3
patients within the groups	500		204		704		500		183		683	

* = bilaterally present: optic atrophy: 20/33; amaurosis: 6/18; abducens nerve palsy: 3/9, 3/19, 6/28 respectively

55, 56, 58], in all groups intracranial hypotension (e.g. after lumbar puncture) and phenytoin intoxication [31] should be considered. Apart from pressure on the incisura tentorii, the VIth nerve crosses under the petrosphenoidal ligament and may be sheared by up- or downward acceleration of the whole brainstem during high velocity accidents. Recovery is as good as after IIIrd nerve impairment [31, 52, 59]. After incomplete recovery, overactivity of the antagonistic muscles may take place as in IIIrd nerve muscle impairment [41] so that the corrective operation should not be delayed too long: the topic should be discussed at least 6 months after the trauma [1, 6, 22, 31, 41, 59, 63].

Oculomotor brainstem signs (Table III) most often are found in groups IV and V. Therefore they are indicators of brainstem damage in the acute phase. Among these signs we observed ocular bobbing, skew deviations, quick alternating horizontal movements ("ping-pong sign") and several forms of central nystagmus. Vertical or horizontal gaze paresis may be present with bulbus positions in dysjugate attitude in most cases of head positioning or vestibular testing as do internuclear ophthalmoplegias [1, 29, 41, 51, 63]. In contrast to these

dysjugate movements and positions, every conjugated gaze, deviation ("déviation conjugée") is in favour of hemispheric supratentorial damage on the side to which the eyes deviate, or of an epileptic seizure spreading from one of the adversive fields [19, 25, 27]. When the patient is at the end of coma, very often dysjugate horizontal eye movements are observed as well as convergence spasms [31, 32]. This period of deviant eye movement control shortens after high dose phenobarbital and neurointensive treatment for days, whereas it was observed for weeks with conventional treatment and lasts for months and even years in the apallic syndrome ("vegetative state") [own observation]. Of the oculomotor brainstem signs, 60% disappear within 6 months (Table III), convergence paresis [6, 20] and partial diplopias may present as "blurred vision" later [22, 29, 51]. These findings 6 months after trauma were found only in patients of group V. They have to be regarded as definite brainstem damage, exactly of the medial longitudinal bundle, which connects the oculomotor nuclei with the vestibular and extrapyramidal motor system [34].

CN V, trigeminal nerve

Trigeminal lesions of different kinds occurred in 4.2% in the acute stage, and in 2.2% they were still present after 6 months. In groups IV—VI, diminished or absent corneal reflex was often found (30 patients), but 6 children of group V had unilalater motor impairment, 4 of permanently. These long lasting effects on trigeminal function are in favour of midbrain damage, whereas after orbital trauma branches of the Vth may be severed: supraorbital division of the ophthalmic nerve was observed in 3 cases, or of the nasociliary part which led to neuroparalytic keratitis in 2 cases. The infraorbital branch which is affected in adults after orbital lesions more often [30, 31, 32, 41, 51] than the supraorbital nerve, was not impaired in any of our patients. Lateral crush injuries to the skull may lead to complete trigeminal impairment [1, 30, 41, 64]; they may be followed by zoster eruption [24] or posttraumatic neuralgia [1, 30, 31]. These crushing traumas are followed by complete anaesthesia of the face if the trigeminal nerve is lesioned, whereas loss of pain and temperature sense with retained feeling of touch are due to upper cervical lesions [30, 41, 64] of the descending trigeminal pathway and nuclei [34].

CN VII, facial nerve

VIIth nerve palsies of the lower motor neurone type occurred in 29 of our patients (4.1%) with 12 minor but permanent sequelae. Of these 8 were of the immediate type, in 13 paresis appeared a few days after the trauma. In 13 patients there was blood/CSF discharge from one ear, and 7 had combined lesions of the facial and acoustic nerve. VIIth nerve lesions are related to petrous bone fractures: transverse fractures extend

from the foramen magnum to the petrous bone and run perpendicularly to its longitudinal axis. These amount to 10—20% of all petrous bone fractures [1, 31, 32, 45, 49, 50 ,64] and are linked with VIIth nerve palsy in 30—50%. Many of these patients have cochlear damage, too [45]. Longitudinal fractures of the petrous bone run from the squama ossis temporalis into the mastoid and the petrous bone. Facial nerve impairment occurs in 15—20% after this type of fracture which is often accompanied by initial ear bleeding [1, 15, 18, 31, 35, 45, 46, 49, 50, 64]. The paresis may be prevented by systemic steroid administration if the X-rays show a fracture of the petrous bone and blood discharge occurs from the ear [2, 32]. Good recovery or even full restitution may be expected in about 75—90% of all cases [1, 24, 31, 35, 41, 64], but even after lesions with delayed onset some permanent sequelae may be found [54]. Peripheral facial palsies should be monitored electromyographically: 7—10 days after the injury one is able to diagnose complete or incomplete denervation [15, 18]. In children one should not perform surgical decompression unless there is still total denervation in EMG-testing after 4—6 months [15]; in adults earlier decompression is advocated by otologists [33, 41, 45, 49, 57]. After complete denervation, recovery starts in the frontal muscle, whereas if it is incomplete, the first reinnervation can be observed in the M. orbicularis oculi [50]. Axonal flow from the nuclear area starts after 8 days, and after 3 weeks it reaches the gap where the nerve is damaged [78]. Resprouting of the axons have a speed of about 1 mm/day, which means 6—9 months for the full length of the facial nerve [57]. Misdirection phenomena [45, 50] are observed: narrowing of the palpebral fissure, evident mainly in upward gaze, chin wrinkling at eye closure, or the crocodile tears phenomenon. They run in a short-circuit through the brainstem nucleus of the CN VII and are no cortical phenomena [23]. The general opinion is that infrastyloid lesions have a bad prognosis [24, 31, 32, 57; three own cases].

CN VIII, acoustic nerve

In most children, posttraumatic loss of hearing is due to haematotympanon (10 of 13 own cases with conductive impairment). Sensory hearing loss (10 of our patients) is often linked with transverse fractures of the petrous bone and VIIth nerve palsy (7 patients). Conductive deafness in children has the tendency to improve [1, 19, 46] except if there is an interruption of the ossicle chain [31]. High tone loss may be due to concussion or contusion of the cochlea, but few children are definitely deaf after trauma [14, 19, 25, 46]. The frequency of minimum hearing impairment after trauma depends on the intensity and methods of testing [31, 32] and is related to age [64].

CN VIII, vestibular nerve

We had 4 patients with impaired peripheral vestibular function, all of them recovered within months. Other

authors found 1/1.015 children with definite vestibular loss after HI [46]. According to Jennet and Teasdale [31], 27% of all patients have an abnormal caloric test until the end of the first year after HI. Of this vestibular impairment 2/3 are due to concussion of the end organ, and 1/3 to brainstem dysfunction; blood within the middle ear might reinforce postconcussional symptoms, especially dizziness and vertigo [41]. In children the "post-commotional syndrome" is encountered rarely (17 of 683 patients; 2.5%); the main complaint in this condition is abnormal fatigue and/or headache but no vertigo or dizzy feeling.

CN IX—XII, the basal group

This kind of CN impairment is more often found after missile injuries in the neck [4, 9, 19, 31, 62] than in blunt injuries with fractures passing the jugular foramen or foramen magnum [1, 10, 41, 64]. Some patients may not survive [51]. Although rare, this clinical entity called Collet syndrome [4] consists of homolateral impairment of the CN IX—XII, Horner syndrome, hearing loss and V_3 (mandibular nerve, if the fracture extends to the foramen ovale) dysfunction and nystagmus to the contralateral side. In some patients cord paralysis may lead to dyspnoea responding only to prompt tracheotomy or intubation [9, 41], while others have tachycardia, dysphagia or excessive salivation, symptoms which improve spontaneously during the following weeks [9, 10]. In one patient oesophageal achalasia was reported which responded well to psychotherapy [10]. In 2 patients after blunt injury, Wallenberg syndrome (of the lateral oblongata) was observed which was due to traumatic thrombosis of the posterior inferior cerebellar artery (PICA) [10, 12].

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A multidimensional threshold model for multiple congenital abnormalities

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Analysis of the data of 2.762 infants with "unidentified" (i.e., no identified as dysmorphological entities) multiple congenital abnormalities from the Hungarian Congenital Malformation Registry, 1970—1976 showed that the majority of congenital abnormalities in newborns with multiple congenital abnormalities was not due to random combinations. The ratio of expected random combination and obseved causal effect was 1: 6, 1: 225-1: 14 000 and 1: 2.10^6 in two, three, four, and five or more congenital abnormalities, respectively. Our multidimensional threshold model supposes specified liabilities for each congenital abnormality, and the correlation structure of these liabilities explains nearly all associations of congenital abnormalities in unidentified multiple congenital abnormalities. According to this hypothesis, the expected and the observed occurrences do not differ significantly either in the sum or in the various possible threefold associations of congenital abnormalities. The biological reasons for the phenomenon are being studied.

A concurrence of two or more different (i.e. different localized errors in morphogenesis) major congenital abnormalities (CAs) in the same person is designated as a multiple congenital abnormality (MCA) [7]. About half of MCAs are recognizable CA-syndromes of monofactorial origin (large effect of mutant major genes, chromosome aberrations, teratogens, or maternal factors) and CA-associations (e.g., VACTERL) of unknown aetiology [10]. The other half of MCAs have, however, not been identified as dysmorphological entities. We refer to these as "unidentified" MCAs.

The purpose of our biostatistical study was to analyse the possible cause(s) of combinations of component CAs in unidentified MCAs. There are three hypotheses;

(*i*) Independence of CAs. Component CAs in unidentified MCAs are random combinations.

(*ii*) Monofactorial origin. As knowledge advances, the majority of unidentified MCAs may be recognized or delineated as CA-syndromes and the minority as random combinations.

(*iii*) A multidimensional threshold model. Specified liabilities are responsible for each CA, and the correlation of these liabilities explains the interrelationships of CAs in unidentified MCAs, i.e. the so-called CA-associations. According to this *a priori* hypothesis, no more CA-syndromes are to be found in unidentified MCAs,

TABLE I

Number of infants affected by a given number of congenital abnormalities (CAs), by category and group

CA category	CA Group	1	2	3	4	5	6	7	Total
X—AN	Anencephaly	984	107	28	2	2	1	0	1.124
EN	Encephalocele	266	46	21	1	3	1	0	338
SP	Spina bifida cystica	1.193	124	35	12	2	0	0	1.366
CL	Cleft lip ± cleft palate	1.091	172	86	20	13	3	1	1.386
CP	Cleft palate	362	66	28	19	13	3	1	492
LR	Limb reduction	388	63	38	8	9	2	0	508
PY	Polydactyly	680	94	52	25	9	4	1	865
SY	Syndactyly	291	65	30	6	5	0	1	398
EX	Exomphalos-omphalocele	222	75	35	10	5	1	0	348
OA	Oesophageal atresia	199	50	21	9	7	3	0	289
AA	Anal atresia	210	72	40	16	13	7	3	361
Y—MC	Microcephaly	146	62	30	15	7	0	0	260
HY	Hydrocephaly	750	131	50	27	11	4	1	974
AM	An-microphthalmia	33	25	14	9	4	0	0	85
CT	Cataract	47	19	4	3	2	0	0	75
EY	Other CAs of eye	146	23	7	8	3	0	0	187
CF	Clubfoot	1.955	435	101	28	16	4	3	2.542
HS	Hypospadias	1.468	163	39	9	4	1	1	1.685
EG	CAs of external genitalia			16	10	7	3	2	104
		43	23	183	67	26	5	3	5.121
HD	Heart defect	4.255	582	28	11	5	1	1	342
DI	Diaphragmatic defect	229	67	22	8	2	2	0	131
RA	Renal agenesis	59	38	30	9	7	1	1	189
CK	Cystic kidney	97	44	4	2	1	1	0	229
AI	Intestinal atresia	193	28	3	2	0	0	0	91
X—ON	Other CAs of nervous system	85	1	30	16	11	3	1	152
FN	CA of face, nose, and skull	67	34	36	27	17	1	0	351
EA	CA of ear	225	45	35	5	2	0	0	399
TC	Torticollis	146	211	1	1	0	0	0	65
BR	Branchial cleft	62	1	68	12	6	0	1	7.760
CD	Cong. dislocation of hip	7.035	638	8	3	1	1	0	131
OL	Other limbs	91	27	19	11	0	2	0	262
RS	Respiratory system	181	49	4	0	0	1	0	422
PS	Pyloric stenosis	395	22						
OD	Other CAs of digestive system	363	55	23	9	2	1	0	453
UT	Undescended testis	689	146	42	19	12	3	0	911
OU	Other CAs of urogenital system	390	90	47	17	6	5	0	555
IH	Inguinal hernia	2.573	247	37	9	3	0	0	2.860
MS	CAs of musculoskeletal system	133	66	33	12	11	1	0	256
EO	CAs of endocrine organs	142	31	10	6	2	0	0	191
ST	Skin, tumours, other CAs	204	19	6	5	1	1	0	236
	Total	28.088	4.256	1.344	488	250	66	21	34.513

and the covariance structure of the individual CAs is due to genetic and environmental factors.

Materials and Methods

The Hungarian Congenital Malformation Registry, 1970—76, shows that 38,023 of the 1 188,529 babies born in that period had CAs [5]. Of these 4 959 (13%) had MCAs. The complex CAs (e.g., tetralogy of Fallot) and CAs with secondary or tertiary anomalies, i.e., sequences [2] were not considered to represent MCAs. The 444 babies who had minor anomalies with one major CA were considered to have one CA. The 5 420 babies with only minor anomalies were excluded from the CA groups studied here. A total of 1 573 (31.7%) of the babies with reported MCAs had recognizable dysmorphic or dysmetabolic syndromes, and in another 180 (3.6%) the CAs in the MCAs were unspecified; these 1 753 children were excluded from analysis. Thus, 6 425 CAs of 2 762 patients with unidentified MCAs were evaluated from 1 186 776 births. In Hungary, autopsy is obligatory in cases of infant death. Suitable postmortem records were accessible in about half of stillborns. Three categories (X, Y, Z) and 40 groups of major component CAs (marked by two capital letters) in those categories were distinguished in the unidentified MCAs (Table I). The categories and groups have been defined elsewhere [9].

The number of MCAs with a given number of component CAs are listed in Table II. Here the diagonal gives the number of children with the corresponding number of CAs, 1—7. The number below the diagonal reflects the observed combinations of CAs, e.g., the number 732 in line 4 and column 2 means the number of CA pairs among the 122 children with four CAs involving six possible CA pairs ($732 = 122 \times 6$). The sums of the individual columns give the total number of CAs, CA pairs etc.

The number of theoretically possible CA pairs is 780. Of these, 358 occurred in the 2 128 children with two CAs, and 204 occurred in the 634 children with three or more CAs. The other 218 theoretically possible CA pairs did not occur. Some of them are only formally possible, because certain CAs are mutually exclusive (e.g., AN and EN, AN and SP, CL and CP, and LR and SY). Among the 448 children with three

Table II

Distribution of number of CAs in infants with unidentified MCAs

Size of subgroups	Number of CAs							Number of different groups ("only" cases)
	1	2	3	4	5	6	7	
Number of CAs								
1	28.088			2.762 MCAs				40
2	4.256	2.128						358
3	1.344	1.344	448					340
4	488	732	488	122				119
5	250	500	500	250	50			50
6	66	165	220	165	66	11		11
7	21	63	105	105	63	21	3	3
							30.850	
Total	34.513	4.932	1.761	642	179	32	3	921
Number of different groups ("total" cases)	40	562	1.141	612	178	32	3	2.568

CAs, 340 of the theoretically possible 9 880 combinations were found. Among the 122 children with four CAs, only 119 of the theoretically possible 91 390 combinations occurred. All of the 64 children with five or more CAs had different combinations. The corresponding numbers of children with at least 1—7 CAs are given in the last column of Table II.

Obviously there is an ascertainment bias in our material with two opposite consequences. On the one hand, if index patients are affected with two or three CAs and one CA is reported, it is more likely that one or two other CAs present will also be reported. On the other hand, if too many CAs occur, physicians are prone to report only some, probably more important, CAs. Additionally, delineation and recognition of MCA entities are the task of clinicians who see and examine the patient. MCAs, however, involve an extremely high number of different CA-combinations and the diagnosis of rare dysmorphological entities is difficult. In order to improve the standard of dysmorphology it is worth-wile trying to use the materials of CA-registries and surveillances for this purpose, too.

Results and Discussion

Preclusion of independent CAs

The first hypothesis, that CAs in unidentified MCAs are random combinations, was rejected in a small sample (232 MCAs) on the basis that two or more CAs were found in the same person more often than they would occur by chance [17]. As a first step, we considered a further study of this hypothesis to be interesting by checking the observation in a large population and characterizing the measure of deviation from random combinations.

If CAs were independent, the expected number of newborns with two CAs would be 304. (The number of newborns having 40 CAs was taken into account in this estimation.) The observed number, 2 128 (Table II) differed from the expected number, indicating that the CAs are not independent. The rate of independence, however, depends on the number of CAs in the MCAs. The ratio of expected random combinations to observed causal effect was 1:6, 1:225, 1:14,000, and $1:2.10^6$ in two (CA pairs), three (CA triplets), and four and five or more CAs, respectively.

Furthermore, if the CAs were independent, their distribution would be Poissonian. According to the logarithm, of the diagonal of Table II, this was not the case (Fig. 1). A mixture of two Poissonian distributions, i.e., a small number of expected isolated CAs (one developmental anomaly only) and a high number of expected CA syndromes could, however, be taken into consideration.

Data on the 40 CAs are summarized in Table I. Column 1 shows the number of infants with isolated CAs, thus the prevalence rates can be calculated. The columns numbered 2—7 show the number of children with the CA listed and with 1—6 other CAs. The relative frequencies (percentages) of CAs in the children with a given number of CAs are shown in Table III. (The number of CA pairs and triplets was published earlier [9].) The column "Mean" shows the weighted average of columns 1—7. The column "Rise" of CAs in the children dem-

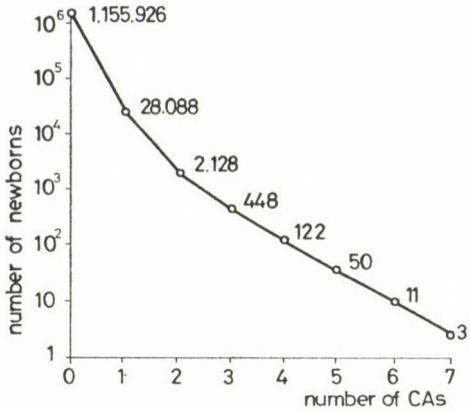


FIG. 1

onstrates how the relative frequencies change with the increasing number of CAs. The column "χ_1^2" shows the differences between the estimated relative frequencies of the mean and of the rise values. For AA and EA, the rise war significantly positive, whereas for CD it was significantly negative.

Table III also shows the homogeneity of the unidentified MCAs derived from Table I. In column 1 (Table III), the first number, 3.50 means, that 3.50% of the 28 088 babies with one CA had AN; the actual number was 984. As Table III indicates, there were no significant differences between the relative frequencies of CAs in newborns with different numbers of CAs except in the above-mentioned cases of AA, EA, and CD. One would expect large differences in the MCAs consisting of CA syndromes. The statistical tests used here and elsewhere did not reflect an exact χ^2 distribution; however, the development of an exact test was beyond our capacities.

We could not test the second hypothesis, because it requires a personal examination by syndromologists. Thus, we shall now discuss the third hypothesis.

A multidimensional threshold model

Let $L = (L_1, \ldots, L_n)$ be an n-dimensional random variable and $T = (T_1, \ldots, T_n)$ a given n-dimensional vector. Let us denote by A_k the kth CA, and let G be an MCA. According to our hypothesis,

$$\{L_k \geq T_k\} \text{ if and only if } \{A_k \in G\},$$

i.e., the coordinates of L exceeding the corresponding coordinates of T indicated the presence of the corresponding CAs. In our study we assumed that the random variable L was a multidimensional Gaussian variable and the Le_k's had an expected value of 0 and a variance of 1. Therefore, the distribution of L was determined by the correlations

$$\varrho_{ij} = E(L_i L_j), \quad 1 \leq i,j \leq n.$$

TABLE III

Relative frequency (per cent) of CAs in newborns with a given number of CAs

CA group	Number of CAs							1—7		χ^2_1
	1	2	3	4	5	6	7	Mean	Rise	
AN	3.50	2.51	2.08	0.41	0.80	1.52	0.00	3.36	—0.79	0.57
EN	0.95	1.08	1.56	0.20	1.20	1.52	0.00	1.01	0.16	0.06
SP	4.25	2.91	2.60	2.46	0.80	0.00	0.00	4.07	—0.91	0.68
CL	3.88	4.04	6.40	4.10	5.20	4.55	4.76	4.07	0.55	0.76
CP	1.29	1.55	2.08	3.09	5.20	4.55	4.76	1.60	0.75	2.00
LR	1.38	1.48	2.83	1.64	3.60	3.03	0 00	1.55	0.46	0.62
PY	2.42	2.21	3.87	5.12	3.60	6.06	4.76	2.59	0.60	1.02
SY	1.04	1.53	2.23	1.23	2.00	0.00	4.76	1.23	0.41	0.44
EX	0.79	1.76	2.60	2.15	2.00	1.52	0.00	1.24	0.60	0.96
OA	0.71	1.17	1.56	1.84	2.80	4.55	0.00	0.98	0.52	0.91
AA	0.75	1.69	2.98	3.28	5.20	10.61	14.29	1.76	1.44	7.95
MC	0.52	1.46	2.23	3.87	2.80	0.00	0.00	1.15	0.77	1.47
HY	2.67	3.08	3.72	5.53	4.40	6.06	4.76	2.89	0.63	1.05
AM	0.12	0.59	1.04	1.84	1.60	0.00	0.00	0.66	0.47	0.42
CT	0.17	0.45	0.30	0.61	0.80	0.00	0.00	0.28	0.15	0.06
EY	0.52	0.54	0.52	1.64	1.20	0.00	0.00	0.58	0.22	0.14
CF	6.96	10.22	7.51	5.74	6.40	6.06	14.29	7.53	0.80	1.60
HS	5.23	3.83	2.90	1.84	1.60	1.52	4.76	5.01	—1.09	1.58
EG	0.15	0.54	1.19	2.85	2.80	4.55	9.52	1.07	0.91	2.48
HD	15.15	13.67	13.62	13.73	0.40	7.58	14.29	14.88	—0.92	1.20
DI	0.82	1.57	2.08	2.25	2.00	1.52	4.76	1.15	0.50	0.74
RA	0.21	0.69	1.64	1.64	0.80	3.03	0.00	0.79	0.52	0.60
CK	0.35	1.03	2.23	1.84	2.80	1.52	4.76	0.99	0.66	1.23
AI	0.69	1.66	0.30	0.41	0.40	1.52	0.00	0.68	—0.02	0.00
ON	0.30	0.02	0.22	0.41	0.00	0.00	0.00	0.30	—0.00	0.00
FN	0.24	0.80	2.23	3.28	4.40	4.55	4.76	1.41	0.98	2.19
EA	0.80	1.06	2.60	5.53	6.80	1.52	0.00	1.68	1.35	5.94
TC	0.52	4.96	2.60	1.02	0.80	0.00	0.00	3.06	1.64	2.15
BR	0.22	0.02	0.07	0.20	0.00	0.00	0.00	0.21	—0.04	0.00
CD	25.05	14.99	5.06	2.46	2.40	0.00	4.76	23.99	—9.18	40.71
OL	0.32	0.63	0.60	0.81	0.40	1.52	0.00	0.42	0.16	0.07
RS	0.64	1.15	1.41	2.25	0.00	3.03	0.00	0.88	0.48	0.55
PS	1.41	0.52	0.30	0.80	0.00	1.52	0.00	1.35	—0.43	0.18
OD	1.29	1.29	1.71	1.84	0.80	1.52	0.00	1.32	0.11	0.03
UT	2.45	3.43	3.13	3.69	4.80	4.55	0.00	2.71	0.55	0.70
OU	1.39	2.11	3.50	3.48	2.40	7.58	0.00	1.82	0.84	2.21
IH	9.16	5.80	2.75	1.84	1.20	0.00	0.00	8.76	—3.03	0.00
MS	0.47	1.55	2.46	2.46	4.40	1.52	0.00	1.27	0.86	1.98
EO	0.51	0.73	0.74	1.23	0.80	0.00	0.00	0.58	0.17	0.07
ST	0.73	0.45	0.45	1.02	0.40	1.52	0.00	0.71	—0.00	0.00

These n(n-1)/2 unknown variables were estimated from the equations

$$N\,P\,(L_i \geq T_i, L_j \geq T_j) = O_T(i,j),$$

where $O_T(i,j)$ is the total number of newborns affected by both A_i and A_j (and perhaps by other CAs, too) in the N births. The probabilities $P_{ij} = P(L_i \geq T_i, L_j \geq T_j)$ were calculated from the following expansion described by Pearson [1]:

$$P_{ij} = \sum_{\nu=0}^{\infty} \frac{\varrho^{\nu}_{ij}}{\nu!} Q^{(\nu)}(T_i) Q^{(\nu)}(T_j),$$

where $Q(t) = \int_t^{\infty} (2\pi)^{-1/2} \exp(-u^2/2)\,du$ and $Q^{(\nu)}$ denotes the νth derivative of Q [20]. The ϱ_{ij} terms are listed in

Table IV with the respective averages for the individual CAs. The variables T_i were estimated from the equation

$$N\ P\ (L_k \geq T_k) = O_T(k),$$

where $O_T(k)$ is the total number of newborns affected by A_k (and perhaps by other CAs, too) in the N births.

Table IV shows a negative correlation in a few cases, e.g., between AN and EN, AN and SP, AN and CP, and CL and CP. The majority of these negative correlations are caused by the definition of the CAs. For example if AN-EN (AN), AN-SP (AN), AN-CP (AN), or CL-CP (CL) occur in the same baby only one CA (the one in parenthesis) is evaluated This is a weak point of our model, because theoretically an impossible event is not the same as an event with low probability.

The multidimensional threshold model is an extension of the GAMT model [6, 21]. L_k is the liability of the newborns to A_k, and T_k is the corresponding threshold. The independence of CAs means the independence of the coordinates of the random vector L. In the original GAMT model, the liability was used for describing the multidimensional configuration on a family tree for one CA. One affected newborn has many liabilities, i.e., one specialized liability to each CA. The relationship of these liabilities indicates the presence or absence of the CAs in newborns. Although in our study we could not evaluate the familial data because they were not available, we still refer to our model as the GAMT model.

Our main objective was to decide whether MCAs with CA triplets followed the structure of the correlation matrix R. (Random combinations are found too frequently in CA pairs, and reporting is more likely to be incomplete when MCAs consist of four or more CAs.) In the GAMT model, the expected number $E_T(G)$ of a given group $G = \{A_i, A_j, A_k\}$ of newborns with three CAs (previously defined) is $NP_T(G)$, where

$$P_T(G) = \sum_{\nu_{12}=0}^{\infty} \sum_{\nu_{13}=0}^{\infty} \sum_{\nu_{23}=0}^{\infty} \times$$

$$\frac{\varrho_{ij}^{\nu_{12}} \varrho_{ik}^{\nu_{13}} \varrho_{jk}^{\nu_{23}}}{\nu_{12}!\nu_{13}!\nu_{23}!} Q^{(\mu_1)}(T_i)\ Q^{(\mu_2)}(T_j)\ Q^{(\mu_3)}(T_k)$$

$$\mu_1 = \nu_{12} + \nu_{13}, \mu_2 = \nu_{12} + \nu_{23}, \quad \mu_3 = \nu_{13} + \nu_{23}.$$

This is the generalization of the Pearson expansion of the two-dimensional normal distribution function. As proven by Taqqu [19], this expansion is convergent if the largest value of R is less than 1/2. [In the s-dimensional case it ought to be less than 1/(s-1).] In our calculations, only the terms larger than 10^{-10} were taken into account. The joint distribution of the expected and the observed occurrences is given in Figure 2. The largest observed occurrence was 26 : CF—TC—CD. This CA combination is compatible with the theory that postural deformities are associated, i.e., Congenital Postural Deformity Association [16]. The expected occurrence of this combination is 30;

TABLE IV

The correlation matrix

CA \ CA	AN	EN	SP	CL	CP	LR	PY	SY	EX
AN	1	—0.02	—0.13	0.42	—0.05	0.29	0.20	0.33	0.47
EN	—0.02	1	0.03	0.40	0.43	0.37	0.39	0.31	0.45
SP	—0.13	—0.03	1	0.19	0.30	0.19	0.23	0.24	0.40
CL	0.42	0.49	0.19	1	—0.07	0.33	0.43	0.38	0.47
CP	0.05	0.43	0.30	—0.07	1	0.35	0.39	0.38	0.17
LR	0.29	0.37	0.19	0.33	0.35	1	0.32	0.00	0.47
PY	0.20	0.39	0.23	0.43	0.39	0.32	1	—0.01	0.29
SY	0.33	0.31	0.24	0.35	0.38	0.00	—0.01	1	0.26
EX	0.47	0.45	0.40	0.47	0.17	0.47	0.29	0.26	1
OA	0.23	0.29	0.24	0.29	0.34	0.41	0.21	0.28	0.33
AA	0.16	0.32	0.41	0.35,	0.41	0.48	0.42	0.30	0.40
MC	0.00	0.00	0.25	0.42	0.38	0.26	0.40	0.42	0.30
HY	—0.10	—0.01	—0.12	0.41	0.40	0.41	0.37	0.37	0.36
AM	0.00	0.32	0.28	0.50	0.53	0.29	0.41	0.47	0.32
CT	0.00	0.00	0.00	0.41	0.43	0.30	0.38	0.00	0.00
EY	0.00	0.00	0.21	0.31	0.30	0.22	0.25	0.31	0.25
CF	0.07	0.09	—0.19	0.29	0.33	0.24	0.30	0.42	0.26
HS	0.04	0.06	0.09	0.07	0.02	0.09	0.02	0.08	0.03
EG	0.36	0.30	0.26	0.35	0.40	0.51	0.44	0.29	0.38
HD	0.16	0.25	0.24	0.34	0.38	0.32	0.33	0.23	0.38
DI	0.35	0.36	0.39	0.27	0.44	0.28	0.27	0.19	0.41
RA	0.39	0.28	0.49	0.17	0.00	0.49	0.28	0.27	0.00
CK	0.42	0.49	0.25	0.36	0.22	0.29	0.49	0.24	0.33
AI	0.00	0.00	0.00	—0.01	0.00	0.36	0.23	0.00	0.23
ON	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00
FN	0.00	0.39	0.33	0.35	0.58	0.46	0.39	0.51	0.34
EA	—0.02	0.27	0.27	0.40	0.50	0.39	0.40	0.44	0.27
TC	—0.03	0.00	—0.05	—0.05	0.00	0.00	0.11	0.00	0.00
BR	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00
CD	—0.12	—0.01	—0.28	0.07	0.07	—0.01	0.09	0.07	—0.07
OL	0.00	0.00	0.29	0.24	0.38	0.00	0.20	0.27	0.36
RS	0.24	0.30	0.25	0.17	0.27	0.26	0.14	0.21	0.38
PS	—0.04	0.00	—0.05	—0.05	0.15	0.00	0.10	0.00	0.18
OD	0.22	0.18	0.12	0.22	0.22	0.14	0.31	0.31	0.00
UT	0.23	0.31	0.19	0.32	0.28	0.27	0.31	0.30	0.31
OU	0.33	0.31	0.37	0.26	0.28	0.24	0.35	0.00	0.38
IH	—0.09	—0.09	0.08	0.13	0.17	0.05	0.06	0.19	0.12
MS	0.00	0.00	0.39	0.18	0.50	0.35	0.30	0.37	0.38
EO	0.27	0.25	0.34	0.13	0.34	0.29	0.17	0.24	0.25
ST	0.00	0.23	0.18	0.18	0.20	0.20	0.33	0.22	0.23
	0.12	0.18	0.17	0.26	0.27	0.26	0.28	0.23	0.26

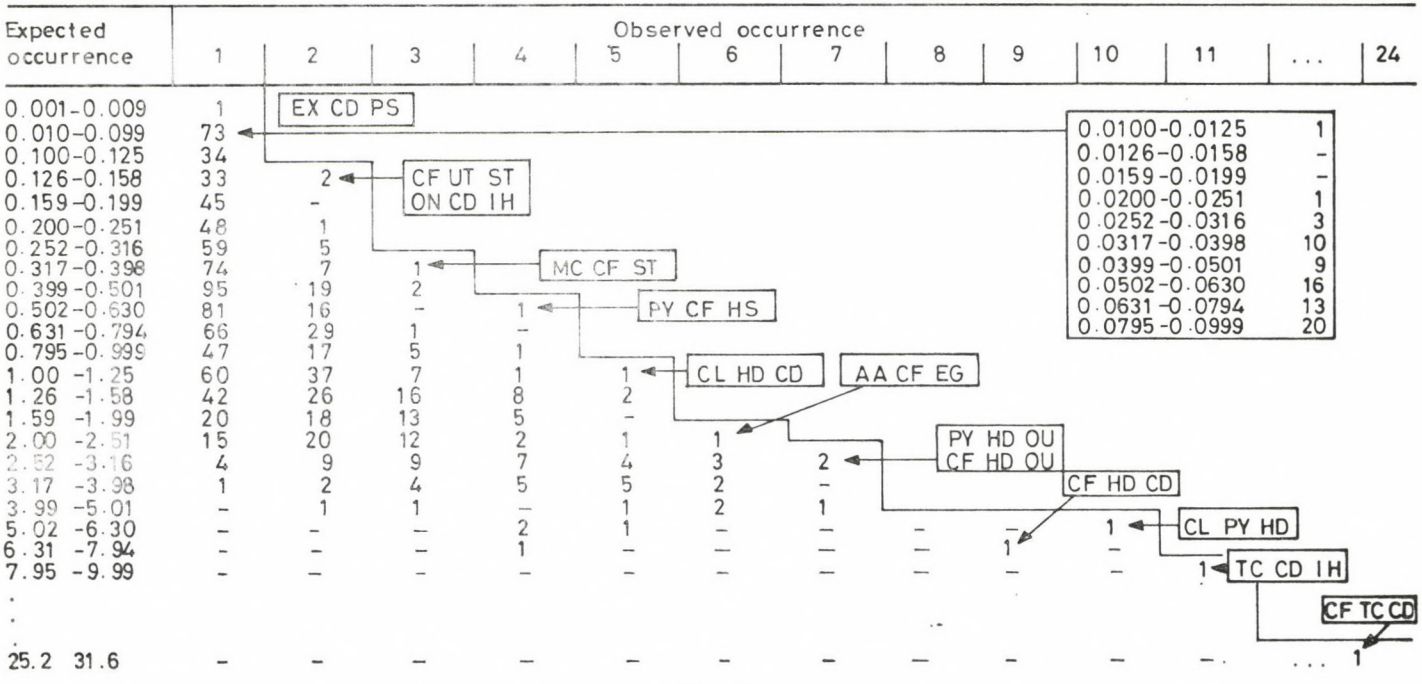
in the GAMT model

OA	AA	MC	HY	AM	CT	EY	CF	HS	EG	HD
0.23	0.16	0.00	—0.10	0.00	0.00	0.00	0.07	0.04	0.36	0.16
0.29	0.32	0.00	—0.01	0.32	0.00	0.00	0.09	0.06	0.30	0.25
0.24	0.41	0.25	—0.12	0.28	0.00	0.21	—0.19	0.09	0.26	0.24
0.29	0.35	0.42	0.41	0.50	0.41	0.31	0.29	0.07	0.35	0.34
0.34	0.41	0.38	0.40	0.53	0.43	0.30	0.33	0.02	0.40	0.38
0.41	0.48	0.26	0.41	0.29	0.30	0.22	0.24	0.09	0.51	0.32
0.21	0.42	0.40	0.37	0.41	0.38	0.25	0.30	0.02	0.44	0.33
0.28	0.30	0.42	0.37	0.47	0.00	0.31	0.42	0.08	0.29	0.23
0.33	0.40	0.30	0.36	0.32	0.00	0.25	0.26	0.03	0.38	0.38
1	0.61	0.00	0.31	0.00	0.34	0.00	0.28	0.05	0.00	0.45
0.61	1	0.38	0.34	0.32	0.41	0.32	0.38	0.07	0.66	0.40
0.00	0.38	1	0.13	0.57	0.48	0.53	0.39	0.00	0.45	0.43
0.41	0.34	0.13	1	0.53	0.47	0.39	0.29	0.07	0.50	0.36
0.00	0.32	0.57	0.53	1	0.00	0.00	0.28	0.01	0.42	0.41
0.34	0.41	0.48	0.47	0.00	1	0.00	0.24	0.00	0.00	0.38
0.00	0.32	0.53	0.39	0.00	0.00	1	0.08	0.00	0.00	0.33
0.28	0.37	0.39	0.29	0.28	0.24	0.08	1	0.09	0.48	0.23
0.05	0.07	0.00	0.07	0.01	0.00	0.00	0.09	1	0.36	0.24
0.00	0.66	0.45	0.50	0.42	0.00	0.00	0.48	0.36		0.41
0.45	0.40	0.43	0.36	0.41	0.38	0.33	0.23	0.24	0.41	1
0.34	0.32	0.22	0.33	0.32	0.00	0.25	0.28	0.21	0.43	0.48
0.49	0.57	0.30	0.45	0.00	0.00	0.33	0.34	0.15	0.46	0.46
0.34	0.37	0.35	0.41	0.50	0.00	0.00	0.48	0.23	0.35	0.44
0.25	0.35	0.26	0.26	0.35	0.00	0.00	0.18	0.25	0.33	0.30
0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.16
0.36	0.45	0.50	0.49	0.61	0.00	0.48	0.41	0.35	0.53	0.45
0.29	0.47	0.53	0.44	0.58	0.41	0.46	0.32	0.27	0.50	0.45
0.00	0.00	0.28	—0.02	0.00	0.00	0.24	0.50	—0.06	0.00	0.11
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.12
0.16	0.08	0.21	0.06	0.05	0.07	0.12	0.44	—0.03	0.15	0.07
0.00	0.40	0.38	0.00	0.00	0.00	0.00	0.40	0.15	0.46	0.29
0.31	0.34	0.37	0.35	0.00	0.00	0.27	0.26	0.20	0.49	0.47
0.20	0.00	0.00	—0.03	0.00	0.00	0.00	0.12	0.11	0.00	0.17
0.37	0.24	0.32	0.15	0.30	0.00	0.23	0.16	0.10	0.36	0.38
0.20	0.43	0.42	0.26	0.32	0.25	0.24	0.28	0.45	0.49	0.28
0.43	0.57	0.26	0.31	0.36	0.27	0.21	0.28	0.13	0.43	0.45
0.10	0.08	0.18	0.10	0.14	0.00	0.07	0.12	0.22	0.00	0.24
0.43	0.43	0.44	0.47	0.47	0.35	0.28	0.35	0.34	0.46	0.44
0.26	0.24	0.00	0.39	0.00	0.38	0.30	—0.04	0.11	0.00	0.35
0.00	0.23	0.38	0.00	0.35	0.00	0.28	0.23	0.17	0.41	0.29
0.23	0.34	0.29	0.26	0.26	0.15	0.19	0.25	0.19	0.32	0.32

TABLE IV *(cont'd)*

CA \ CA	DI	RA	CK	AI	ON	FN	EA	TC	BR
AN	0.35	0.39	0.42	0.00	0.00	0.00	—0.02	—0.03	0.00
EN	0.36	0.28	0.49	0.00	0.00	0.39	0.27	0.00	0.00
SP	0.39	0.49	0.25	0.00	0.00	0.33	0.27	—0.05	0.00
CL	0.27	0.17	0.36	—0.01	0.00	0.35	0.40	—0.05	0.23
CP	0.44	0.00	0.22	0.00	0.00	0.58	0.50	0.00	0.00
LR	0.28	0.49	0.29	0.36	0.00	0.46	0.39	0.00	0.00
PY	0.27	0.28	0.49	0.23	0.24	0.39	0.40	0.11	0.00
SY	0.19	0.27	0.24	0.00	0.00	0.51	0.44	0.00	0.00
EX	0.41	0.00	0.33	0.23	0.00	0.34	0.27	0.00	0.00
OA	0.34	0.49	0.34	0.25	0.00	0.36	0.29	0.00	0.00
AA	0.32	0.57	0.37	0.35	0.31	0.45	0.47	0.00	0.00
MC	0.22	0.30	0.35	0.26	0.00	0.50	0.53	0.28	0.00
HY	0.33	0.45	0.41	0.26	0.00	0.49	0.44	—0.02	0.00
AM	0.32	0.00	0.50	0.35	0.00	0.61	0.58	0.00	0.00
CT	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.00	0.00
EY	0.25	0.33	0.00	0.00	0.00	0.48	0.46	0.24	0.00
CF	0.28	0.34	0.48	0.18	0.15	0.41	0.32	0.50	0.00
HS	0.21	0.15	0.23	0.25	0.00	0.35	0.27	—0.06	0.21
EG	0.43	0.46	0.35	0.33	0.00	0.53	0.50	0.00	0.00
HD	0.48	0.46	0.44	0.30	0.16	0.45	0.45	0.11	0.12
DI	1	0.45	0.33	0.36	0.00	0.43	0.27	0.00	0.00
RA	0.45	1	0.00	0.00	0.00	0.47	0.00	0.00	0.00
CK	0.33	0.00	1	0.00	0.00	0.53	0.37	0.00	0.00
AI	0.36	0.00	0.00	1	0.00	0.30	0.23	0.00	0.00
ON	0.00	0.00	0.00	0.00	1	0.00	0.31	0.00	0.00
FN	0.43	0.47	0.53	0.30	0.00	1	0.61	0.25	0.00
EA	0.27	0.00	0.37	0.23	0.31	0.61	1.00	0.30	0.34
TC	0.00	0.00	0.00	0.00	0.00	0.25	0.30	1.00	0.00
BR	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	1.00
CD	—0.01	0.01	0.09	0.03	0.17	0.10	0.18	0.71	—0.04
OL	0.00	0.44	0.00	0.32	0.00	0.00	0.28	0.27	0.00
RS	0.00	0.50	0.51	0.00	0.34	0.29	0.30	0.00	0.00
PS	0.00	0.00	0.00	0.22	0.00	0.25	0.00	0.00	0.00
OD	0.00	0.42	0.48	0.00	0.00	0.24	0.25	0.00	0.00
UT	0.33	0.20	0.24	0.15	0.00	0.37	0.38	0.10	0.00
OU	0.40	0.46	0.46	0.27	0.27	0.41	0.31	0.00	0.00
IH	—0.09	—0.02	0.14	—0.06	0.21	0.26	0.20	0.31	0.24
MS	0.35	0.30	0.35	0.26	0.00	0.63	0.45	0.21	0.00
EO	0.41	0.50	0.49	0.00	0.00	0.39	0.00	0.00	0.00
ST	0.23	0.00	0.28	0.00	0.00	0.29	0.30	0.00	0.00
	0.25	0.25	0.28	0.14	0.06	0.35	0.33	0.08	0.03

CD	OL	RS	PS	OD	UT	ON	IH	MS	EO	CT
—0.12	0.00	0.24	—0.04	0.22	0.23	0.33	—0.09	0.00	0.27	0.00
—0.01	0.00	0.30	0.00	0.18	0.31	0.31	—0.09	0.00	0.25	0.18
—0.28	0.29	0.25	—0.05	0.12	0.19	0.37	0.08	0.39	0.34	0.18
0.07	0.24	0.17	—0.05	0.22	0.32	0.26	0.13	0.18	0.13	0.18
0.07	0.38	0.27	0.15	0.22	0.28	0.28	0.17	0.50	0.34	0.20
—0.01	0.00	0.26	0.00	0.14	0.27	0.24	0.05	0.35	0.29	0.20
0.09	0.20	0.14	0.10	0.31	0.31	0.35	0.06	0.30	0.17	0.33
0.07	0.27	0.21	0.00	0.31	0.30	0.00	0.19	0.37	0.24	0.22
—0.07	0.36	0.38	0.18	0.00	0.31	0.38	0.12	0.38	0.25	0.23
—0.16	0.00	0.31	0.20	0.37	0.20	0.43	0.10	0.43	0.26	0.00
0.08	0.40	0.34	0.00	0.24	0.43	0.57	0.08	0.43	0.24	0.23
0.21	0.38	0.37	0.00	0.32	0.42	0.26	0.18	0.44	0.00	0.38
0.06	0.00	0.35	—0.03	0.15	0.26	0.31	0.10	0.47	0.39	0.00
0.05	0.00	0.00	0.00	0.30	0.32	0.36	0.14	0.47	0.00	0.35
0.07	0.00	0.00	0.00	0.00	0.25	0.37	0.00	0.35	0.38	0.00
0.12	0.00	0.27	0.00	0.23	0.24	0.21	0.07	0.28	0.30	0.28
0.44	0.40	0.26	0.12	0.16	0.28	0.28	0.12	0.35	—0.04	0.23
—0.03	0.15	0.20	0.11	0.10	0.45	0.13	0.22	0.34	0.11	0.17
0.15	0.46	0.49	0.00	0.36	0.49	0.43	0.00	0.46	0.00	0.41
0.07	0.29	0.47	0.17	0.38	0.28	0.45	0.24	0.44	0.35	0.29
—0.01	0.00	0.00	0.00	0.00	0.33	0.40	—0.09	0.35	0.41	0.23
0.01	0.44	0.50	0.00	0.42	0.20	0.46	—0.02	0.50	0.50	0.00
0.09	0.00	0.51	0.00	0.48	0.24	0.46	0.14	0.35	0.49	0.28
0.03	0.32	0.00	0.22	0.00	0.15	0.27	—0.06	0.26	0.00	0.00
0.17	0.00	0.34	0.00	0.00	0.00	0.27	0.21	0.00	0.00	0.00
0.10	0.00	0.29	0.25	0.24	0.37	0.41	0.26	0.63	0.39	0.29
0.18	0.28	0.30	0.00	0.25	0.38	0.31	0.20	0.45	0.00	0.30
0.71	0.27	0.00	0.00	0.00	0.10	0.00	0.31	0.21	0.00	0.00
—0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.00
1.00	0.25	0.01	0.01	0.03	0.18	0.01	0.20	0.22	—0.13	0.02
0.25	1.00	0.00	0.00	0.26	0.20	0.32	0.10	0.47	0.00	0.00
0.01	0.00	1.00	0.00	0.35	0.00	0.36	—0.07	0.41	0.40	0.00
0.01	0.00	0.00	1.00	0.23	—0.02	0.21	0.22	0.00	0.00	0.00
0.03	0.26	0.35	0.23	1.00	0.29	0.37	0.17	0.27	0.38	0.00
0.18	0.20	0.00	—0.02	0.29	1.00	0.33	0.34	0.29	0.00	0.22
0.01	0.32	0.36	0.21	0.37	0.33	1.00	—0.13	0.30	0.37	0.19
0.20	0.10	—0.07	0.22	0.17	0.34	—0.13	1.00	0.30	—0.05	0.05
0.22	0.47	0.41	0.00	0.27	0.29	0.30	0.30	1.00	0.40	0.33
—0.13	0.00	0.40	0.00	0.38	0.00	0.37	—0.05	0.40	1.00	0.00
0.02	0.00	0.00	0.00	0.00	0.22	0.19	0.05	0.33	0.00	1.00
0.07	0.17	0.22	0.05	0.21	0.25	0.29	0.11	0.32	0.19	0.15


Expected occurrence
Observed occurrence
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2
3
4
5
6
7
8
9
10
11
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24
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0.010-0.099 73
0.100-0.125 34
0.126-0.158 33
0.159-0.199 45
0.200-0.251 48
0.252-0.316 59
0.317-0.398 74
0.399-0.501 95
0.502-0.630 81
0.631-0.794 66
0.795-0.999 47
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1.59 -1.99 20
2.00 -2.51 15
2.52 -3.16 4
3.17 -3.98 1
3.99 -5.01 -
5.02 -6.30 -
6.31 -7.94 -
7.95 -9.99 -
25.2 31.6 -
EX CD PS
CF UT ST ON CD IH
MC CF ST
PY CF HS
CL HD CD
AA CF EG
PY HD OU CF HD OU
CF HD CD
CL PY HD
TC CD IH
CF TC CD
0.0100-0.0125 1
0.0126-0.0158 -
0.0159-0.0199 -
0.0200-0.0251 1
0.0252-0.0316 3
0.0317-0.0398 10
0.0399-0.0501 9
0.0502-0.0630 16
0.0631-0.0794 13
0.0795-0.0999 20

FIG. 2

therefore, this occurrence follows the model. (The corresponding correlations were, CF—TC = 0.50; CF—CD = 0.44; TC—CD = 0.71.) As the observed occurrences become smaller, the distribution of the expected occurrences becomes wider with each step. The deviation, however, is not significant because of the extremely large number of theoretically possible combinations.

Each column of Figure 2 shows a consistent under-estimation of the expected values. In cases marked individually (EX—CD—PS, CF—UT—CT, ON—CD—IH etc.), the difference is obvious because other triplets with nearly the same expected occurrence were not observed. If in a study we see one instance in which the expected occurrence is 1/1,000, we may be surprised. If, however, there are 1,000 rare births in which the expected occurrence is 1/1,000, then we should not be surprised to see at least one of them. That is why our main concern was not the individual value for the expected occurrence of an observed triplet, but it was the expected number of triplets with a given expected occurrence. We calculated the expected occurrences for all the theoretically possible. 9 880 triplets, and we found no significant deviation between the sums of the observed and the expected occurrences (1 761 vs. 1 876).

If we denote by $E_c^3(k)$, respectively $E_c^3(i,j)$, the sum of the expected total numbers fo triplets containing A_k, or A_j and A_i and by $|G|$ the number of CAs in G, then

$$E_c^3(k) = \sum_{GA_k \in G\ |G|=3} E_T(G),\ E^3(i,j) = \sum_{G:A_i,A_j \in G|G|=3} E_T(G).$$

[The corresponding observed numbers were denoted $0_c^3(k)$ and $O_c^3(i,j)$.] The sum of the terms $E_c^3(i,j)$, and of $O_c^3(i,j)$ for all (i,j) pairs was 5 627 and 5 283, respectively. The numbers $E_c^3(k)$, $O_c^3(k)$ are given in Table V, with the usual χ^2 statistics $(O-E)^2/E$. The distribution is not χ^2, however, because the summands in $O_c^3(k)$ are not independent.

In CA triplets, the multidimensional threshold model could fit the observed values on the basis of distribution of CAs within CA pairs; thus the third hypothesis may be valid, and our model may explain the combination of CAs in MCAs. Although not being able to reject a null hypothesis does not make it correct, our model seems to answer an important question. From the teratological point of view, it is difficult to understand why no explanation has been found for the combination of CAs in the extremely high number of unidentified MCAs. Our model confirms that it is worth-while to consider a third type of MCAs besides CA syndromes and random combinations: CA-associations [18]. Our data do not, however, reject that there are further CA-syndromes to be found. The findings of the Hungarian nation-wide follow-up evaluation of unidentified MCAs [11] are consistent with this hypothesis and the proportion of recognized CA-syndromes and CA-associations was increased to 50%.

TABLE V

Observed and expected cumulative occurrences of CAs in the at-least threefold cases

CA	Observed	Expected	χ^2
AN	56	71.08	3.20
EN	52	66.76	3.26
SP	83	121.84	12.38
CL	269	255.69	0.69
CP	208	186.67	2.44
LR	136	151.55	1.60
PY	236	215.47	1.96
SY	93	108.50	2.21
EX	105	133.89	6.23
OA	120	115.45	0.18
AA	281	268.80	0.55
MC	117	160.11	11.61
HY	252	255.50	0.05
AM	65	101.20	12.95
CT	25	30.74	1.07
EY	49	47.96	0.02
CF	366	341.25	1.80
HS	115	133.95	2.68
EG	148	156.75	0.49
HD	635	632.55	0.01
DI	116	125.55	0.73
RA	78	105.56	7.19
CK	124	139.93	1.81
AI	26	26.21	0.00
ON	9	3.92	6.58
FN	189	231.05	7.65
EA	229	234.11	0.11
TC	62	66.99	0.37
BR	4	1.40	4.82
CD	155	163.80	0.47
OL	33	37.51	0.54
RS	72	84.77	1.92
PS	14	8.40	3.74
OD	72	77.96	0.46
UT	201	194.08	0.25
OU	184	197.80	0.93
IH	82	96.42	2.16
MS	145	194.77	12.72
EO	40	50.25	2.09
ST	37	30.64	1.32

The biological background of CA-associations has not yet been determined. Roberts and Powell [16] suggested that [17] "there is 'one single cause' for most human malformations and that this is likely to be 'intrinsic' rather than 'extrinsic' "; Cohen [4], however, reported some additional aetiological possibilities. The overlapping of polygenic systems and/or environmental triggering factors in the pathogenesis of common CAs [3], the susceptibility and interrelationship of some organs by critical time of impact, the joint intrinsic regulatory system [8], and development of the field effect theory [15] could explain the validity of the

multidimensional threshold model in at least some of the unidentified MCAs. According to the multidimensional threshold model, the different CAs may be manifestations of a developmental spectrum; thus, beyond their unidimensional aetiological background they may have a basic underlying interrelationship. It follows that the present practice of providing genetic counselling only regarding the recurrence of a specific malformation should be modified. For example, after delivery of a baby with oesophageal atresia or diaphragmatic defect, the counsellor should be able to estimate not only the recurrence of oesophageal astresia and diaphragmatic defect but also the occurrence of neural tube defects, in the next pregnancy [13, 11].

For identifying and detecting CA-syndromes and CA-associations, we used multidimensional scaling, factor and cluster analysis, loglinear model [14], and latent structure analysis [20]. Investigators hope to find the final clue to this problem through the study of families with cases of unidentified MCAs [12]. Perhaps the family study of our data for 1970—1980, involving more than 1000 cases will provide some progress in this field.

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Immunoglobulin levels in bronchoalveolar lavage fluid of children with recurrent obstructive bronchitis

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Bronchoalveolar lavage was performed in 22 children with recurrent obstructive bronchitis and the recovered lavage fluid samples were analysed for concentration of IgA, IgG, IgM, IgE and C_3. Previously a significant influx of exudate macrophages and persistence of bacteria on the bronchoalveolar surface were detected in these patients and a severe mucosal inflammation was observed bronchoscopically. The relative lavage fluid levels of immunoblobulins to albumin were significantly higher than in serum, indicating a local production of these proteins. The elevated levels of C_3 indicated a high activity of the macrophages and the complement system. It is concluded that the mucosal inflammation in patients with recurrent obstructive symptoms cannot be attributed to a deficiency of immunoglobulins either in blood or in bronchial secretions.

Bronchoalveolar lavage (BAL) fluid contains a wide variety of cells and proteins which play an integral role in lung host defence and inflammatory reactions [7]. In children with recurrent obstructive bronchitis we have determined the morphological, cytochemical and functional characteristics of the lavaged cells [6]. In these cases a severe chronic mucosal inflammation was observed bronchoscopically with a high frequency of positive bacteriologic cultures (80% of all lavages) in the recovered fluid, and a considerable influx of exudate macrophages were found without any sign of acute inflammation even in symptom-free periods.

Immunoglobulins (Igs) may protect against inhaled proteins or viral and microbial organisms in the lung. So, local levels of immunoglobulins in the lung lining fluid have a pathogenetic importance as their absence predisposes to infection or to persistence of bacteria on the alveolar surface [7]. There are scattered reports of patients with recurrent pulmonary infections having selective IgA deficiency detected in bronchial fluid [5].

Therefore, levels of immunoglobulins were measured in the lung lining fluid and serum of children with recurrent obstructive bronchitis and compared with data of normal healthy volunteers.

Materials and Methods

Twenty-two children from 2 to 6 years of age with recurrent obstructive bronchitis were selected on the basis of various criteria as described previously [16]. The children were subjected to bronchoscopy

for the diagnostic purpose to exclude bronchial malformations and other disorders which might be associated with obstructive symptoms. BAL was performed after bronchoscopic examination in each patient. The procedure has been described in detail earlier [16].

The recovered lavage fluid was centrifuged at 800 $\times$ *g* for 10 min at 4°C after having been seperated from the mucus. The fluid supernatant was decanted and stored at —4°C until assayed.

Total protein concentration of each specimen of bronchial washing was determined by the method of Lowry et al [10]. Concentrations of IgA, IgG, IgM and C_3 were determined in bronchial fluid and serum samples by Beckman Immunochemistry Analyser II ICS (USA). IgE measurements were made with paper stabilized radioimmunoassay kit (IgE PRIST; Pharmacia Diagnostics, Uppsala, Sweden). The assay was sensitive to nanogram amounts of IgE (2 ng/ml $\approx$ 1 U/ml) [1, 21]. Albumin contents of the recovered fluid and serum were quantified by bromcresolgreen photometry [19, 20] and used as a reference standard. The bronchial fluid samples were left unconcentrated in view of the very sensitive methods used. The mean immunoglobulin and C_3/albumin ratios in bronchial fluid and serum were compared to reported data of young healthy adults [14, 17, 18]. Because of ethical problems it was not possible to have a better control group of children.

Results

The average recovered volume was 46 $\pm$ 4.6% of the instilled fluid. BAL was performed in all patients without difficulty or complications. No fluid samples had blood contamination.

Quantitative values for total protein, albumin, C_3 and immunoglobulins found in serum and bronchial lavage fluid were not compared directly to other results (Table I) because procedures used by different authors were different; so the amount of recovered protein depended on the lavage volume.

The Ig content in BAL expressed as Ig/albumin ratio, each of them was significantly greater than that present in serum (Table II). Assuming that albumin in BAL is derived exclusively from serum by nonspecific transudation [13], this disproportionate increase in IgA and IgG/albumin ratios was strong evidence of these immunoglobulins being locally synthesised in the lung, and that transudation was partially responsible for the Ig content in BAL.

IgM in BAL was also present to a significantly greater extent than in serum. Because of its large molecular weight, IgM molecules do not move freely from serum to the alveolar surface under normal conditions [5, 17, 18, 23]. In our cases signs of severe chronic mucosal inflammation were found bronchoscopically, so we had to reckon with greater fluxes of immunoglobulins across epithelial and alveolar membranes than in normal individuals.

Levels of IgE in the lavage fluid were increased compared to their serum levels.

C_3 levels in bronchial specimens may be the result of an increased local production but the source of the complement components within the lung is not known [3]. Complement activity was certainly lost with the

TABLE I

Concentrations of total protein, albumin, IgA, IgG, IgM and C_3 are expressed as g/l in serum and mg/l in lavage fluid. All concentrations of IgE are expressed as U/ml $\approx$ 2 ng/ml
Data represent mean $\pm$ SE.
Concentrations of protein species in serum and lavage fluid

	Total protein	Albumin	IgA	IgG	IgM	IgE	C_3
Children with recurrent obstructive bronchitis n = 22							
Serum	70.3±2.0	45.0±0.6	1.92±0.1	13.75±0.2	1.1±0.02	110±4.4	1.32±1.2
Lavage	93.8±4.5	26.6±2.6	22.8±2.0	41.5±4.8	9.9±1.2	0.92±0.1	11.0±0.8

TABLE II

Relative concentrations of protein species in serum and lavage fluid
Values represent mean concentrations of protein species divided by albumin concentration. Values for Ig species and C_3 are mg/mg albumin, and values for IgE are expressed as ng/μg albumin

		IgA/alb.	IgG/alb.	IgM/alb.	IgE/alb.	C_3/alb.
Children with recurrent obstructive bronchitis n = 22	Serum	0.042	0.30	0.024	0.0048	0.029
	Lavage	0.85	1.56	0.37	0.091	0.41
Merill et al. (14) Young healthy subjects n = 17	Serum	0.04			0.0045	
	Lavage	0.319			0.017	
Rankin et al. (17) Healthy volunteers n = 11	Serum	0.042	0.189	0.018		
	Lavage	0.28	0.195	0.006		
Reynolds and Newball (18) Healthy volunteers n = 5	Serum	0.05	0.23			
	Lavage	0.72	0.12			

lavage but the sensitivity of our assay was high, thus the concentrating procedure with its harmful, denaturing effect could be omitted. The C_3/albumin ratios in serum were not significantly different from results of other authors [18].

DISCUSSION

Of the major immunoglobulins, IgA, IgT and IgE are all present on the bronchoalveolar surface in normal individuals but IgM is not detected or is present in very low amounts using

a sensitive assay technique [4, 7, 9]. There are at least two potential sources for immunoglobulins found in the lung lining fluid: transudation from serum and in situ production by lung lymphocytes in the submucosa and the airway lumina [2, 7, 8, 15, 17, 18, 23]. The alveolar structures are permeable to molecules of low molecular weight and are comparatively impermeable to very large molecules [18]. The leakage of immunoglobulins is probably more significant during an inflammatory process due to changes in alveolar, epithelial membrane permeability.

In various inflammatory lung diseases immunoglobulin production is markedly increased at sites of disease activity but not in blood [5, 6, 8]. Rankin et al reported on some correlation between the number of IgG secreting cells and the IgG/albumin ratio in BAL fluid of patients with sarcoidosis, and the ratio of IgG/albumin was significantly greater in BAL than in serum [17]. This suggests that the increase in local Ig production in sarcoidosis is primarily responsible for the elevated IgG levels in BAL fluid. Both sources are important and contribute to the maintenance of the immunoglobulin levels in the airspaces.

The present study was done on children with recurrent obstructive bronchitis with protracted cough and wheezing without signs of acute infection. Diseases which cause obstructive symptoms were previously excluded but signs of chronic mucosal inflammation were bronchoscopically observed throughout months in the symptom-free periods, too. A previous study showed the persistence of bacteria on the bronchoalveolar surface in 80% of cases, and a significant influx of exudate macrophages was detected without polymorphonuclear leukocyte accumulation [16]. This inflammation might be intensified by various products of exudate macrophages, and changes of the inflamed mucosa were considered to be the morphological basis of the frequent and permanent obstructive symptoms [22, 24].

The present results have shown that the levels of all major immunoglobulins are increased in BAL fluid of children; this was markedly supported by the ratios of Ig/albumin in bronchial lavages and serum. It is therefore reasonable to assume that the local production of immunoglobulins predominates. We cannot distinguish between monomeric and dimeric forms of IgA by the method used but most of the IgA in lavage is certainly in the dimeric form [12]. It may be assumed that at least some of the lung monomeric IgA is derived from serum [18].

The point of view that children with recurrent obstructive bronchitis have a local deficiency of secretory IgA and so an increased susceptibility to lung infections and atopic diseases [24] cannot be accepted.

Both the classical and alternative complement pathways are represented on the alveolar epithelial surface of normal individuals [3, 7, 8]. The complement components are probably

derived from serum and their local production is also possible [7, 18]. The levels of C_3 synthesised by mononuclear phagocytes were increased in our patients indicating the activity of macrophages and the complement system.

The relative lavage levels of IgE were increased compaired to their serum concentrations. The patients had no positive skin test to environmental antigens nor a history of atopy, and their serum IgE levels were normal. This chronic inflammation of the mucosa may play an important role in the early hypersensibilization altering the affinity of the mast cell membranes for IgE [11], but answers to this question await further study.

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Characterization of cells in bronchoalveolar lavage fluid of children with recurrent obstructive bronchitis

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In children 1 to 6 years of age with recurrent obstructive bronchitis bronchoalveolar lavage was performed to obtain material to allow characterization of the cell-types present on the bronchoalveolar surface. Recurrent infections may produce a chronic mucosal inflammation which was observed bronchoscopically in the symptom-free periods, too. Two main components of the lavaged cells were alveolar and NAML macrophages with morphological, cytochemical and functional features of mononuclear phagocytes. It seemed that the persistence of bacteria in the respiratory tract induces an increased influx of macrophages without PMN accumulation. This inflammation may constitute the morphological basis of frequent relapses which sometimes occur without any sign of respiratory infection.

Recurrent obstructive bronchitis in infants and children is a symptom-complex consisting of a chronic or recurrent "wet" cough, increased phleg production and wheezing that may be associated with evidence of inflammation or hyperinflation of airways on the chest roentgenogram [18]. Many diseases may cause symptoms of recurrent obstructive bronchitis in childhood, such as malformations of bronchi, of the heart and large vessels, cystic fibrosis, foreign body, tuberculous bronchoadenitis, gastro-oesophageal reflux, etc [18, 19, 21].

Some patients may develop from infancy a protracted cough and wheezing after every minor recurrent respiratory infection or sometimes without any sign of infection. The role of allergy, atopic status, and immunodeficiencies in children with this kind of bronchitis is not completely understood. In every case signs of chronic inflammation of the mucosa can be observed bronchoscopically in symptom-free periods, too. This protracted inflammatory condition may be the cause of the frequent relapses [19, 21].

In order to reveal the role of infections and normal and pathologic mechanisms of immunoreactivity in this selected group of patients, we performed bronchoalveolar lavage (BAL) and characterized the cell populations collected by the lavage using morphological, cytochemical and functional criteria and methods. The study on the features and functions of the lavaged inflammatory and immunologic effector cells may reveal important data concerning the pathomechanisms of this frequent disease.

Materials and Methods

Patients

We performed bronchoalveolar lavage on 22 children 2 to 6 years of age, suffering from recurrent obstructive bronchitis, undergoing bronchoscopy for diagnostic indications. Focal bacterial infections, allergic hypersensibilization and other disorders which could be associated with obstructive symptoms, such as malformations of bronchi, heart and large vessels, cystic fibrosis, foreign body, etc., were excluded, and signs of chronic inflammation of the mucosa were observed bronchoscopically. Hypersecretion of usually viscous adhering material was almost always present which formed actual strings or many cones above orifices of mucus glands, reducing the calibre of bronchi. The mucus membrane of granulomatous appearance was thickened and rough with dilated vessels. This was due to a combination of fragmented particles of mucus, mucosal oedema and the dilated openings of the submucosal glands. The pronounced longitudinal corrugations in the pars membranacea were also characteristic. These changes in the mucosa appeared even though there was no recent history of any attack or clinical symptom at least during a month before the examination.

ALB procedure

The lavage procedure was fully explained to parents and informed written consent was obtained. Considering that only rigid tube bronchoscopes can be used in infants and small children [19] because of the difficulty of ventilation and the poor cooperation of the patients, lavage was carried out under anaesthesia according to Friedel (Bronchoscope model MLW Medizinische Geräte, Berlin, GDR). The right lower lobe was lavaged in each patient. After passing the tube, isotonic, sterile sodium chloride solution at 37°C was injected into the bronchus through a Thal tube.

The volume of lavage fluid varied from 45 to 100 ml, in about 15 ml boluses. The residual volume of this zone was calculated by using physiological data (5) for each age group, as shown in *Table I*. The fluid was immediately aspirated with suction tube using 50 to 100 mm Hg negative pressure. The lavage took approximately 5 min from the first instillation until the last withdrawal. All patients tolerated the lavage well and without any side effect.

Mucus strands were removed by filtration through several layers of very loose cotton gauze. The samples were centrifuged at 800 × *g* for 10 min at 4°C and the supernatants were immediately frozen at —30°C for future analysis. The cells were resuspended in 1 ml of Hank's balanced salt solution and the cell count was determined in a standard haemocytometer. Viability by trypan blue exclusion, and differential cell counts on a Giemsa stained smear were done. The morphology of cells obtained by lavage was studied after glass adherence on coverslips. The preparations were washed with Medium TC 199 containing 20% heat-inactivated newborn calf serum, 2000 U/ml penicillin G, and 50 μg/ml streptomycin, dried in air, fixed in methanol for 10 min or in formalin vapour for 60 sec, and stained with Giemsa stain for 5—7 min.

Sterile and siliconized glassware and plastic instruments were used throughout all procedures.

Cytochemistry

Peroxidase activity was determined according to Kaplow (10) and esterase-1 activity was investigated according to Ornstein et al (13).

Cell surface receptors

The presence of Fc and C_3 receptors was detected on glass adherent cells with rabbit IgG-coated sheep red blood cells (SRBC) and by the use of SRBC coated with IgM fraction of rabbit anti-SRBC serum and complement from fresh mouse serum (7, 8).

Before lavage, some bronchial fluid was aspirated for bacteriological culturing.

TABLE I

Lung volumes in infants and children

Volume (ml)	AGE					
	Newborn	6 mo.	1 year	3 year	6 year	14 year
V_T	15	41.7	68.7	107.4	120.8	263
IRV	90	291.9	480.9	751.8	845.6	1841
ERV	35	83.4	137.4	214.8	241.6	526
RV	35	104.2	171.7	268.5	302	662.5
VC	140	417	687	1074	1208	2630
TLC	175	521	858	1342.5	1510	3290
V_D	5	13.9	22.9	35.8	40.3	87.6
*TLC	48.1	143.2	236.9	369.1	415	630
*RV	9.6	28.6	47.2	73.8	83	182

* Calculated volumes of the lavaged right lower lobe

$$\text{Right lung TLC} = \text{whole lung TLC} \cdot \frac{55}{100}$$

$$\text{Right lower lobe TLC} = \text{right lung TLC} \cdot \frac{50}{100}$$

$$\text{Right lower lobe TLC} = \text{whole lung TLC} \cdot \frac{27.5}{100}$$

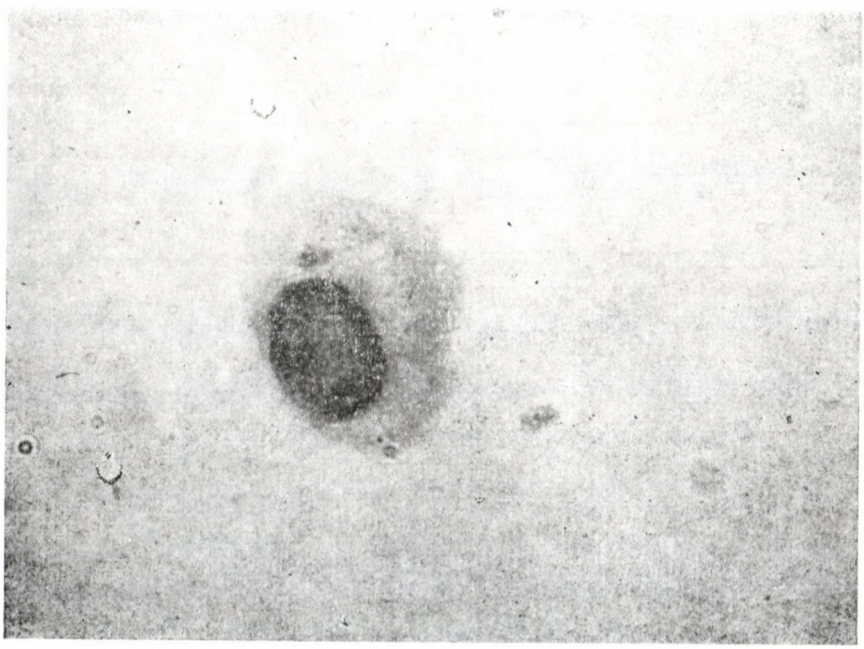

FIG. 1. Alveolar macrophage with black inclusions in cytoplasm (Giemsa staining, ×1000)

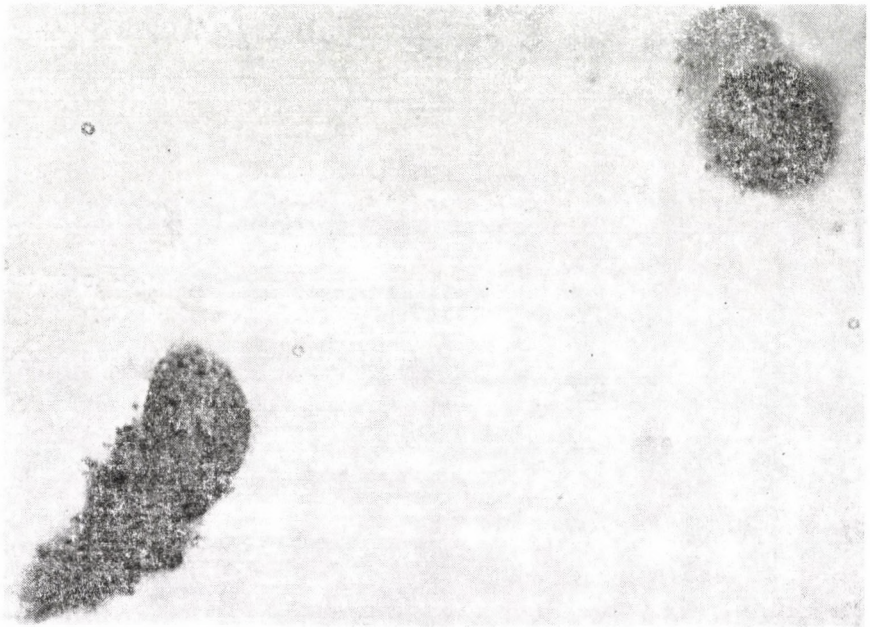

FIG. 2. NAML macrophage stretched out on glass surface (peroxidase + Giemsa staining, ×1000). The cytoplasm is peroxidase positive whereas the alveolar macrophage (right above) has no positive granules

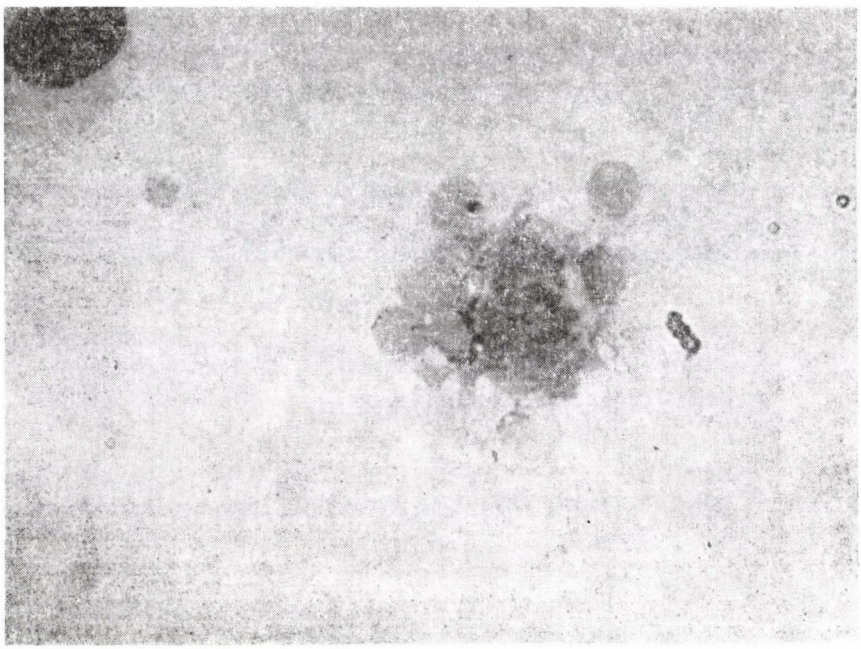

FIG. 3. Rosette formation of SRBCs coated with IgG around an alveolar macrophage (Giemsa staining, ×1000). Intensive phagocytosis

Results

Fluid and cell recovery

The average recovered volume was 46 ± 4.6% of the instillated fluid. No fluid had blood contamination. Recovered volume of at least 15% of the infused fluid was considered the lower limit below which the specimen was not analysed. The total cell yield was $15.4 \pm 4.3 \times 10^6$ per patient (Table II) with a viability of 96.4 ± ± 2.0% by trypan blue exclusion. The data could only be compared with findings in young, healthy non-smokers or animals, because lavage should not be performed in healthy children.

Significantly larger number of cells were obtained from children with obstructive bronchitis than in cases of normal, young volunteers in studies by other authors. The difference was much more striking considering the 0.63 ± 0.26 cell count per ml of recovered lavage fluid samples. The large majority of cells observed in the Giemsa-stained preparations from all subjects were macrophages, 93.8 ± 3.6% (Table III). The alveolar macrophages were fairly homogeneous in size and oval or round in shape, with a basophilic cytoplasm often containing black inclusions, and an excentric oval or bean-shaped nucleus, and a cytoplasm : nucleus ratio > 1. These cells did not form large pseudopods on glass surface (Fig. 1). There were other cells with some divergent feature, but these were certainly macrophages because they adhered to glass, were positive on nonspecific esterase-1 staining, and often contained intracytoplasmic particles such as fragments of various dead cells and bacteria. These cells were usually smaller than the alveolar macrophages; they had a lower cytoplasm : nucleus ratio, were sometimes stretched out or bore large pseudopods and they mostly contained a bean-shaped nucleus (Fig. 2). These macrophages are referred to as non-alveolar-macrophage-like macrophages (NAML) (3). Among the macrophages 21.7 ± 3.6% were of the NAML type in the preparations. Multinucleate macrophages comprised 2.4 ± 1.9% of the total counts and over 97% of these had only 2 or 3 nuclei.

Polymorphonuclear leukocytes (PMN) were rarely found in the lavage fluid samples, only in 1.3 ± ± 2.2%. The percentage of lymphocytes was 4.3 ± 2.0%, and other cell types such as mast cells or eosinophils were occasionally present. The high percentage of ciliated epithelial cells referred to chronic mucosal inflammation.

Histochemistry and receptors of macrophages

Almost all of the alveolar macrophages and the NAML macrophages were positive for esterase-1, with varying activity. Peroxidase staining showed macrophages containing positive granules in 23.7 ± 9.2% (Table IV). Fc receptors were present on both types of macrophage and ingestion of sheep red cells coated with IgG was

TABLE II

Received volume, cell counts and viability in bronchoalveolar lavage fluid

	Lavage fluid recovery per cent	Total cell yield, $\times 10^6$	Cell count/ml $\times 10^6$/ml	Cell viability per cent
Own data n = 22	46±4.6	15.4±4.3	0.63±0.26	96.4±2.0
Reynolds and Newball (15) n = 5	66.4±9.4	12.8±2.7		94.2±1.1
Davis et al (6) n = 14	77.5±2.2	13.2±3.3	0.069±1.8	
Pingleton et al. [14] n = 11	64±7.0	8.3±2.0	0.13±0.04	94.2±1.1

All data are mean ± SEM

TABLE III

Cell-type in bronchoalveolar lavage fluid in children with recurrent obstructive bronchitis. Own data are compared to those of young healthy volunteers

	Macrophages per cent	Lymphocytes per cent	PMN per cent	Mast cells per cent	Multinucleate macrophages per cent	NAML per cent
Own data n = 22	93.8±3.6	4.36±2.0	1.3±2.2	0.36±0.7	2.4±1.9	21.7±3.6
Davis et al. [6] n= 14	94.9±1.3	3.2±0.9	1.9±0.7			
Pingleton et al. [14] n = 11	92±6.0	7.3±5.6	0.7±1.1			
Michel et al. [11] n = 11	79.5±3.3	18.4±2.6	0.7±0.2	0.2±0.1		

All data are mean ± SEM

Abbreviations: PMN = polymorphonuclear cell
NAML = non-alveolar macrophage-like macrophage

TABLE IV

Esterase and peroxidase activity, and membrane surface immunoreceptors on the lavaged macrophages

	Esterase activity per cent	Peroxidase activity per cent	Fc Receptors per cent	C_3 Receptors per cent
Own data n = 22	98.8±1.6	23.7±9.2	90.5±5.7	20.1±3.7
Blussé van Oud Alblas and van Furth [2] (normal mice) n = 24	100	0.9	72.4	2.2

Own data are mean ± SEM

high, as shown in Fig. 3. Complement receptors were found on macrophages (especially on NAML macrophages) in 20.1 ± 3.7%; ingested IgM-complement-coated red blood cells were rarely seen.

The recovered fluid was bacteriologically positive in 80%, mainly streptococcus and staphylococcus strains were identified.

Discussion

The present study was undertaken to characterize the cells in the bronchoalveolar portion of the lower respiratory tract, and to determine what kind of inflammatory process persisted in the respiratory tract of children with recurrent obstructive bronchitis in symptom-free periods. The question arose whether the long-term mucosal inflammation was caused by recurrent viral and/or bacterial infections or the first appearance of bronchial asthma. Presumably, the bronchial status may constitute the morphological basis of the frequent relapses, occurring without any signs of respiratory infection [19].

Endoscopy alone reveals the endobronchial appearance and allows bronchoalveolar lavage to be employed [9, 11]. The lavage was perfectly tolerated by all the patients. The anatomic size of the lavaged lobe was estimated, and we used lavage fluid volumes considering the residual volume and total lung capacity. It seemed that about 15 ml aliquot was adequate to give a representative sample; smaller volumes did not suffice because these never reached the alveoli. Lavage was performed with a small total volume in order to avoid artifacts and to keep the risk of the procedure at a minimum. So there was no blood contamination in the samples, nor were there side effects such as bronchoconstriction, fever or atelectasis and, for the same reason, the percentage of recovered fluid volume was lower than in other studies [6, 9, 14, 15].

The total cell yield was significantly higher than in young healthy volunteers, considering particularly the cell count per ml of recovered fluid [9, 11]. Our data can only be compared to those of healthy young nonsmoker adults or data of animals, but these are not true controls; because of ethical considerations, better control data cannot be found in the literature.

In the differential counts, macrophages were predominant, and the percentage of lymphocytes and PMNs were similar to those of healthy individuals [6, 12, 15]. Morphological characterization of the macrophages provided an approach for two subdivisions. The NAMLs which are virtually absent in normal lungs in steady state [2, 3] increased to 21.7%.

The macrophages resembled exudate macrophages and originated from blood monocytes [4, 8]. They migrate through the interstitial lung tissue during inflammatory reactions induced by various stimuli ranging from inert particles to pathogenic microorganisms [17]. They had C_3

receptors and other characteristics of mononuclear phagocytes, and changed into alveolar macrophages entering the alveolar space [7, 8]. During this process morphological and functional changes develop, so alveolar macrophages have less C_3 receptors and a lower peroxidase activity [16, 20].

Esterase-1 activity and carrying of Fc receptors were similar in both types of macrophages, but peroxidase activity was different. Exudate and resident macrophages could be distinguished cytochemically, too [1]. Exudate macrophages showed reaction products in a varying number of cytoplasmic granules (lysosomal vesicles), and the Golgi system and lysosomes of resident cells were negative.

In our patients, chronic mucosal inflammation was observed bronchoscopically in the symptom-free periods, too. In spite of the fact that signs of acute inflammation were absent, bronchoalveolar fluid samples were bacteriologically positive in 80% of the patients, but the concentration of microorganisms was probably not high enough for the mobilization and influx of PMNs. This chronic inflammation may constitute the morphological basis of the frequent relapses occurring with or without signs of respiratory infection.

On the basis of the results it seems that the persistence of bacteria in the respiratory tract induced an increased influx of exudate macrophages without PMN accumulation. The severe inflammatory process in the lungs helps to direct and recruit inflammatory cells into extravascular spaces. Their synthetic products, such as lysosomal enzymes, oxygen radicals, complement factors and leukotriens, may play an important role in the persistence of the inflammatory reaction, bronchus constriction and pathological changes of mucus production.

Further investigations will be needed to reveal the causes of persistence of bacteria and thus the initiation of mucosal inflammation.

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5-Hydroxyindole acetic excretion in newborns, infants and children

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The 5-hydroxyindole acetic acid excretion of healthy prematures, term newborns, infants, and children up to 15 years of age was investigated. Comparison of the results with healthy adult values suggests that more exact information can be obtained on excretion values of children if they are calculated for bodyweight. The results obtained in the different age groups point to an increased activity of serotonin metabolism in newborns, infants and children. During the important phases of somatic and psychomotor development an increased urinary output of 5-hydroxyindole acetic acid was found as compared with adult values.

In man, serotonin (5-hydroxytryptamine, 5-HT) is produced from tryptophane by several enzymic steps. 5-hydroxyindole acetic acid (5-HIAA) is a final product of the catabolism of this important biogenic amine. Serotonin is first transformed to 5-hydroxytryptophan acetaldehyde by monoaminooxidase, and this in turn to 5-HIAA by aldehyde dehydrogenase [11, 23, 29]. This product of catabolism is not stored by the cells, consequently it appears in the plasma and is excreted by the kidneys [4, 12, 13, 18, 28]. It is noteworthy that 5-HIAA was found in urine well before the discovery of serotonin [10]. The daily output of healthy adults is 2—8 mg 5-HIAA [11, 23]. The excretion rate is increased in pregnancy, an even more marked elevation can be observed in toxaemia, and extremely high excretion may be encountered in carcinoidosis [3, 11, 14, 16, 20, 21, 22, 24]. There is little information about its excretion in infants and children and data referring to 5-HIAA excretion of term and preterm newborns are even more scarce [1, 2, 19, 27]. As no normal values for infants and children are available, this has prompted us to perform the present study.

Patients and Methods

Healthy term babies, symptom-free prematures, infants and children treated in the corresponding departments of the Hospital of County Bács-Kiskun participated in the study. All patients below 4 years were boys, both sexes were represented in the age group 4—14 years. Urine was collected on the first five consecutive days from newborns, and from infants of 1, 2, 6 and 12 months of age. Beyond infancy the data were grouped for each completed year of age. Infants or children affected by malabsorption, metabolic disease, epilepsy,

mental or motor retardation of any origin or having been treated by any drug suspect of interfering with serotonin metabolism before admission, were excluded. The newborns and infants received a diet normal for their age. No cheese or tomatoes were given to older infants or children, these foods having a high serotonin content. Results obtained in 10 healthy adults served as control values. All age groups comprised 15 patients each.

The 5-HIAA content of the 24 h urine specimens was determined according to the method of Lynch et al [17]. For statistical analysis Student's *t*-test was used.

Results

5-HIAA excretion of preterm babies was significantly less on each day within the first five days than that of term infants (Fig 1). Newborns and infants had a significantly lower excretion than adults ($p < 0.01$). Healthy newborns had a higher excretion on the 4th day of life than ever during infancy. If the peak of the fourth day is disregarded, there was a gradual slow increase from the first day up to 12 months of age. At one year the mean value of 5-HIAA output was 1.3 mg $\pm$ 0.24 mg daily. During the four years following the first one, there was a marked increase, about 1 mg per year (Fig 2). The total increment from 5 to 9 years was as small as 1 mg. There was a sharp increase in daily output around the 14th year of age, more than 2 mg from the 13th to the 14th year. The mean excretion of children of 14 years was 7 $\pm$ 4.2 mg per 24 hours 5-HIAA. This was higher than in adults, but the difference was not significant statistically. In children one year of age, the daily 5-HIAA excretion was significantly lower than that of the healthy adult controls ($p < 0.01$).

If excretion is related to weight and expressed in mg/kg/24 h, the highest values for children were encountered

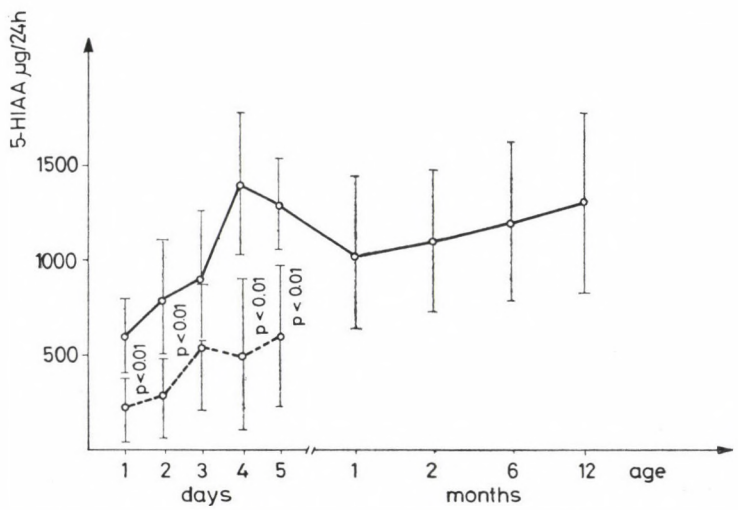

Fig. 1. Daily 5-HIAA output of newborns and infants: ○—○ of premature newborns: ○- - -○ on the first five days of life. Each group comprised 15 infants

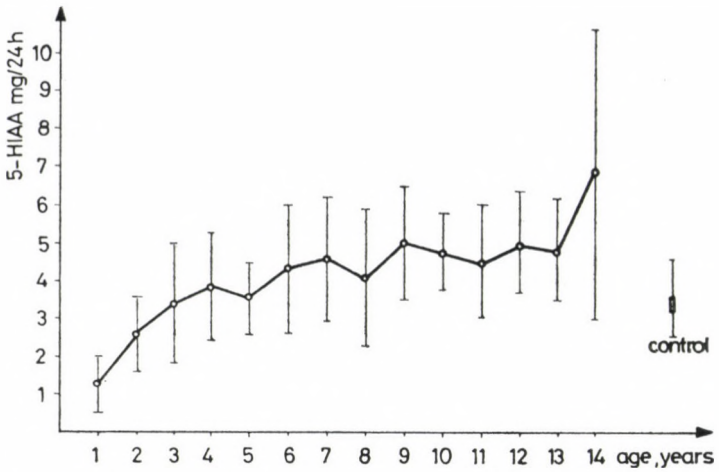

FIG. 2. Daily 5-HIAA outpout expressed in mg/24 h, of children compared with healthy adult control values. The number of adults was 10, each group of children comprised 15 individuals

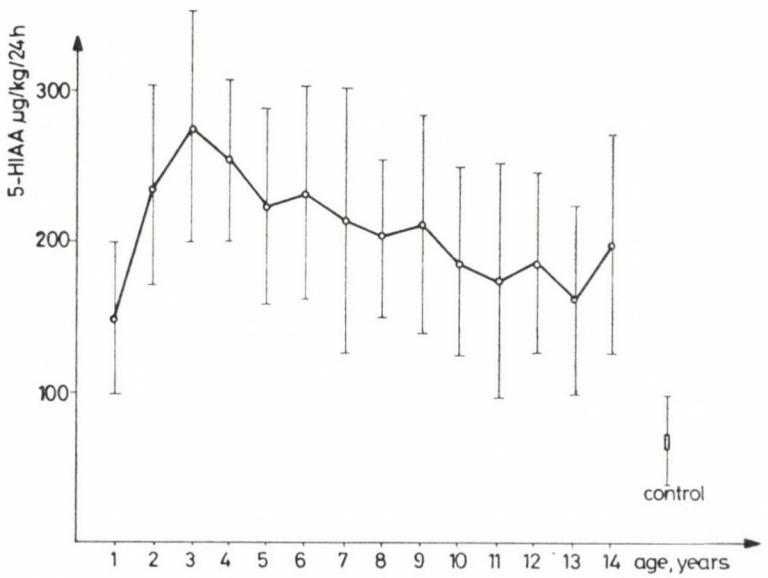

FIG. 3. Comparison of weight-related daily 5-HIAA excretion of children and adults, expressed in 5-HIAA µg/kg/24 h. The number of control individuals was 10, each age group comprised 15 children

in individuals of 2,3 and 4 years of age (Fig 3). Their mean values were significantly higher than the adult control values ($p < 0.01$). After 4 years, there was a gradual decrease in the weight-related 5-HIAA excretion. In general, children had higher values than adults, but by 13 years of age the difference disappeared. Children of 14 years of age showed a moderate increase again, but the difference was not significant statistically.

Discussion

Serotonin is an important biogenic amine and is one of the transmitter compounds playing an important role in the regulation of the central nervous system, especially in vertebrates [9]. 5-HIAA can be much easier measured than serotonin. Erspamer has shown that metabolism of 0.92 mg serotonin results in 1 mg 5-HIAA [5]. Therefore, excretion of 5-HIAA is a fairly good indicator of serotonin metabolism.

In a previous study [25] we showed that serotonin plays a great role in the adaptation of newborns to extrauterine conditions. Also, we compared the plasma serotonin level and 5-HIAA excretion of prematures affected by the idiopathic respiratory distress syndrome: there was a strong correlation between the two values and both changed with the severity of stress of adaptation [26].

The present results have confirmed our previous findings. Tu and Wong followed the 5-HIAA excretion of newborns up to the tenth days of life; they found a markedly increased excretion rate during the first 4 days, exceeding the mean values of healthy children of school-age [27]. Their results are only partly in agreement with our results. It is true that after the period of adaptation we found a gradual increase up to the end of the first year of life. The daily output of newborns calculated for 1 kg of bodyweight, however, did not exceed the corresponding mean values observed in school-children. The increased values of the first few days of life may be attributed to adaptation to extrauterine life. In our experience the daily output of 5-HIAA is parallel to the degree of adaptation stress and the increase is a good indicator of commensurate adaptation.

After completion of the first year, there is a considerable increase under normal conditions. In subsequent years the increase is less pronounced, only at about 14 years of age is there a rapid steep increase. The value of daily output of 5-HIAA is a crude indicator of serotonin metabolism, the weight-related value gives much more information. There is a strikingly high output between 2 and 4 years as compared to the adult values. In addition to somatic development, serotonin also plays an important part in learning and memory processes [9, 30]. It has been shown in many animal experiments that if serotonin metabolism is inhibited in the brain, the animal learns with difficulty and there will be a reduction in memory capacity [6, 7, 8, 15]. Our data showed

that in addition to adaptation to extrauterine conditions serotonin may also play an important part in the biological maturation process of the organism. Its presence may be determinant in somatic development, in learning the motion process, in learning and retaining data in the memory.

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Granulocyte viability test in children from an environment with heavy metal pollution

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In a group of 43 school-children living near a zinc plant, the test of granulocyte viability by the method of Cocchi et al, the lead level by flame atomic absorptiometry, and the level of erythrocyte zinc-protoporphyrin by the fluorimetric method were determined in peripheral blood.

The mean result of the granulocyte viability test was 6.49%. In 18 children, the proportion of abnormal granulocytes was raised abnormally above 6.0%. The lead level was in the range from 14.1 to 53 μg/dl, and the level of erythrocyte ZPP was from 0.3 to 2.1 μg/g Hb. No correlation was found between the results of the granulocyte viability test and the blood lead level. The result of a comparison with the ZPP value was of borderline significance ($p = 0.05$).

The criteria of lead poisoning of children with environmental exposure are not sufficiently clear. New laboratory diagnostic tests are being introduced and the greatest advance in the field was achieved by introducing determinations of the blood lead level and tests for its toxic effects on biochemical processes [3].

Lead poisoning may not manifest itself clinically and the symptomless period may precede for a long time the appearance of clinical symptoms. Heme biosynthesis is particularly sensitive to the effects of lead [6, 8]. One of the earliest signs of damage to erythrocyte functions is a rise in the level of zinc-protoporphyrin (ZPP) due to a direct action of lead on mitochondria [4, 6, 8].

The aim of this work was to evaluate the disturbances of intracellular metabolic processes in granulocytes, using the method of granulocyte viability test. Studies on the life span of the circulating leucocytes allow the detection of abnormalities in their function [2]. In addition, the cause of the increased incidence of respiratory infections in children living in a highly industrialized area was also studied [1, 4, 8].

Material and Methods

The study was carried out in 43 healthy children aged 8—10 years living in a town 0.5 to 2 km from a zinc plant. Samples of 6 ml venous blood were taken into heparinized test tubes, and peripheral blood cell counts were done.

The granulocyte viability test was carried out by the method of Cocchi et al [2]. A drop of 1% aqueous eosin was placed on a slide and left to dry, then a drop of blood

was placed on it and mixed. Then the slide was put into a humid chamber at room temperature for 2 minutes. The proportion of granulocytes containing the red dye was calculated by microscopic examination of the sample. Since damaged granulocytes are unable to remove the absorbed eosin in its ionised form, this remained in the cell and stained diffusely its cytoplasm. The acceptable proportion of abnormal granulocytes should be 0 to 6% according to Cocchi et al [2].

The lead level in whole blood was determined by flame atomic absorptiometry according to Whiteside [9]. The measurements were performed with a Sp-2900 Pye Unicam atomic absorption spectrophotometer. The results were expressed in μg/dl. Values above 30 μg/dl were regarded as raised blood lead level. Levels of blood lead from 30 to 69 μg/dl suggested an increased absorption of lead. Lead poisoning was diagnosed when the blood lead level exceeded 70 μg/dl [8].

Determination of zinc-protopophyrin (ZPP) in the erythrocytes was based on measurements of the fluorescence of zinc-protoporphyrin III complex, using a Manual Hematofluorometer Model ZPP Meter 210 (Aviv) apparatus. The relationship of ZPP to haemoglobin in the tested blood sample was expressed as μg/g Hb. The meannormal result according to Stankiewicz and Frydrych [6] was 1.1 ± 0.2 μg/g Hb. and the acceptable value in children was 4.4 μg/g Hb.

The results were subjected to statistical analysis using Student's t test and the chi square test with Yates' modification.

Results

The mean proportion of granulocytes with abnormal viability was $6.49 \pm 10.3\%$ in peripheral blood of the children (Table I). The children were then divided into two groups, group I with the proportion of abnormal granulocytes exceeding 6% (the normal value of viable granulocytes), and group II with that proportion below 6%. The first group comprised 18 children with raised proportion of abnormal granulocytes, mean value $13.3 \pm 12.98\%$ (Table II). In the second group of 25 children, granulocyte viability was normal, the mean value was $1.64 \pm 1.76\%$ (Table II).

The serum lead level was from 14.1 μg/dl to 53.0 μg/dl, with a mean of 29 ± 49 μ/dl (Table I). In the group with abnormal result of the test the blood lead level was $25.81 \pm \pm 6.91$ μg/dl, while in the group with a normal result of the test the mean was 31.3 ± 10.51 μg/dl. The difference was not significant statistically.

The mean zinc-protoporphyrin level measured in the erythrocytes was in these children 1.23 ± 0.55 μg/g Hb, with a range of 0.3 to 2.1μg/g Hb (Table I). The results in the first group compared with the second group showed a difference of borderline statistical significance, being 1.42 ± 0.53 μg/g Hb, and 1.1 μg/g Hb in the first and second groups, respectively ($p=0.05$) (Table II).

The blood cell count was normal in all the 43 children, the mean haemoglobin concentration was 13.53 ± 1.36 g/dl, the mean white blood cell count was 4.200 ± 1.100 cu, mm.

Physical examination failed to demonstrate in the studied children any abnormalities in the osteoarticular, respiratory, circulatory and nervous systems.

TABLE I

Mean results of granulocyte viability test (g. v.), blood lead level, and erythrocyte zinc-protoporphyrin (ZPP) in children with environmental lead exposure

n = 43	g.v.%	Pb μg/dl	ZPP μg/g Hb
$\bar{x}$	6.49	29	1.23
SD ±	10.3	9.49	0.55
range	0—62	14.1—53.0	0.3—2.1

TABLE II

Granulocyte viability test (g. v.) blood lead level and erythrocyte zinc-protoporphyrin (ZPP)

Group	No of patients		Pb μg/dl	ZPP μg/g Hb	g. v. per cent
			1/	2/	
normal g. v.	25	$\bar{x}$	31.3	1.1	1.64
		SD ±	10.51	0.53	1.75
		range	14.1—53	0.1—2.0	0—5
abnormal g. v.	18	$\bar{x}$	25.81	1.42	13.3
		SD ±	6.91	0.53	12.98
		range	17.5—37.2	0.4—2.1	6—62

1.t = 1.94 0.1 p 0.05
2.t = 2.0 p = 0.05

DISCUSSION

The results showed that in children living under environmental exposure to lead the number of granulocytes with reduced viability is increased in the peripheral blood. The mean proportion of granulocytes with reduced viability (6.49 ± 10.3%) was significantly higher than the mean value of 1.7 ± 1.5% obtained by Cocchi et al in a group of healthy children aged 1—6 years [2]. Impaired function of the granulocytes was observed in the neonatal period during septicaemia or malnutrition, and the toxic effect of lead on biochemical processes is also known; of particular importance for the assessment of these effects is the determination of zinc-protoporphyrin. After excessive absorption of lead the blood level rises to 30—69 μg/dl and biochemical disturbances develop. These changes may appear without any clinical symptoms or signs [8].

In our material the mean blood lead level was 29 ± 9.49 μg/dl (from 14.1 to 53.0 μg/dl). In 17 children the level ranged from 31.0 to 53.0 μg/dl. The results obtained showed a wide

range of blood lead values which may be still within the physiological variability or may be caused by severe exposure to lead from time to time [7, 8].

In children lead is absorbed mainly through the digestive tract with soil, wall paints and from the surface of toys. The blood lead level corresponds to the degree of exposure. One of the important indicators of lead poisoning are changes of erythrocyte zinc-protoporphyrin. Its level in the children with environmental exposure was in the range from 0.3 to 2.1 μg/g Hb, with a mean of 1.23 $\pm$ 0.55 μg/g Hb, and in 17 children with raised serum lead level it ranged from 0.3 to 1.8 μg/g Hb.

Many authors stressed the lack of a correlation between ZPP and the blood lead level [4, 6, 8]. A raised ZPP level indicated a toxic effect of lead on heme synthesis, confirming the presence of disturbances of intracellular metabolism. It may be assumed then that demonstration of a toxic effect of lead on blood elements in children is sufficient for considering them to be at risk of lead poisoning. A moderate rise of the blood lead level (30—69 μg/dl) does not seem sufficient for this purpose [8]. The absence of a significant correlation between the serum lead level and the increase in the proportion of abnormal granulocytes and a simultaneously demonstrated correlation between these values and the results of ZPP determinations might confirm this concept.

The presented results suggest that during excessive absorption of lead by the organism of a child, despite the absence of clinical signs of lead poisoning, certain metabolic disturbances develop. The value of ZPP in the erythrocytes rises, the survival of granulocytes is shortened.

Summing up, in children living in an area with atmospheric lead pollution the test of granulocyte viability is often abnormal. Besides, a correlation exists between the erythrocyte zinc-protoporphyrin concentration and the result of the granulocyte viability test in these children.

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Severe hypertension in a ten-year-old boy secondary to an aldosterone-producing tumour identified by adrenal sonography

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Severe hypertension discovered incidentally in a 10 year-old boy was associated with persistent hypokalaemia and metabolic alkalosis. Primary hyperaldosteronism was diagnosed by demonstrating elevated plasma aldosterone levels and increased urinary aldosterone excretion with concomitant depressed plasma renin activity. Adrenal sonography identified a left adrenal adenoma which was removed surgically; normotension and normalization of plasma renin and aldosterone values ensued. This appeared to be the first use in children of sonography to identify adrenal adenoma and it is suggested to be the first step in the differential diagnosis of primary hyperaldosteronism

It is now well recognized that hypertension in childhood is not as rare as previously thought. The majority of children with mild or borderline hypertension have essential or primary hypertension, whereas children with severe symptomatic hypertension are likely to have underlying disorders including endocrine diseases with overproduction of hypertensive hormones.

We report on a child with severe hypertension discovered incidentally, who had a large aldosteronoma which was located by ultrasound and surgically removed.

Methods

Blood pressure was measured with the widest cuff that would fit between the axilla and antecubital fossa. For blood pressure measurements over the legs a large adult-size cuff (width, 16 cm) was used.

Plasma renin activities (PRA) were measured by Phadebas RIA-kit and plasma aldosterone levels by Aldok RIA-kit.

Report of Case

In a ten-year-old boy hypertension was recognized incidentally during physical examination before appendectomy. Blood pressures at admission were 170/130 mm Hg, 170/120 mm Hg, 150 mm Hg and 160 mm Hg measured over the right arm, left arm, rigth leg and left leg, respectively. No family history of hypertension or hypertension-relateddisease could be detected. Nothing abnormal could be found during routine physical and ophthalmoscopic examination. The chest X-ray was also normal, but the ECG showed signs of hypertrophy of the left ventricle.

Urinanalysis was normal, bacterial cultures of the urine were sterile.

TABLE I

Plasma renin activities and plasma aldosterone levels measured in supine and upright position before (1—3) and after (4) removal of the aldosterone-producing adrenal adenoma

No.	Sodium intake (mmol/day)	Potassium intake (mmol/day)	Plasma renin activity (ng/ml/h) in supine position	Plasma renin activity (ng/ml/h) in upright position	Plasma aldosterone level (pg/ml) in supine position	Plasma aldosterone level (pg/ml) in upright position
1.	130	50	0.02	0.022	218	291
2.	98*	80*	0.03	0.14	340	480
3.	153	80	0.02	0.045	375	360
4.	130	50	0.2	0.9	30	20

* Average of the preceding five days

Endogenous creatinine clearance was within physiological limits. Intravenous pyelography revealed normal kidneys and urinary tract.

Routine laboratory investigations showed persistent hypokalaemia (serum potassium 3.5—3.6 mmol/l) and metabolic alkalosis (pH 7.42—7.50; base excess 6.7—7.5 mmol/l) suggesting primary hyperaldosteronism as a cause of hypertension.

Plasma aldosterone level and plasma renin activity were measured at three levels of sodium intake in both upright and supine position (Table 1). On a standard hospital diet (sodium intake: 130 mmol/day), plasma aldosterone level was high (normal 50—175 pg/ml) and plasma renin activity was depressed (normal 0.3—2.0 ng/ml/h). After 5 days on low sodium intake (98.7 mmol/day) plasma aldosterone level increased further and plasma renin activity remained low. The non-suppressibility of the elevated plasma aldosterone was documented by measurement made on a high sodium intake (153 mmol/day) (Table I). The lack of physiological response to postural changes on normal and high sodium diet also suggested an "independent" aldosterone hypersecretion.

The ratio of daily aldosterone excretion to surface area is plotted against sodium excretion in Figure 1. Despite the high urinary sodium excretion (high sodium intake), aldosterone excretion remained high, suggesting inappropriate aldosterone hypersecretion.

On the basis of this information, the diagnosis of primary hyperaldosteronism (Conn-syndrome) was made; it was further corroborated by demonstrating the normalization of blood pressure after 3 weeks of spironolactone therapy (200 mg/day).

Normal plasma cortisol level, normal urinary cortisol excretion and normal VMA excretion indicated intact glucocorticoid and adrenomedullary functions.

Adrenal sonography (Picker International LS 3000) showed a round mass of approximately 4 cm in diameter over the left kidney. The mass could clearly be distinguished from both the kidney and the spleen (Fig. 2). No similar mass could be

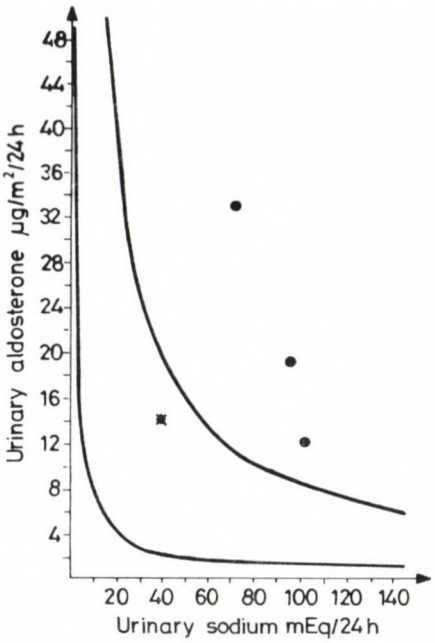

FIG. 1. Daily aldosterone excretion per surface area plotted against sodium excretion (5th and 90th percentile curves of normal subjects have been taken from New et al. [17]). The values of three subsequent preoperative determinations are shown by circles while the postoperative value by asterisk

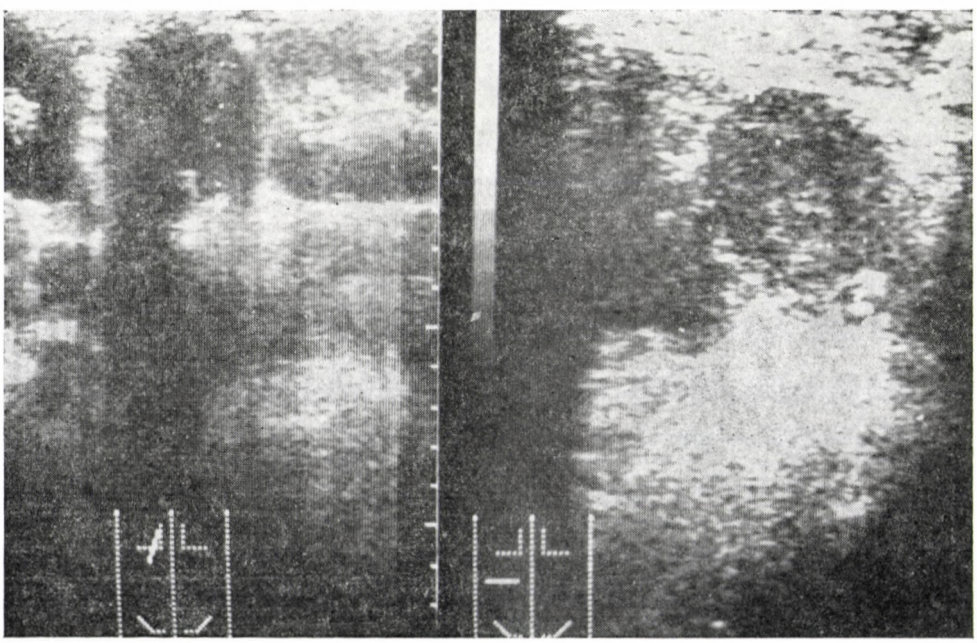

FIG. 2. Ultrasound scan of the region of the left suprarenal gland. A round mass of about 4 cm in diameter can clearly be distinguished from the kidney and the spleen

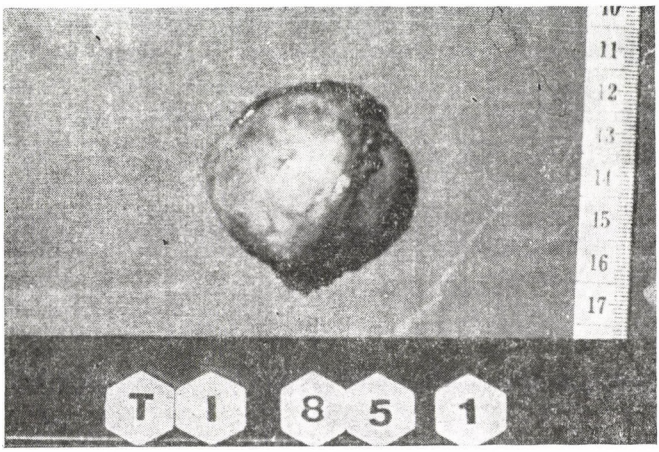

FIG. 3. Adenoma of removed left suprarenal gland. Weight: 27 g, diameter 4 cm

detected at the region of the right suprarenal gland.

At surgery a solid tumour weighing 27 g was removed from the left suprarenal gland (Fig. 3). Histological analysis of the tumour showed the characteristics of an adenoma. No sign of malignancy could be seen by histological examination.

The postoperative course was uneventful, neither the hypertension nor the hypokalaemia and metabolic alkalosis could be observed any more. PRA and aldosterone plasma levels (Table 1), as well as aldosterone excretion (Fig. 1) and also the physiological response of PRA to postural changes (Table 1) all returned to normal values.

DISCUSSION

Aldosterone overproduction is a rare but curable form of hypertension in paediatric age. It is suspected in a hypertensive patient who has persistent hypokalaemia and metabolic alkalosis. Hypokalaemia may remain asymptomatic as in our case, but it may also produce a variety of signs and symptoms which include fatigue, muscle weakness, paraesthesias, "periodic paralysis", polyuria, polydipsia, short stature, nocturia, etc. [8].

The diagnosis of primary hyperaldosteronism was readily established by the presence of elevated, nonsuppressible plasma and urine aldosterone and depressed plasma renin activity (low-renin hypertension).

The predominant adrenal pathology in childhood is bilateral adrenal hyperplasia [5, 6], aldosterone-producing tumour being exceedingly rare in children. To the best of our knowledge seven cases have been described up to now [4, 9]. As a subgroup of bilateral adrenal hyperplasia, a rare familiar form of hyperaldosteronism has also been described. The unique feature of this "dexamethasone suppressible hyperaldosteronism" is the com-

plete suppression of aldosterone secretion with dexamethasone administration.

There are several approaches to distinguish between adrenal hyperplasia and adenoma: photoscanning of the adrenal glands after ^{131}I-19-Iodocholesterol administration [1, 2, 16], determination of serum 18-hydroxycorticosterone level [10], computed tomography [11] and venography with determination of aldosterone level in samples obtained directly from the adrenal vein [7, 9]. Adrenal sonography has also been used to identify the adrenal lesion but has been considered difficult and often unsuccessful even in adults [11]. To the best of our knowledge there is no previous report of this method being used in children to identify adrenal adenoma. Furthermore, ultrasonic identification of the adrenal tumour made other complicated and not entirely harmless [12] diagnostic procedures superfluous.

On the other hand, it has to be emphasized that the tumour removed was unusually large even when compared with adrenal tumours in adults [1, 17]. It is probable that much smaller tumours could not always be detected by ultrasound technique. Nevertheless, since ultrasound evaluation is an entirely noninvasive method we suggest to use it as the first step in the differential diagnosis of primary hyperaldosteronism in childhood.

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Effect of a calcium-binding gluten fraction on the superprecipitation of actomyosin

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The Ca^{2+}-binding gluten fraction isolated by the authors [34] has been shown to prolong the clearing phase and to shorten the physiological contraction phase of ATP activated natural actomyosin suspension in the presence of physiological potassium chloride in a dose dependent manner. The mean ATPase activity of myosin and actomyosin was calculated for each phase from the actual ATP concentrations, measured at 30 sec intervals, and from the phase lengths. The preparation was found markedly to inhibit the ATPase activity during the clearing and physiological contraction phase. Since both myosin and actomyosin ATPase is Ca^{2+}-dependent, it is assumed that the inhibitory effect of the gluten fraction on ATPase activity may be mediated by its free Ca^{2+}-binding capacity, resulting in modified phase lengths of actomyosin superprecipitation.

On the basis of these experimental results a hypothesis is put forward of the part of the Ca^{2+}-binding gluten fraction played in the pathomechanism of coeliac disease.

Since Dicke's observation made in 1950 [11] the causal role of wheat and rye gluten in the aetiology of coeliac disease has been accepted, although the exact pathomechanism has not been clarified. There are strong arguments for a biochemical [5, 6, 7, 8, 21, 27, 28, 31, 32, 33, 34] and an immunological mechanism [4, 10, 13, 14, 15, 17, 18, 19, 20, 23, 30, 31] alike. In either concept the initial step in gluten toxicity is binding of this protein to the mucosa. This binding either acts directly on the enterocytes perhaps by gluten endocytosis into the lysosome [27, 28], or provokes an immunological reaction to gluten or its fragments [10, 23, 30]. Such a reaction might be ascribed to abnormal permeability of the intestinal mucosa [1, 2, 3] and to the unique amino acid composition of gluten, rendering this protein resistant to proteases [30]. It is known that certain peptide bindings, e. g. glycyl-glycine, prolyl-peptides (we have found 17—20% proline in gluten on a molecular basis) are resistant, while other bindings like lysyl-glutaminic acid, arginyl-glutaminic acid, etc., are partly resistant to pancreatic and intestinal proteases [24].

We have recently isolated some gluten fragments that possess the capacity of altering the concentration of certain compounds acting under physiological conditions [32, 33, 34]. These gluten components may play a

secondary or even a primary role in the pathomechanism of coeliac disease.

In a previous paper [34] we reported on a gluten fraction capable of binding calcium; we termed this compound as gluten ES. This preparation may influence the level of free Ca^{2+}; thereby, it may be anticipated that it can inhibit biological processes needing the regulatory effect of free Ca^{2+}. In fact, we have demonstrated that gluten ES inhibited the $Ca^{2+} + Mg^{2+}$-dependent ATP-ase activity and Ca^{2+} uptake of the fragmented sarcoplasmatic reticulum isolated from rabbit striated muscle. More recently, we have studied the quantitative effect of gluten ES on another Ca^{2+}-mediated system regulating the length of individual phases and the $Ca^{2+} + Mg^{2+}$-dependent ATP-ase activity during these phases of actomyosin superprecipitation. In the experiments we used the model of phasic superprecitation of natural actomyosin induced by ATP in the presence of 140 mm l/l potassium chloride [9, 16, 36].

Material and Methods

Actomyosin was isolated from rabbit striated muscle according to Ebashi's method [12]. Gluten ES was prepared as described in a previous paper [34].

Follow-up of the superprecipitation was carried out as follows.

Composition of the reaction mixture of 3 ml: 140 mmol/l KCl, 20 mmol/l TRIS-HCl, pH = 7.0, 0.77 mmol/l $MgCl_2$, 0.077 mmol/l $CaCl_2$, 1 g/l actomyosin, 1 mmol/l ATP.

The reaction was initiated by addition of ATP. Changes in turbidity were continuously registered at 660 nm wave-length by a compensograph OH-814/1 (Radelkisz) connected with a spectrophotometer Spektromom 195 (MOM, Budapest); the reaction mixture was continuously mixed by a magnetic mixer in a cuvette of 1 cm depth, its temperature was kept at 25°C.

The time course of ATP concentrations during superprecipitation was studied as follows.

20 μl samples of the reaction mixture were removed at 30 sec intervals and added to 180 μl 100 g/l trichloroacetic acid in order to stop the reaction. The samples were neutralized by 0.2 mol/l TRIS, then buffered in 0.1 mol/l TRIS-acetate-EDTA buffer (pH = 7.75), luciferin luciferase (LKB) was added and the bioluminescent light intensity (I) dependent of the concentration of ATP was measured in a Packard TRI-CARB 3320 liquid scintillation spectrometer. The system was calibrated for the concentration range 10^{-5}—10^{-8} mol/l ATP [26]. The calibration line was fitted to a function of $lg(ATP) = a_0 + a_1(lg\ I) + a_2(lg\ I)$ type. Protein was measured by the method of Lowry et al [22], bovine serum albumin (SERVA) was used as the standard.

Results

Gluten ES prolonged the clearing phase of actomyosin superprecipitation in a dose-dependent manner (Figure 1). For better understanding tangential lines were drawn to each section of the registrate and their section points were projected to the abscissa representing the time axis. Thereby we obtained the time span of each period. The approximately horizontal phases showing no appreciable change in the turbidity of the

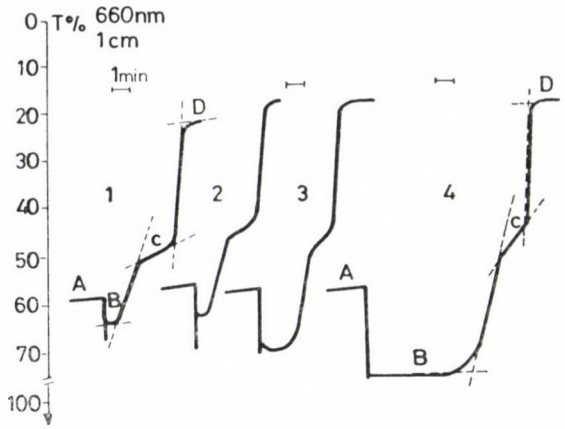

FIG. 1. Effect of bovine serum albumin (BSA) and various quantities of gluten ES on the superprecipitation of actomyosin. Changes in light transmittance of actomyosin in controls (1), after addition of 100 μg BSA (2), 30 μg gluten ES (3) resp. 80 μg gluten ES (4). A: Associated state of actomyosin; B: clearing phase; C: phase of physiological contraction; D: supercontraction phase

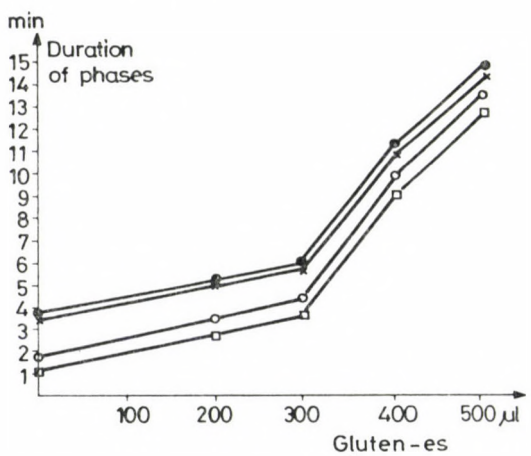

FIG. 2. Effect of various quantities of gluten ES on the length of phases of actomyosin superprecipitation. □——□: clearing phase; ○—○: onset of physiological contraction; x — x: end of physiological contraction phase; . —.: onset of supercontraction

actomyosin suspension were labelled A, B, C and D; A: actomyosin in the state of association; B: clearing phase (actomyosin is dissociated); C: phase of physiological contraction; D: phase of supercontraction.

Further, we studied the effect of increasing concentrations of gluten ES on the phase lengths within superprecipitation. Figure 2 demonstrates that the higher gluten ES content in the reaction mixture prolongs the clearing phase (B). On the other hand, the period extending between the beginning and the end of the physiological contraction shortens

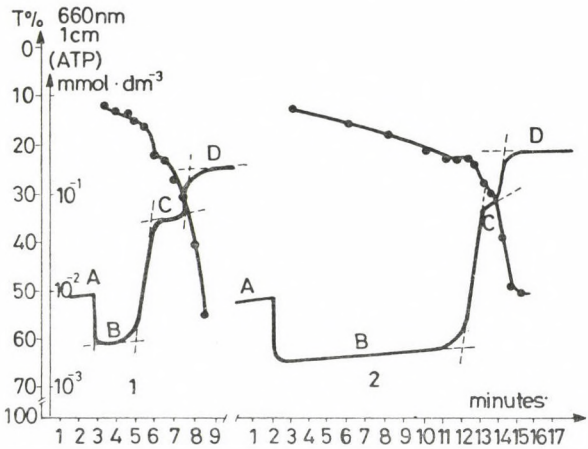

FIG. 3. Changes in ATP concentration and light transmittance in the course of actomyosin surperprecipitation. Controls (1); 80 μg gluten ES (2). —: change in transmittance; -•-•-•-•- change in ATP concentration

with increasing gluten ES concentrations (phase C).

To have a better insight into the effect of gluten ES on superprecipitation, we also studied the actual ATP concentrations during the individual phases. It is known from the literature [35] that actomyosin is in the state of association before starting the reaction by addition of ATP (Figure 3, A). In the moment of initiation of the reaction by ATP 1 mmol/l, this compound splits actomyosin to actin and myosin (clearing phase, in Figure 3: B). The changes in the actual ATP level observed from the beginning of the clearing phase are illustrated in Figure 3. In the control experiment this value is 0.576 mmol/l at the end of the clearing phase (Figure 3, 1), i.e.

TABLE I

Mean ATP-ase activity during each phase of superprecipitation versus gluten ES quantity

Water added to the reaction mixture, μl	Gluten ES	ATP-ase activity, nmol ATP · protein mg^{-1} · min^{-1}			
		Phase B	Transition from phase B to phase C	Phase C	Transition from phase C to phase D
		(phase length, min /activity (actual ATP concentration at the end of the phase, μmol ATP/l)			
500	—	(2.30)	(0.70)	(1.50)	(0.35)
		184	411	133	114
		(576)	(288)	(88)	(48)
200	300	(2.80)	(0.70)	(1.25)	(0.47)
		174	360	115	162
		(512)	(260)	(116)	(40)
—	500	(9.9)	(0.90)	(0.75)	(0.45)
		76	100	80	124
		(242)	(152)	(92)	(36)

the decrease is 0.424 mmol/l. In the presence of 500 μl (80 μg) gluten ES, 0.758 mmol/l ATP is consumed during a clearing phase 4.3 times longer than in the control experiment. From the phase length and the serial ATP measurements we calculated the mean ATP-ase activity of myosin and actomyosin during the individual phase. In the presence of gluten ES ATP-ase activity had markedly changed during the clearing phase and in the transitional period between the end of the clearing phase and the beginning of the physiological contraction phase (Table I). Since actomyosin dissociated at the starting point of the clearing phase and myosin ATP-ase is Ca^{2+}-dependent and actomyosin ATP-ase is $Ca^{2+} + Mg^{2+}$-dependent, it seems likely that gluten ES inhibits ATP-ase activity by reducing the free Ca^{2+} level.

Discussion

It is generally accepted that contraction of glycerol treated muscle fibres, myofibrils and actomyosin need not only Mg^{2+} and ATP but also calcium [25, 37]. In other words the state of contraction and relaxation of the contractile system is regulated by the free calcium ion concentration of the sarcoplasm. In relaxed muscle this has a value of 10^{-7} to 10^{-8} mol/l. An increase of the free Ca^{2+} concentration to $10^{-5} - 10^{-6}$ mol/l triggers contraction [25, 29, 37]. The gluten fragment isolated by us may influence muscular function by virtue of its calcium-binding capacity, the function of intestinal smooth muscles included, resulting in hypotonia of the intestine observed in coeliac disease.

To scrutinize this hypothesis we studied the effect of gluten ES in detail on a system representing an elementary process of muscle contraction. It has been long known that the so-called natural actomyosin suspension prepared from striated muscle tissue clears up if a high concentration of ATP (1—5 mmol/l) is added; the protein precipitates again if the ATP level decreases to 0.1 mmol/l as a result of enzymic cleavage of ATP. This phenomenon was termed as superprecipitation [35]. In the presence of physiological potassium chloride (140 mmol/l) the superprecipitation of actomyosin shows several phases [9, 16, 36], the clearing phase is followed by the physiological contraction phase, this in turn by a second phase of precipitation, the supercontraction phase. This phasic process was used in our experiments. In addition to the protein of the thick filament, myosin, our actomyosin preparation also contained actin, tropomyosin and the troponin complex, proteins of the thin filament. Tropomyosin binds the troponin complex to the thin filament. The troponin complex consists of three components: troponin-T, a compound responsible for binding to tropomyosin; troponin-C, a compound showing calcium ion binding property; and troponin-I, which inhibits the interaction between action and myosin. The key

step of contraction in this system is hydrolysis of ATP, a process carried out by Ca^{2+}-activated myosin ATP-ase and/or $Ca^{2+} + Mg^{2+}$-activated actomyosin ATP-ase.

In our experiments gluten ES prolonged the clearing phase, thus delaying the onset of physiological contraction, and shortened the latter phase (Figures 1 and 2). In our opinion, this prolongation of the clearing phase induced by the presence of gluten ES, cannot be fully explained by mere calcium ion detraction causing inhibition of myosin and actomyosin ATP-ase activity. It can be seen in Table I that in the presence of gluten ES markedly larger quantities of ATP are broken down by the end of the clearing phase than in the control; it is true that this more intensive ATP degradation occurs during a markedly prolonged phase. It may be anticipated that the decreased free calcium ion concentration induced by gluten ES not only inhibits ATP-ase activity but also leads to a delay in saturation of troponin-C with Ca^{2+}, an important prerequisite of contraction. We think that gluten ES probably shortens the phase of physiological contraction because, as it can be seen in Table I, the actual ATP concentration at the beginning of the physiological contraction is lower (260 resp. 152 μmol/l) than in the control experiment (288 μmol/l).

We have thus shown that the fraction of gluten isolated by us is capable of influencing muscle function. Although the experiments were carried out on actomyosin preparations taken from striated muscle tissue, it appears plausible that the same mechanism may act in intestinal wall hypotonia, a phenomenon characteristic of coeliac disease. In addition to the decreased muscle tone, reduced peristalsis may also result, as a consequence of the action of purine and pyrimidine derivates bound to another fraction of gluten [32, 33] and inhibiting cholinergic transmission and intestinal peristalsis if released.

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Active immunization of children exposed to varicella infection in a hospital ward using live attenuated varicella vaccine given subcutaneously or intracutaneously

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Active immunization using Takahashi OKA live attenuated varicella vaccine was carried out fire 5times to prevent the spread of "imported" varicella in a hospital ward. Susceptibility was previously tested by serological examinations: 14 children were vaccinated subcutaneously, the other 19 received the vaccine intracutaneously. Vaccination within a few days following exposure provided complete immunity in the great majority of cases. Intracutaneous administration was nearly as protective as the subcutaneous one.

Although an effective varicella vaccine has existed for ten years now [16] the problem of active immunization is still unsolved. This may partly be explained by the fact that varicella has been considered as a mild illness by both doctors and patients. Investigations in the past few decades revealed, however, that chickenpox can be dangerous and even lethal in immunosuppressed patients and in children in a poor condition due to a malignant disease [7, 8].

The first effective method to prevent varicella mortality was passive immunization by zoster immunoglobulin (ZIG) introduced by Brunnel et al [7, 8] and Gerson et al [9] to protect against varicella infection of patients with malignant disease treated with cytostatics. ZIG has a moderate therapeutic efficacy in actual and more serious varicella.

Passive immunization was a great step forward. It is, however, all the same important, especially in the above mentioned endangered cases, to investigate the possibilities of an active immunization. Takahashi et al [16] passaged varicella virus 11times on human embrional lung tissue, then 12times on guinea pig embryo cells and 2—5times on WI-38 type cells and the vaccine developed in this way has proved safe against varicella in susceptible children. The live attenuated vaccine was injected subcutaneously. Leukaemic and tumourous children were vaccinated with good results [11, 12, 13] with a similar vaccine, as it was shown by a series of detailed immunological examinations. Antileukaemic treatment was suspended for one week before and after vaccination.

The first reports were followed by a great number of vaccinations in hospitalized children suffering from malignant and different other diseases and in healthy children living in

closed communities or with their families [1, 2, 3, 4, 5, 10, 14, 15, 18]. They all proved that the Takahashi vaccine was harmless and produced nearly 100% seroconversion and immunity in healthy children susceptible to varicella. At least 80% safety could be achieved in case of recent contact with the virus in a hospital ward even after a few days incubation. Moreover, vaccinated patients developed no or only a mild form of the disease which means 100% protection against lethal manifestations. Recent studies have indicated that there is hope for the vaccine to be used with good results in the prevention of herpes-zoster, too.

Several decades ago a pioneering work was done in the field of active immunization against varicella by Ferencz, who recognized the intracutaneous immunizing effect of the fluid content of varicella vesicles and proved it by a number of appropriate epidemiological and complement binding methods. He called his procedure "varicellization". After the first results with live attenuated virus had been published, we called attention to those important early investigations [6].

The present examinations were focussed on the possibilities of active immunization to prevent the spread of varicella infection in a hospital ward.

Moreover we felt compelled to compare the results of the modern methods to those of Ferencz's examinations.

Patients and Methods

The investigations were carried out during five hospital epidemics due to imported varicella between December 22th, 1983, and July 3rd, 1984. They involved 39 children ranging in age from 3 months to 12 years, suffering from different diseases. None of them had malignant disease or leukaemia.

When varicella occurred in a ward, blood was taken from all the patients with a negative history for the illness to prove susceptibility by serological tests. Patients with no antibody response were vaccinated, the latest 36 hours after the manifestation of the first imported varicella. These children were divided into two randomized groups: parallel to each subcutaneous vaccination where 0.5 ml of Takahashi vaccine was used, one or two patients of the randomized group received 0.1 ml of the vaccine intracutaneously. No previous serological tests were done in nine cases and the vaccine was given only on the basis of the history. Later serological examinations showed that six of these nine patients had been exposed to chickenpox on an earlier occasion. Clinical follow-up examinations were done on the 10th post-vaccination day and one month later; blood samples were taken in some cases on the 10th day, otherwise one month after vaccination for antibody determinations. (Discharged patients were asked to come back for control.)

The vaccine used was the one labelled LOT 7906 containing the OKA strain live attenuated varicella virus developed by Takahashi et al [16] sent to us with prescriptions of control examinations. The vaccine arrived frozen in dry ice and was stored in the deep-freezer until assay.

The antibody titre was determined by ELISA (Enzygnost Varicella/Zoster Solid Phase Enzyme Immunoassay, Hoechst OSMK 03, Behringwerke AG, Marburg) using a Flow Titertek Multiscan (Eflab OY, Helsinki). The investigations were authorized by the Scientific Council and

the Research Ethical Committee of the Hungarian Ministry of Health. No vaccination was done without the informed consent of at least one parent.

Results

During the different hospital epidemics in our department a total of 96 children were found with a negative history for varicella. Real susceptibility was confirmed by serological examinations only in 33 cases. The other patients, although they were not vaccinated, did not get the disease even if they had contacted varicella patients.

Vaccination results are shown in Table I.

Neither local nor general reactions were recorded after subcutaneous vaccination.

Intracutaneous vaccination was followed, in some of the cases, by local reaction 7—10 days after injection, which corresponds to Ferencz's observations. Two patients developed erythema 2—3 mm in diameter, two others had vesicles of the size of a pinhead. In these latter cases no varicella vesicles were observed at any other area neither at that time nor later. None of the patients seroconverted on the 10th day following vaccination. One of the three children showed seroconversion at another test one month later. When immunity was checked one month after vaccination, the antibody response in the two groups was satisfactory, although the results of the subcutaneously vaccinated group were somewhat better (Table II).

At the same time none of the patients without elevation of antibody titre following vaccination developed varicella during the one month period of follow-up.

As it can be seen in Table I, varicella occurred in two subcutaneously vaccinated and in three intracutaneously vaccinated patients, on the 10th, the 12th, the 16th and two on the 17th day after vaccination.

The first subcutaneously vaccinated child who developed varicella on the 10th day must have been infected earlier. The other intracutaneously

Table I

Results of Immunization with Live Attenuated Varicella Vaccine

	Number of cases	
	subcutaneous vaccination	intracutaneous vaccination
No of vaccinated patients	14	19
Local reaction	0	4
General reaction	0	0
Seroconversion on 10th day	0/1	0/2
Seroconversion one month later	12/13	10/13
Mild or moderate varicella	2	3
Abortive varicella	0	2

Table II

Seroconversion following active immunization against varicella

Antibody titre	subcutaneous application	intracutaneous application
0	1	3
10×4^1	1	3
10×4^2	7	4
10×4^3	2	—
10×4^4	—	2
10×4^8	2	1
No of examined cases	13	13

vaccinated child presented with varicella on the 12th day had certainly been previously exposed to the disease since his brother who had not been hospitalized also contracted it. Traditional varicella was observed in one case in each group. It was considered to be mild in the intracutaneously vaccinated patients with only a few but definitely varicella vesicles. One patient of the intracutaneous group manifested abortive varicella first with sudamina-like vesicles, another one with several small papular eruptions. The only relationship between these signs and the vaccination was, however, the fact that we were extremely attentive to any such manifestation. Otherwise the incidence rate was the following: one case of varicella occurred in both vaccinated groups with each epidemic, which was an important factor proving that the children were in fact exposed to infection.

The six patients with earlier exposure to varicella (three vaccinated subcutaneously and three intracutaneously) had no local or general reaction and they did not develop manifest varicella. Their immune titre did not rise after one month, in one case it fell from 1 : 160 to 1 : 40.

Discussion

Special emphasis has been put on the need of an effective vaccine by the recent discovery of the possible complications of varicella. The great number of examinations reviewed have proved that the OKA vaccine of Takahashi is up to all such requirements. It has been demonstrated to be safe in patients with leukaemia and other malignant diseases. Still, as immunosuppressive cytostatics decrease the degree of immunization, it has been agreed that such patients must be tested serologically for immunity, and in case of susceptibility they should be given a temporary ZIG treatment until remission when active immunization can be done.

A great advantage of the vaccine is that, although it should be administered the soonest possible, it is effective even after exposure. Thus it can be of use for diminishing compli-

cations arising from imported infections regularly occurring in hospital wards. Unfortunately the chain itself cannot be broken, as one case of manifest varicella was always observed when immunization was done after exposure. The same consequence could be drawn from our rather limited investigations.

Administering the vaccine intracutaneously was not a novel idea but rather the reintroduction of Ferencz's examination, using now a well-tested vaccine and up-to-date serological methods. Our findings have confirmed the original observation in that a satisfactory degree of protection could be achieved with the virus given intracutaneously with one occasional vesicular eruption occurring at the injection site. No generalized symptoms appeared, and long term immunity was obtained.

The limited number of cases involved in the present investigation did not allow definite conclusions about the value of intracutaneously applied vaccination. In any case, intracutaneous vaccination seemed to be somewhat less effective than subcutaneous one. This lesser degree of protection does not necessarily ensue from the intracutaneous administration itself but rather from the smaller dose applied. In our latest examinations [17] 100% safety and seroconversion two weeks after vaccination were achieved using Ferencz's original vaccine derived from chickenpox vesicles. Further studies will decide on the degree of protection that might be obtained with a larger dose of vaccine given intracutaneously. It must also be considered whether any advantage may be achieved from the virus dermotropism manifesting itself at intracutaneous administration.

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Oral L-carnitine supplementation in low-birth-weight newborns: a study on neonates requiring combined parenteral and enteral nutrition

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Effect of L-carnitine supplementation on plasma ketone body (KB) and triglyceride (TG) concentrations was studied in ten premature infants requiring combined enteral and parenteral nutrition. At the second week of life (9 to 14 days of age) the infants were randomly divided into two groups. Five of them (plasma carnitine value, 33.77 ± 2.48 μmol/l; mean ± SEM) received oral L-carnitine supplementation (60 μmol/kg daily) added to pasteurized pooled human milk for seven consecutive days; additional five (plasma carnitine value, 36.70 ± 5.19 μmol/l) served as controls. Composition of the daily diet was nearly constant in the study period. On the seventh day, prior to an Intralipid infusion, plasma carnitine and ketone body levels were significantly increased in the supplemented group as compared to controls or to previous values of the same group. In response to lipid infusion the fat load induced ketone body production was significantly higher in the supplemented group as compared to controls, whereas the triglycerides reached higher levels in the control group. It is suggested that L-carnitine supplementation in low-weight newborns promotes ketone body formation from endogenous stores as well as from exogenous fat supply, and thus may enhance triglyceride utilization.

L-carnitine, β hydroxy-γ-N-trimethylammonium butyrate plays an essential role in the oxidation of fatty acids by facilitating their transport across inner mitochondrial membrane via carnitine acyltransferases and translocases [8, 18]. Low-weight newborns nourished parenterally on L-carnitine-free intravenous regimens are at risk to develop carnitine deficiency [7, 19, 23] leading to impairment of mitochondrial fatty acid oxidation and ketogenesis [24]. Recently, extensive interest has been focussed on the different metabolic consequences of parenteral L-carnitine supplementation during total parenteral nutrition (TPN) [1, 16, 17, 21, 25].

The needed quantity of L-carnitine is not known, but human milk may be regarded as its natural source for the newborn. On the basis of different carnitine contents of selected formulas and breast milk it is not unreasonable to suggest that a subnormal carnitine intake may occur in neonates and particularly in premature infants maintained on combined enteral and parenteral nutrition or during transfer from TPN to oral feeding [2, 6, 22]. Variations of the carnitine content of human milk in different stages of lactation and the high carni-

tine levels in liquid formulas and special diets based on cow's milk or beef, raised the issue of supplementation of collected pooled human milk [6, 22].

The aim of the present work was to study whether L-carnitine supplemented human milk was capable of elevating the plasma and probably the tissue carnitine levels in partially fed premature infants. If so, potentially more lipids could be utilized via mitochondrial β oxidation and then a better tolerance of exogenous fats could be achieved.

Materials and Methods

Ten appropriate for gestational age infants with feeding difficulties were selected for the study. Mean gestational age at birth was 31.75 weeks (range 29 to 34 weeks). The infants were 1 to 2 weeks of age at the start of the study (mean 10 days, range 9 to 14 days), their mean body weight was 1573.75 g (range, 1180 to 1860 g) on the first day of the experiments. All infants were considered to have no major medical problem and were in stable clinical condition. The clinical diagnoses included prematurity, perinatal asphyxia and gastroesophageal reflux. Each infant received nearly the same volume of pooled, pasteurized human milk on each day of the study period (mean, 85.73 ml/kg; range 71.2 to 107.8 ml/kg bw). Parenteral solutions consisted of glucose (5 w/v%), fat (Intralipid, 10%), electrolytes, trace elements and vitamins, the calculated total caloric intake ranged approximately from 60 to 90 Kcal/kg bw. The daily dose of administered L-carnitine was 60 μmol (9.6 mg), divided equally in the feeds.

The blood samples for plasma metabolite determinations were taken on the first and 7th day of the study from a peripheral vein precisely 3 h after the last oral feed. On the seventh day, blood sampling followed by Intralipid infusion (0.25 g/kg) lasting 20 minutes and further blood samples were taken at 20, 40 and 60 minutes. The samples were centrifuged immediately and the plasma was stored at —20°C until analysis. Ketone bodies (KB), defined here as the sum of β hydroxybutyrate (BOB) and acetoacetate (AcAc), and triglycerides (TG) were determined by enzymatic methods [14, 26, 27]. Plasma total carnitine (TC), representing the sum of free and acylcarnitine, was determined by the radiochemical method of Cederblad and Linstedt with some modifications described previously [4, 11]. Statistical significance of results was calculated by Student's t test.

Results

Prior to L-carnitine supplementation the plasma values of TC, KB, BOB AcAc and TG were normal in both groups (Table I.). In response to carnitine supplementation the plasma levels of TC, total KB and BOB were significantly increased as compared to controls, but the increase of AcAc fraction and the decrease of TG was not significant statistically. Comparison of the first day values of TC, KB, BOB and AcAc with those obtained on the seventh day of supplementation revealed a significant elevation in the group which had received the supplement. A moderate, but statistically not significant increase in TC, KB and BOB concentrations were observed in the controls during the study period.

The sequential changes in the plasma KB and TG levels during the post-infusion period are shown in Fig. 1.

TABLE I

Plasma carnitine, ketone body and triglyceride levels in the two groups of newborns before and after oral L-carnitine supplementation

	efore supplementation			After supplementation		
	control	supplemented	significance	control	supplemented	significance
TC+	36.70±5.19	33.77±2.48[a]	NS	38.80±4.98	90.15±9.09[a]	$p<0.01$
KB+	89.28±3.50[e]	84.48±12.63[b]	NS	130.87±19.35[e]	356.98±79.31[b]	$p<0.05$
BOB+	46.38±3.60[f]	58.79±7.09[c]	NS	90.02±20.29[f]	297.15±83.56[c]	$p<0.05$
AcAc+	42.89±5.83	25.68±6.07[d]	NS	40.85±3.25	59.81±12.15[d]	NS
TG+	1.93±0.19	1.79±0.49	NS	1.69±0.16	1.01±0.10	NS

Values are means ± SEM; expressed as +: μmol/l; *s*: mmol/l
[a]: $p < 0.005$; [b]: $p < 0.02$; [c,d] $p < 0.05$; [e,f]: NS

There was a striking elevation in KB and TG levels, as it is generally expected in response to an intravenous fat load [16, 17, 25]. The increase of KB values was greater in the supplemented than in the control newborns, reaching a significant difference at 40 minutes after the termination of Intralipid infusion (584.18 ± 90.13 versus 292.93 ± 53.5 μmol/l, respectively; $p < 0.02$). The TG levels of the nonsupplemented infants exceeded those of the supplemented group, but the difference remained statistically not significant throughout the postinfusion period.

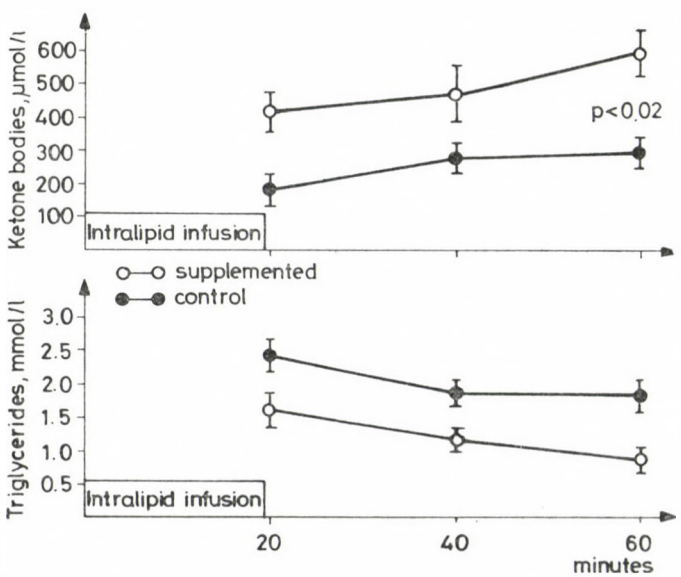

FIG. 1. Plasma ketone body and triglyceride levels during the postinfusion period (Intralipid 10%, 0.25 g/kg bw) in the two groups of neonates. (values are means±- SEM)

Discussion

As shown previously, the carnitine content of breast milk varies in different stages of the postpartum period [22]. In the present study it was found that the carnitine content of pooled milk, despite of its variability, was sufficient to maintain the plasma level in the control group during the observation period. In contrast, in the supplemented group a pronounced elevation in the plasma carnitine values was observed. The mechanism and the time course of carnitine absorption in neonates is not known. According to animal studies a still rising blood level was found 2 h after intraluminal injection of carnitine into the proximal intestine [9], thus the high plasma carnitine levels found in the supplemented newborns might represent an elevated postabsorption state rather than a steady state equilibrium condition between the carnitine pools of the body. Since there is no evidence of a significant degradation of carnitine in mammalian tissues [3], it seems reasonable to assume that the use of supplemented breast milk might be a natural route of exogenous supply, nevertheless an intestinal loss may occur via bacterial catabolism [20].

The widespread concept of exogenous carnitine dependency of newborn infants had prompted several groups to investigate different effects of supplementation in parenterally fed children [16, 17, 21, 25]. With the exception of a recent work of Orzali et al [16, 17] most studies using parenteral supplementation demonstrated an enhanced tolerance to exogenous fats in the newborn. Helm et al in a preliminary communication also reported an improved utilization of fat following oral carnitine intake [10]. In the present study the low triglyceride levels found after seven days of supplementation (Table I) as well as the value obtained 40 minutes after a fat load (Fig. 1) raised the possibility of an incrased elimination of triglycerides due to the improved carnitine availability.

The regulation of hepatic fatty acid oxidation and ketone body production at different levels is a complex process [12]. It is generally accepted that under certain conditions the fluctuation in the tissue carnitine concentration is an important element in the control of ketogenesis. It has been shown that addition of carnitine to medium-perfused liver [13] and to isolated hepatocytes [5] increases ketone body production. It has been shown in vitro that carnitine enhances lipolysis in neonatal adipose tissue [15]. In the present study the higher basal KB levels in the carnitine supplemented newborns might be a metabolic consequence of enhanced fat utilization from endogenous stores permitted by enhanced carnitine availability. The higher KB levels observed in the supplemented infants in response to an exogenous fat load also point toward the potential regulatory role of tissue carnitine con-

centration in the newborn's ketogenetic capacity. To support the above results, further experiments are in progress.

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Early postnatal growth in preterm infants

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Changes in weight of 50 preterm infants (gestational age 32.7 ± 0.3 weeks, birthweight 1772 ± 49 g) were studied during the period of the 0—4 postnatal weeks. Intrauterine weight gain of fetuses with equivalent gestational age, weight percentile position and sex was calculated and used as a control. Study infants achieved significantly less weight by age of 4 weeks (116.2 ± 1.2%) than it could have been expected theoretically (144.7 ± ± 1.0%). Growth performance did not correlate significantly with calorie intake, but was closely related with gestational age.

The outlook for survival of low birthweight and/or preterm infants with major pathology has been improving remarkably due to recent progress in intensive perinatal care. The successful treatment of severe acute disorders of neonatal adaptation, however, results in new challenges to the clinician. Among these the clarification of the criteria of optimum postnatal growth and nutrition of preterm infants [4] seems to have a special importance from the point of view of long term prognosis [2].

Our study was undertaken to investigate the growth performance of preterm newborn infants during the highly critical period of the first four postnatal weeks, on the one hand. On the other hand, an attempt was made to look for factors which determine or influence early postnatal growth in preterm infants.

Patients and Methods

During the period from 1/1/1983 to 31/12/1983 all preterm (gestational age ≤ 36 weeks) babies admitted to our Neonatal Intensive Care Unit within 24 h of birth were enrolled in the study, provided that full or partially oral feeding could be established within 7 days postnatally and hospital care was necessary for 4 weeks at least. Those with congenital malformations were excluded but not the ones with any other kind of pathology. After all, the data of 50 preterm babies (33 males and 17 females) with gestational age and birthweight (mean±SEM, range in parentheses) of 32.7 ± 0.3 (28—36) weeks and 1772 ± 49 (1050—2500) g, respectively, could be used for further analysis. Of the 50 study subjects 14 were considered to be small for dates both by physical characteristics and their weight for gestational age (≤ 10 percentile).

In 15/15(30%) cases the pregnancy was complicated with pathology; 6/50(12%) newborn infants suffered from severe prepartal or sub partu asphyxia and needed resuscitation. 35/50(70%) babies developed

hyaline membrane disease or asphyxia-related cardiorespiratory disorders; all of them needed oxygen or ventilation therapy. Antibiotics were administered to 21/50(42%) patients because of proved or suspected infections of various origin. In 4 of the newborn infants the postnatal course was completely uneventful.

For feeding, banked human milk was used primarily. 8 infants received only 5—10% glucose in water drip infusion throughout the first 3.1 ± 0.5 days of life. In 41 babies human milk feeding had to be completed with 5—10% glucose in water infusion for 4.6 ± 0.4 days, before full oral feeding could be established. From day 13.7 ± 1.2 onwards, 28 infants received a humanized formula milk (Robebi-A EGYT) supplementation. All babies were fed either by bottle or gavage. They were weighed daily to an accuracy of 10.0 g. Total calorie intake was calculated every day in each infant, considering the daily volume and quality of food and/or infusion fluid administered. The energy content of the banked human milk and the formula milk was determined by using reference data as to their nutrient composition.

For statistical analysis standard mathematical methods were used. Changes in weight were expressed both in g/kg and per cent of birthweight, considering the weight at birth as being 100%, in the latter case. In an attempt to qualify the postnatal growth it was assumed that it should closely correspond to the growth rate of fetuses of the same gestational age and sex. Therefore, for comparison the 4 weeks time growth rate of fetuses matched by gestational age, sex and weight percentile position, was calculated by using our local intrauterine growth charts.

Results

Table I shows the weekly changes in calorie intake, body weight and the incremental changes in weight of the 50 preterm babies studied. It can be seen that most of the infants regained their birthweight by the age of 2 weeks, from which time onwards a steady rise in weight could be observed (Fig. 1). In fact, however, by the end of the 4th postnatal week the study infants gained significantly less weight than their theoretical in utero controls (116.2 ± 1.2% vs 144.7 ± ± 1.0%, $p < 0.001$; birthweight considered to be 100%). On correlation analysis no significant relationship was found between mean total energy intake (120.8 ± 2.2 kcal/kg/day) and percentual rise in body weight throughout the 4 weeks study period, and no significant correlation existed between the weekly incremental changes in weight and weekly mean calorie intake either.

Table I

Weekly calorie intake, percentual changes in weight and incremental changes of body weight in 50, 0—4 weeks old preterm babies (mean ± SEM). Birthweight considered to be 100%

	Calorie intake kcal/kg/day	Body weight in per cent of birthweight	Weekly incremental changes in weight, g
1st week	83.5±3.1	96±0.6	— 66.2±13.3
2nd week	131.2±3.1	99±0.8	+ 69.8±10.0
3rd week	133.9±2.7	106±0.9	+ 125.0± 8.5
4th week	133.8±3.1	116±1.2	+ 172.2±10.8

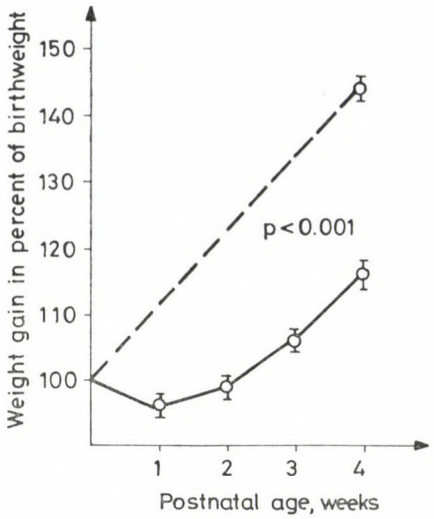

FIG. 1. Postnatal weight gain (mean ± SEM) in 50 preterm infants (——) expressed in percentage of birthweight (= 100%), compared with calculated weight gain of fetuses with equivalent gestational age, weight percentile and sex (— —), during a period of 4 weeks

In studying the relationship of gestational age and birthweight to postnatal growth performance, a significant positive correlation was found between the maturity of the infants and the percentage increase in weight, attained by 4 weeks age ($r = 0.43$, $p < 0.01$). Correspondingly, a strong negative correlation ($r = -0.66$, $p < 0.001$) existed between gestational age and the difference between the optimum (control) and the *de facto* growth performance produced by the end of the study period (Δ OPG). Birthweight was not related with early changes in weight.

In order to eliminate the effect of the initial weight loss and stagnation, the weight gain of each infant between his or her minimum and peak value has also been calculated and expressed as a function of birthweight kg. Figure 2 shows that minimum-maximum weight increase/kg birthweight correlated significantly ($r = 0.53$, $p < 0.001$) with gestational age, but again, not with calorie intake. Furthermore, no close relationship was found between calorie intake and gestational age either.

Early postnatal growth of newborn infants may well be influenced by the duration of weight stagnation and/or the extent of the initial weight loss as well. Therefore, we examined whether or not the gestational age correlated with the extent of maximum weight loss and the postnatal age of babies when they began to thrive steadily, after having regained their birthweight. No significant correlation could however be detected between these parameter-pairs.

Table II shows some growth characteristics of the 14 small for dates preterm infants when they were con-

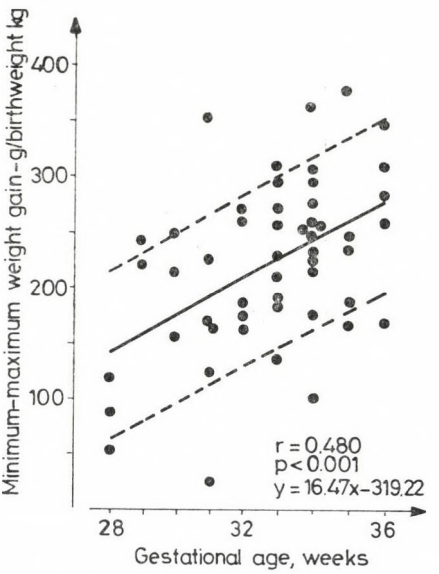

FIG. 2. Minimum-maximum weight gain (g/birthweight kg) by the age of 4 weeks against gestational age in 50 preterm newborn infants

sidered separatedly from normally grown babies. Despite of a closely similar mean calorie intake over the 4 weeks long study period, small for dates infants had significantly less initial weight loss (2.7 $\pm$ 0.6 vs 8.0 $\pm$ 0.7% of birthweight, $p < 0.001$) and began to thrive much sooner (11.6 $\pm$ 0.8 vs 16.5 $\pm$ 0.8 postnatal days, $p < 0.01$) than the normal for dates babies. This may well explain that by the age of 4 weeks, growth retarded infants had gained significantly more weight, expressing it as a percentage of birthweight (24.9 $\pm$ $\pm$ 2.4 vs 12.3 $\pm$ 1.0%, $p < 0.001$) or

TABLE II

Characteristics of early postnatal growth in 4 weeks old small for dates and normal for dates preterm infants (mean $\pm$ SEM)

	SFD (14)	NFD (36)	p
Birthweight (g)	1668$\pm$73	1813$\pm$62	ns
Gestational age (week)	34.3$\pm$0.4	32.1$\pm$0.3	**
Calorie intake (kcal/kg/day)	121$\pm$4.8	119$\pm$5.3	ns
Maximum weight loss (per cent)	2.7$\pm$0.6	8.0$\pm$0.7	***
Thriving begins (day)	11.6$\pm$0.8	16.5$\pm$0.8	**
Weight gain (per cent of birthweight)	24.9$\pm$2.4	12.3$\pm$1.0	***
Minimum-maximum weight gain (g/kg birthweight)	282$\pm$19	196$\pm$10	***
ΔOPG (per cent)	17.5$\pm$3.1	32.8$\pm$1.5	***

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

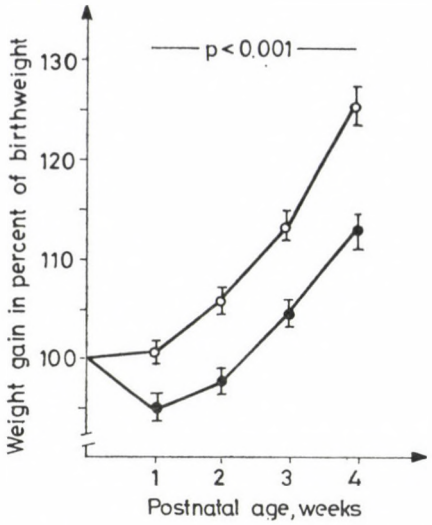

FIG. 3. Early postnatal changes in weight in 36 preterm normal for dates (●—●) and 14 small for dates (○—○) infants (mean ± SEM). Birthweight considered to be 100%

as a weight gain/birthweight kg ratio (282 ± 19 vs 196 ± 10 g, $p < 0.001$). When postnatal growth curves of normal and small for dates infants were compared to each other (Fig. 3) from the age of 2 weeks onwards they seemed to run remarkably parallel. Thus the conclusion was drawn that the growth delay in normally grown preterm infants is probably due to some factor(s) interfering temporalily with food digestion, absorption or utilization. Both maturity per se or gestational age-related perinatal pathology could have a role in this phenomenon, but the present observations provided ground only for speculations.

DISCUSSION

Being borne before term is obviously nonphysiologic, therefore the question arises whether it is possible at all to delineate the normal early postnatal growth of such babies. In spite of well grounded considerations against it, the principle has widely been adopted that the postnatal weight gain of preterm babies should be measured against growth curves of fetuses with equivalent gestational age, percentile position and sex [1, 9].

When comparing the postnatal percentage increase in weight of 50 preterm infants to the percentage rise in weight of 50 fetuses matched for parameters indicated above, a significant delay in growth was observed at the postnatal age of 4 weeks. In contrast with the expected gain in weight of 144.7 ± 1.0% their postnatal weight accumulation was only 116.2 ± 1.2%, on the average. Postnatal slow-down of growth in preterm infants may be caused by a reduced nutrient supply or transient and

partial insufficiency in the utilization mechanisms, or both. As for the first possibility, in accordance with others [3, 7] we could find no relationship between total calorie intake and weight gain of infants, considering the whole study period. From the beginning of the second postnatal week onwards, a calorie intake of around 130 kcal/kg/day was provided to the babies, but the weekly incremental changes in body weight seemed not to be related to the infants' energy supply, neither in the period of weight stagnation (1st— 2nd week), nor in the period when weight gain has already begun (3rd—4th week).

On further search for growth regulating factors we found a very strong positive relationship between gestational age and early postnatal growth. The more mature the infant at birth a higher increase in weight was achieved by the postnatal age for 4 weeks, independently of whether the weight gain was expressed in percentage of birthweight or calculated as a minimum-maximum weight increase per birthweight kg. At the same time, calorie intake, the extent of initial weight loss and the duration of postnatal weight stagnation were not related with the gestational age of the babies.

Gestational age-related early growth delay may be due to perinatal pathology more frequently occurring to more premature babies and interfering in a way with utilization and metabolism of nutrients. A more plausible explanation is, however, that the growth promoting effect of the food supplied in our present clinical practice decreases nearly parallel with the diminishing gestational age of the infants. Consequently, in support of previous reports [3, 5, 6, 7, 8] it may be concluded that calorie intake by itself does not have a primary role in determining early postnatal growth performance. Certainly, much depends on the nutrient composition of the food and also the gestational age-related functional maturity of the newborn infant.

Comparison of the growth parameters of normal and small for dates newborn infants revealed a significantly faster weight gain in retarded than in normally grown babies, in spite of a closely similar mean calorie intake over the whole study period. The explanation for the difference most likely is that small for dates infants being more mature functionally had more efficient food utilization and less severe weight loss, after which they began to thrive much sooner than the normal for dates babies. The notably parallel postnatal weight gain curves of normal and small for dates infants from the time onwards when they had begun to thrive, reflect, however, a similar growth rate capacity in the two groups of infants, if once the period of initial weight stagnation is over. But it is to be emphasized that interpretation of this finding needs caution since the links between fetal nutritional status and postnatal growth are fairly complex, and to investigate it was not the primary aim of the pres-

ent studies. For the reason that small for dates infants had more advanced gestational age than the normally grown babies, the question remains unanswered, whether the difference in growth performance was due to the different maturity or to the different body composition of infants in the two groups.

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Cytogenetic studies on peripheral blood cultures of neonates treated in an intensive unit

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The possible causes of an increased rate of structural chromosomal aberrations and sister chromatid exchange in peripheral blood cultures of mechanically ventilated newborns in an intensive care unit were investigated.

No cytogenetic abnormalities were found in low-birth-weight babies affected by hypoxia and acidosis during their first week of life. The rate of chromosome breakage and sister chromatid exchange was increased in blood cultures of neonates continuously ventilated with 70—80 vol% oxygen for a long period of time. The incidence and degree of chromosomal damage, although showing wide individual variations, was related to the duration of oxygen treatment.

In addition to high oxygen tension, other environmental factors of intensive care therapy like antibiotic and chemotherapeutic agents may be responsible for the mutagenic effect. The results indicate once again the importance of continuous pO_2-monitoring of ventilated newborns.

The incidence of structural chromosomal aberrations in peripheral blood cultures of term babies is 0.8—3.2% during the first fifteen days of life [4, 7, 18]. Some authors [3, 15] demonstrated a 4—10-fold increase of chromosome breakage in blood cultures of low-birth-weight neonates, while others [16] found no chromosomal abnormalities in newborns weighing less than 1000 g.

Hatcher and Hook [10] produced evidence that chromosomal breakage develops postnatally in both preterm and term newborns; this points to environmental mutagenic factors.

During the first days of life the homoeostasis of newborns is menaced by hypothermia, hypoxia, hypoglycaemia, infection and resulting acidosis. The question emerges whether the therapy directed against these conditions was responsible for damage to the chromosomes.

Patients and Methods

1. Fourteen preterm babies with a gestational age less than 36 weeks, admitted to the neonatal intensive care unit in hypothermia, hypoxia and severe acidosis were included into the study. The number of chromosome breaks per cell in peripheral blood cultures was determined during the first eight days of life (Table I).

2. The rate of chromosome breakage and sister chromatid exchange (SCE) was determined in blood cultures of 10 newborns on 1—4 weeks uninterrupted intermittent positive pressure breathing (IPPB) or positive expiratory end pressure (PEEP) after termination of ventilatory treatment.

TABLE I

Cytogenetic finding encountered in prematures affected by hypoxia and acidosis

Patient No.	Birth weight g	Age, days	Diagnosis	Blood pH	pO_2 mmHg	Blood glucose m/dl	Body temperature °C	No. of evaluated metaphases	No. of structural aberrations
1.	1250	3	Centralis lesion	7.07	—	90	35.8	30	
2.	1400	4	Perinatal infection	6.8	—	140	36	50	
3.	1600	5	IRDS I—II	7.2	—	45	36.4	30	1
4.	1000	8	Perinatal infection	7.0	—	90	36.7	30	
5.	1200	2	Prematurity	7.2	—	175	36.4	30	
6.	1830	7	Oesophageal atresia	7.1	32	150	36.4	30	1
7.	1440	4	Oesophageal atresia	7.0	28	200	36	40	1
8.	1730	1	Asphyxia in utero	7.24	36	100	35.5	30	
9.	1080	2	IRDS, intracerebral haemorrhage	7.19	39	130	36.4	30	
10.	2100	2	Sepsis, jaundice	7.09	14	250	35.4	30	2
11.	1200	1	IRDS III—IV	7.15	16	90	35.5	30	
12.	660	3	Cerebral haemorrhage	7.32	53	130	36	30	1
13.	1550	4	Hyperbilirubinaemia	7.3	44	—	36.5	30	
14.	950	8	Pneumonia	7.14	42	90	36.2	30	
								450	6

The gas mixture used for therapy contained more than 60 vol% O_2. pO_2 values were either monitored transcutaneously or measured in arterial blood samples by the Astrup method. Cytogenetic studies were performed simultaneously in O_2-treated neonates and newborns of comparable age admitted to the same unit but needing no oxygen therapy.

In addition to oxygen treatment, the patients received parenteral glucose, electrolyte and amino acid solutions, vitamins and antibiotics. The control group was treated with these substances in a similar way, as demonstrated in Table II.

Newborns treated with phototherapy, or with caffeine because of apnoeic spells were excluded from the study, as these factors are supposed to possess a chromosome damaging action [8, 21].

Chromosome studies were carried out on cultured peripheral blood by the method of Sumner et al (24); the breakage points were evaluated by light-microscopy after traditional staining. The SCE phenomenon was studied by the method of Perry and Wolff [19], as modified by Raposa [20].

RESULTS

1. The number of breaks per metaphase was 0.013 immediately after admission in prematures with acidosis and hypoxia (normal value for this laboratory: 0.010 ± 0.013) as shown in Table I.

2. Most pO_2 values of the patients treated with continuous mechanical ventilation were higher than 80 mm Hg. After termination of ventilatory treatment they exhibited a mean rate of aberration of 0.16 as contrasted to the value of 0.05/metaphase, found in newborns cared for in the same unit but not treated with oxygen ($p < 0.05$). The mean rate of SCE amounted to 7.1 in the oxygen treated group and to 5.7 ($p > 0.40$) in the control group (Table III). The highest breakage rates were encountered in the cul-

TABLE II

Clinical data of newborns receiving long-term oxygen therapy

Serial number of patient	Diagnosis	Drugs administered	Type of oxygen treatment	Duration in days
1.	Pneumonia, sepsis, central lesion	Tobramycin, meticillin, oxacillin, bromohexine	IPPB—CPAP	26
2.	Acute fetal distress, pneumonia	Tobramycin, azlocillin, polymyxin furosemide, dopamine, dobutamine, phenobarbital, human lyophil Ig	IPPB	11
3.	IRDS, bronchopulmonary dysplasia	Tobramycin, penicillin, oxacillin, bromohexine, furosemide, dopamine dobutamine, tolazoline	PEEP—CPAP IPPB, nasal gavage	4 30
Control	Ileal atresia	Tobramycin, penicillin, cephamandol, ampicillin, oxacillin, azlocillin, dexpanthenol, human lyophil Ig	—	—
4.	Connatal tuberculosis	Tobramycin, penicillin, azlocillin, cephamandol, furosemide, dexpanthenol, human lyophil Ig	PEEP—CPAP PEEP—IPPB	18 28
5.	In utero infection	Tobramycin, penicillin	PEEP—CPAP	7
Control	Hydronephrosis	Tobramycin, ampicillin,	—	—
6.	E.coli meningitis	Tobramycin, oxacillin, azlocillin, cephamandol, chloramphenicol, human lyophil Ig	IPPB-incub. O_2	12
7.	Intrapartum asphyxia, infection	Tobramycin, ampicillin, diazepam, phenobarbital	PEEP—CPAP	7
8.	Intrauterine growth retardation, bronchopulmonary dysplasia	Tobramycin, oxacillin, human lyophil Ig, heparin	IPPB—CPAP	16
9.	Perinatal infection	Tobramycin, oxacillin, dopamine, dobutamine	CPAP-incub. O_2	13
10.	IRDS III—IV, cerebral haemorrhage	Penicillin, ampicillin, cephamandol furosemide, phenobarbital, diazepam, dopamine, dobutamine	CPAP-PEEP	18
Control	Neonatal pyelonephritis	Tobramycin, penicillin	—	—

tures of newborns treated with 90—100 vol% oxygen for a considerable time. The two cytogenetic parameters showed mostly no parallelism and there was considerable individual scatter within the results obtained by either method. Only three patients had a SCE rate exceeding the mean, i.e. these patients exhibited excessively high values. In one patient the increased breakage rate was not accompanied by SCE induction (Table III). It appeared that the duration of oxygen therapy was of importance. In an illustrative case, a baby needing continuous positive airways pressure (CPAP) resp. PEEP because of intrauterine infection and serial

TABLE III

Cytogenetic findings of newborns receiving prolonged oxygen treatment

Patient No.	Oxygen concentration of inhalation mixture, vol%	Duration of oxygen therapy, day	Number of metaphases studied	Aberration per cell	Type of chromosome aberration		SCE per cell
					chromatid	chromosome	
1	100—70	26	50	0.28	12	2	9.7
2	100—90	11	50	0.14	4	3	not investigated
3	100—70	14	50	0.24	11	1	8.3
Control			50	0.10	5		5.7
4a	100—90	18	50	0.06	2	1	5.5
4b	100—95	28	50	0.18	8	1	6.3
5	60—50	7	50	0.06	2	1	not investigated
Control			100	0.02	1	1	5.7
6	50—40	12	50	0.12	5	1	5.2
7	60—40	7	50	0.02	1		6.0
8	70—60	16	50	0.24	9	3	5.0
9	65—30	13	50	0.12	5	1	not investigated
10	100—60	18	50	0.20	7	3	7.7
Control			50	0.06	3		5.0
Total/mean			550	0.16	66	17	7.1
Control values			200	0.05	9	1	5.7

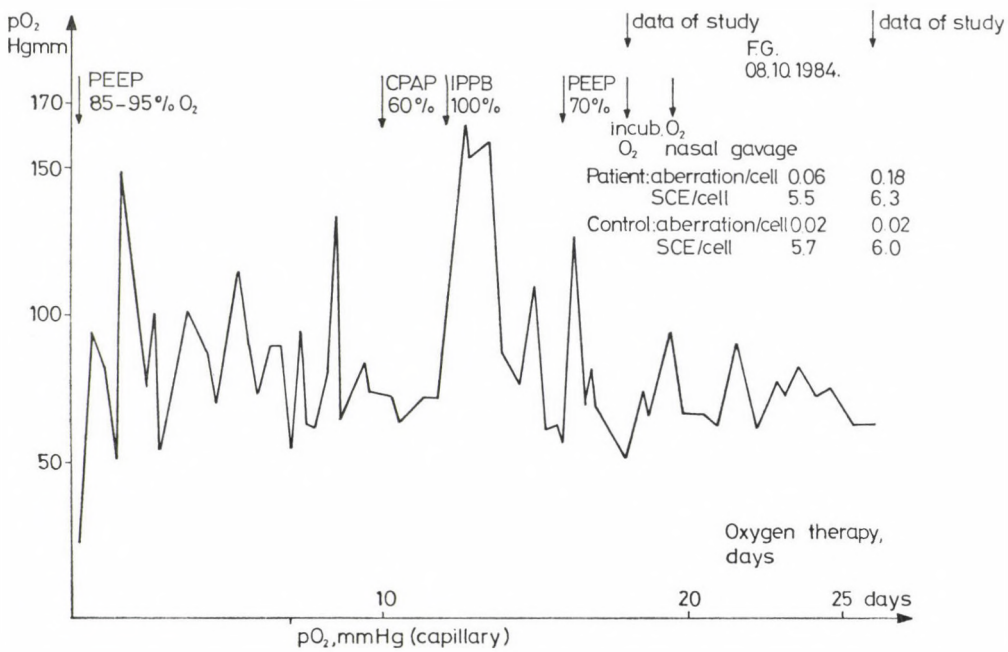

FIG. 1

apnoeic spells over 18 days had 0.06 aberrations and 5.5 SCE per cell; 40 days later, after a period of uninterrupted PEEP-IPPB treatment, these values increased to 0.18 and 6.3 respectively (Figure 1).

Discussion

Environmental mutagenic effects must play a role in the development of chromosomal aberrations observed in newborns since there are no structural aberrations or SCE in the cord blood of neonates born of healthy mothers [10]. There is an elevated rate of chromosomal breakage in newborns under intensive care or those exhibiting signs of intrauterine growth retardation [10].

Oxygen seems to play an outstanding part among the environmental factors leading to mutagenesis [25]. Within certain limits, the degree of free oxygen pressure may increase the concentration of peroxy radicals and thereby exert an indirect mutagenic effect. It is known from culturing human lymphocytes that free oxygen radicals produced in the medium by light or enzymes cause chromosomal breakage or induce SCE [5].

The genotoxic effect of high oxygen pressure was shown in bacterial tests [14] while others [23] observed cell death, micronuclear positivity, chromosomal gaps and breaks in Chinese hamster fibroblast cultures. The aberration rates observed on exposure to oxygen showed a dose and duration dependent character. Increased oxygen tension causes elevation in the rate of SCE in Allium cepa Linné cell cultures [9]. In other words, molecular oxygen may cause genome changes in both procaryote and eucaryote organisms.

Several defense mechanisms against free radicals have been developed by all kinds of live matter (nuclear membrane, reducing enzymes, catalases, peroxidases, etc.). It was assumed [6] that all aerobic organisms possess an enzymic defense system protecting the cells from oxygen induced damage. A similar role has been ascribed to plasma uric acid, an antioxidant compound capable of protecting the erythrocytes from lipid peroxidation [1].

In phytohaemagglutinin stimulated blood cell cultures of patients with Fanconi anaemia, elevation of the O_2 concentration provokes a commensurate increase in the rate of chromosomal breakage. This oxygen dependent chromosomal instability shows interindividual fluctuations which are further enhanced by the addition of mitomycin C [13]. In Fanconi anaemia a faultive defense mechanism has been assumed to be at work [12].

Clinical cytogenetic studies cannot help in deciding whether the increased rate of breakage and SCE, observed in lymphocytes of newborns ventilated with 60—100 vol% oxygen should be ascribed to the indirect damaging effect of oxygen itself or to a failure of the assumed enzymic defense mechanism.

The rate and degree of chromosomal damage is appreciably influenced by

the duration of oxygen therapy. Importance of the time factor is corroborated by data collected from several perinatal intensive care centres [17], who showed no cytogenetic aberrations in blood cultures of neonates exposed to ventilation therapy not exceeding 4—5 days.

In evaluating the individual variability, the possible role of mutagenic factors other than oxygen also emerges. When selecting the control group, patients comparable in respect to antibiotic and chemotherapeutic treatment were chosen since these drugs may cause cytogenetic aberrations [2, 11, 22]. The present data do not allow an evaluation of a possible relationship between acquired chromosomal aberrations and specific antibiotic regimens. However, in a patient of the control group, affected by ileal atresia, who had had to be treated with numerous antibiotics in addition to cephamandol, a markedly increased breakage rate (0.10 aberration per cell) was observed.

It may be speculated that pathological chromosomal phenomena have a multiple cause; beside a high oxygen pressure, antibiotics and other hospital pollutants (detergents, chemicals used for disinfection or cleaning, etc.) may be suspected. It appears that exact registration of the oxygen content of the gas used for ventilation and continuous monitoring of the patients' pO_2 values are indispensable in proper oxygen dosage and in attem pts to reduce the cumulative effect of deleterious factors present in intensive care units.

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Book reviews

B. H. ATHREYA, B. K. SILVERMAN, A. R. SPITZER: *Pediatric Physical Diagnosis.* Appleton-Century-Crofts, East Norwalk, CT 1985. Price $ 29.95

In this world of sophisticated and expensive diagnostic tools a question of increasing importance is how to select the right patient for the right diagnostic intervention. Quite obviously, physical examination is, or should be, the first step of the diagnostic chain, still, many of us do not resist the temptation to skip it or at least to postpone detailed physical examination after the evaluation of special tests or procedures ordered after superficial history taking. It is therefore refreshing to take this book in one's hands and to see how much orientation can be obtained from reasonable history taking and physical examination. But all this is nicely dealt with in a Clinician's Lament, itself a faultless summary of nowadays faultive clinical thinking of; it follows immediately the preface of this excellent book.

The small section introducing the reader to the concept of normality already accentuates the importance of common sense, disciplined and economic thinking. The most difficult task is to teach good history taking. By asking the patient or his/her parents about the complaints or symptoms that have coerced them to seek the physician's help you may, and probably must, confront the unpredictable. To be prepared to the problem how to pursue the fugitive diagnosis needs much experience, knowledge and wisdom; the beginner can save a lot of time by adopting the geniality of the author concealed in this section.

The bulk of the book describes the methods of physical examination and sound interpretation of the findings. Special attention is conveyed to the nervous system, so frequently neglected at common paediatric examination.

It was a useful idea to include the chapter of specialised examinations dealing with developmental and behavioural assessment, physical examination of the newborn infant and that of the adolescent.

This book should be read by every student, medical teacher in paediatrics and every doctor dealing with healthy and sick babies, children and adolescents.

P. CHOLNOKY

Nutrition for special needs in infancy. Protein hydrolysates. Ed: F. LIFSHITZ. 336 pages with 47 figures and 48 tables. Marcel Dekker Inc., New York 1985. Price $ 59.75 (except U. S. and Canada where it is $ 49.75)

This book is the fourth volume of the Clinical Disorders in Paediatric Nutrition series. The earlier volumes were dealing with questions of carbohydrate intolerance in infancy, the feeding of low-birthweight infants, the important nutritional deficiencies, the variety of intestinal

problems in infancy including diarrhoea and malnutrition, intolerance to food proteins, and the mineral and vitamin requirement in preterm infants. This volume is designed to summarize the recent theoretical and clinical knowledge on protein hydrolysates in special needs of infant nutrition.

The first part of the volume is dealing with theoretical considerations, namely the absorption of proteins and its derivatives during early life, the role of protein breakdown products in the absorption of essential trace elements, the morphological alterations in small intestinal epithelial surfaces during nutrient transport, the mechanisms of allergic reactions and local antibody production in infancy, the regulation of immunoglobulin delivery into the gut and the processing and evaluation of the antigenicity of protein hydrolysates.

The second part of the book summarizes the questions of clinical use of protein hydrolysates. The main problems which are discussed in this part are the preclinical and clinical evaluation of casein hydrolysate products, use of casein hydrolysate formulas in the diagnosis and management of gastrointestinal food sensitivity, carbohydrate and fat malabsorption, dietar management of postinfectious chronic diarrhoea in malnourished infants, comparison of protein hydrolysates and elementary diets in the intractable diarrhoea syndrome of infancy, the use of continuous naso-gastric feeding in malabsorption syndromes, feeding of premature infants, the infantile colic and the role of casein hydrolysate formulas, the problems of offsprings of high-risk allergic families and the role of protein hydrolysates in cystic fibrosis.

The appendix is very useful for the neonatologist because it contains the correct components of human milk and milk-based infant formulas, soy-based infant formulas, protein hydrolysate infant formulas and the special infant formulas (blenderized whole protein nutritional supplements, hypertonic lactose containing nutritional supplements, hypertonic and isotonic lactose-free whole protein nutritional supplements, hypertonic high calorie high nitrogen whole protein formulas, etc.

The book is an excellent summary of up-to-date knowledge on nutrition for special needs in infancy and it should not be missing from the library of all neonatologists interested in the care of high-risk preterm newborns and infants.

I. RUBECZ

N. O'DOHERTY: *Atlas of the newborn* 2nd edition, 413 pages. MTP Press Limited, Lancaster, England 1986. Price £ 29.95

I have opened this book of O'Doherty with great pleasure and interest. My first reaction was admiration, but it was envy that grew in me when I have repeatedly read this fascinating work. I cannot get rid of the thought that this Atlas could have been composed by myself. How much we observe and experience in our medical practice, still, we are unable to hand it over to generations to come since the case remains our personal property because not all interesting, striking and instructive pictures guarded in our memory can be expressed in words. But this should not necessarily be so. Only a good camera and sharp eye are needed. The author did possess both. He realized that a good picture tells the reader much more than a good many words. This is especially true ever since we have good cameras with adequate colour films and our world is rapidly shaping according to the prophecies of McLuhan.

The Atlas consists of a large series of photographs of sick newborns, and the excellent pictures are accompanied by short explanations. They are in six chapters: normal neonates, low-birthweight newborns, obstetrical injury, infection, congenital malformations and skin defects.

This seems a bit arbitrary but the series of photographs does cover the whole of neonatology.

Who should use this book? All paediatricians and obstetricians, all candidates in neonatology and — last but not least — all nurses caring for newborn babies. Everybody who uses this atlas with open eyes will find new features. The book should be opened many times, it will then be helpful in the practical work necessary to all those who have to do with newborn babies.

Judit SZOLNOKI

Berner Datenbuch der Pädiatrie. Herausgegeben von der Medizinischen Universitäts-Kinderklinik, Inselspital Bern. 2., durchgesehene Auflage. XVIII + 772 Seiten mit 60 Abbildungen und zahlreichen Tabellen. Gustav Fischer Verlag, Suttgart—New York 1985. Preis DM 38.—

Die zweite Auflage des ausgezeichneten Berner Datenbuches ist Prof. E. Rossi von seinen Mitarbeitern zum 70. Geburtstag gewidmet.

Gegenüber der ersten Auflage weist die neue Version nur wenige Neuerungen auf. Diese haben aber die schon ursprünglich erhebliche Informationskraft noch weiter verstärkt. Besonders das Kapitel Pneumologie wurde stark revidiert und verbessert. Einige Tabellen sind wesentlich übersichtlicher geworden, wie z. B. die Plasma-Glukose und Insulin-Referenzwerte bei oraler Glukosebelastung (S. 97), oder die hämatologischen Referenzwerte (S. 730). Die Richtigstellung einiger Einzelheiten wie z. B. das Weglassen der unnötigen Behandlung der Akne neonatorum (S. 80), oder der Adrenalin-Verabreichung aus der Sofortmaßnahmen beim Astmaanfall (S. 605) ist auch zu begrüßen.

Der reichhaltige Inhalt bietet dem Leser eine schnelle und einfache Orientierung in der Diagnostik und Therapie der häufigsten Krankheiten. Das Buch enthält viele wichtige Angaben, die sonst in diesem Ausmaß zusammengebracht kaum zu finden sind. Es ist nur bedauerlich, daß die morphologischen Aspekte wieder völlig vernachlässigt werden, obwohl für die Syndromdiagnostik wichtigen anthropometrischen Messungen und Normwert-Tabelle schon recht präzis ausgearbeitet worden sind.

Trotz dieser Bemerkung kann der Rezensent nur seine frühere Meinung wiederholen: das Berner Datenbuch bietet eine moderne, rasche Nachschlagemöglichkeit und damit eine wertvolle Ergänzung der pädiatrischen Lehr- und Handbücher.

K. MÉHES

M. von DITTRICH, H.-M. STRASSBURG, E. DINKEL, B.-J. HACKELÖER: *Zerebrale Ultraschalldiagnostik in Pädiatrie und Geburtshilfe.* XI + 144 Seiten mit 182 Abbildungen in 323 Einzeldarstellungen, Springer-Verlag, Berlin—Heidelberg—New York—Tokyo 1985. Preis DM 98,—

Die Monographie entstand in gemeinsamer Arbeit von vier in verschiedenen pädiatrischen, radiologischen und gynäkologischen Zentren tätigen Autoren. Bereits dieser Umstand deutet auf die stürmische Entwicklung der Ultraschalldiagnostik und folglich die Abtrennung der einzelnen Gebiete hin.

Nach einem technischen Überblick werden die normalen intrakraniellen Sonogramme in den von den Verfassern angewandten und international anerkannten Schnittebenen dargestellt. Überzeugende schematische Darstellungen illustrieren ferner die frontalen und sagittalen Schnittebenen. Die Gyrierung des Hirnparenchyms, Einzelheiten der Ventrikel und Gefäßstrukturen treten auf den ausgezeichneten Aufnahmen gut in Erscheinung. Das Bildmaterial der hinteren Schädelgrube ist demonstrativ, etwas weniger jenes der Zysternen; letzterer Umstand dürfte auf die Grenzen der Untersuchungsmethode hinweisen.

Die fetale Sonographie eignet sich zum Nachweis von Schädeldeformitäten, Hydrozephalus, Myelodysplasie, Enzephalozele; ultraschallgeführte Eingriffe bei hydrozephalen Feten (Shunt) werden von den Autoren zur Zeit als problematisch betrachtet.

Die angeführten Normkurven zur Schwangerschaftsalterbestimmung und die Kephalometriemaße bieten äußerst nützliche Stützpunkte.

Hinsichtlich der postnatalen transfontanellaren Untersuchungen vertreten die Verfasser die Meinung, daß diese sich derart genau erwiesen haben, daß z. B. beim Fehlen von feinen Strukturen wie das Septum pellucidum, dieser Zustand als eindeutig beweiskräftig betrachtet werden konnte, wenn es sich auf dem Sonogramm nicht abzeichnete. Selbstverständlich sind bedeutende Fehlbildungen wie Zysten in der Mittellinie oder die Agenesie des Corpus callosum klar erkennbar. Das Vorkommen eines Aneurysmas der Vena magna Galeni ließ sich mittels Doppler-Sonographie und Angiographie nachweisen und dokumentieren.

Ein allgemein verbreitetes Anwendungsgebiet der intrakraniellen Ultraschalldiagnostik ist der Nachweis von subependymalen, intraventrikulären und zerebralen Blutungen; ein über reiches und methodologisch gut erwogenes Bildmaterial verfügendes Kapitel ist diesem Thema gewidmet. Ähnlicherweise soll das Kapitel über den Hydrocephalus hervorgehoben werden; man vermißt hierbei jedoch die den Kliniker unterstützenden postoperativen Untersuchungen der Maßveränderungen.

Weitere Kapitel befassen sich mit der Sonographie von dem Subduralerguß, Hirninfarkt, Hirnödem, den intrakraniellen Infektionen und Hirntumoren, mit den möglichen ergänzenden oder andersartigen Untersuchungsverfahren (CT, Angiographie).

Abschließend findet man die Besprechung der ultraschallgezielten Punktionen und der klinischen Anwendung der Doppler-sonographischen Registrierung intrakranieller Gefäße.

Am Ende jedes Kapitels findet sich eine 1984 abgeschlossene Zusammenstellung der weiterführenden Literatur.

Das Buch bietet eine umfassende Darstellung der sonographischen Zerebraldiagnostik einschließlich Dopplersonographie und dürfte somit dem pädiatrisch und gynäkologisch tätigen Kliniker beim Erlernen der Methode oder zur Vertiefung seiner Kenntnisse als Leitfaden dienen.

G HARMAT

S STENGEL-RUTKOWSKI, P SCHIMANEK: *Chromosomale und nicht-chromosomale Dysmorphie-Syndrome.* VIII + 216 Seiten mit 230 Abbildungen und 4 Tabellen, Ferdinand Enke Verlag, Stuttgart 1985. Preis DM 68,—

Die gut aufgebaute, übersichtliche Monographie gliedert sich in vier Kapitel.

Das erste — Genetische Grundlagen — erörtert diejenigen genetischen Begriffe, deren Kenntnis zur Erkennung der Ätiologie einer Genetik bedingten Krankheit unentbehrlich ist. Besonders nützlich ist hier die angegebene Nomenklatur der Chromosomenaberrationen.

Das zweite Kapitel — Systematik klinisch-genetischer Untersuchungen — ist für den praktizierenden Kinderarzt der verwertbarste Teil des Buches. Hervorzunehmen sei hierbei die anderswo bisher nicht auffindbare Bestimmung und Beschreibung des Begriffes Dysmorphie. Außer der Definition werden genau und ausführlich und auch entsprechend illustriert die Charakteristika vor allem der craniofacialen Dysmorphien sowie der Abweichungen der Extremitäten und anderen Körperegionen erläutert. Die Autoren haben es versucht, den weiten und subjektiven Begriff Dysmorphie womöglich mit exakten anthropometrischen Angaben zu konkretisieren. Die benötigten Methoden und Geräte wurden auch dargestellt. Das Kapitel schließt mit einer, vielleicht zu einge-

henden, Schilderung der Hautleisten auf Handteller und Sohle, und einer kurzen Besprechung der Cytogenetik.

Im dritten Kapitel — Fallbeispiele — findet man die Richtlinien, wie das im vorangehenden Teil Besprochene in der Praxis angewandt werden soll. Es handelt sich um Beispiele und nicht um einen Syndromenatlas. Die Verfasser behandeln aus eigenem Material 13 seltene Chromosomenaberrationen, 12 bekannte Dysmorphie-Syndrome und die Alkoholembryopathie. Bei einem dysmorphischen mentalretardierten Kind ist der erste Schritt zur Diagnose der Ätiologie die Erkennung der augenfälligen Veränderung des Phenotyps der craniofacialen Dysmorphie. Dies wird mit en face und Profil-Photos illustriert. Eigene und andere Daten vergleichende Tabellen, ein Stammbaum, Dermatoglyphe und gegebenenfalls die Angabe vom pathologischen Karyotyp ergänzen die Falldarstellung. Es sei bemerkt, daß die Qualität der Bilder nicht gleichförmig gut ist, teilweise wegen der Aufnahme und teilweise aus Reproduktionsgründen; so sind z. B. die Portraits der Russell bzw. Lange Fälle nicht entsprechend überzeugend. Ferner dürfte die X-dominante Heredität des Coffin—Lowry Syndroms in Frage gestellt werden, da in den meisten Handbüchern dieses Syndrom als eine an X gebundene rezessiv erbliche Genschädigung betrachtet wird. Vielleicht wäre es vorteilhaft gewesen, anhand der Besprechung der angeführten Fälle und Syndrome auch auf die Differentialdiagnose der aufgrund der Phenotype in Frage kommenden anderen Syndrome einzugehen.

Das vierte Kapitel ist ein Anhang, in dem die Autoren ihre Untersuchungsprotokolle, ferner einen Katalog der in den einzelnen Körperregionen vorkommenden Dysmorphien in alphabetischer Reihenfolge darbieten.

Die kurze Monographie ist für den Pädiater und klinischen Genetiker deshalb sehr wertvoll und nützlich, weil sie ihn der Gedankenreihe entlangführt, mit deren Hilfe er von den Dysmorphie ausgehend zur Diagnose der Ätiologie gelangen kann. Letztere ist wohl die Grundlage der Prognose und Prophylaxe.

P Kiss

M Reissig, *Körperliche Entwicklung und Akzeleration Jugendlicher*. 120 pages with 31 tables and 16 figures, Verlag Volk and Gesundheit, Berlin 1985. Price DM 26,—

This book is focussed on the phenomenon of growth acceleration whereby children are becoming taller and heavier at an early age. Unfortunately, the author restricts her review of the literature to German sources rather than the whole of the literature on human biology. Moreover, the sources cited are often inadequately documented.

Nevertheless, any elaboration of longitudinal growth is a contribution and the description of the growth of 550, 12—16-year-old Leipzig boys and girls between the years 1968 and 1972, is a welcome addition to the available information. Description of growth of ten anthropometric variables, and some physiological traits such as blood pressure and vital capacity are further studied in terms of subsampling as "small", "normal", "tall", and "accelerated", "normal", "retarded" as well as in terms of categorization of "physiological start-endowment in early childhood" and "frequency of illnesses" related to pubertal growth and maturation.

The author's conclusions are consistent with observations made elsewhere that the maturation rate of girls is in advance of that of boys by two years at peak height velocity. She reported a harmonic growth in height and weight. She also attempted to provide some indication of a possible secular trend by comparing her contemporary data with those assembled by Koch on Leipzig children in the 1930s. In general, she found a positive secular trend of increased height and weight. Thus the book enriches the literature on growth studies with East-German data.

O. G. Eiben

K JÄHRIG: *Das Kind in der Allgemeinpraxis*. 608 Seiten mit 71 Abbildungen und 79 Tabellen. Gustav Fischer Verlag, Jena 1985. Preis DM 79,—

Der Autor geht von der Tatsache aus, daß der Allgemeinarzt zur Zeit und in der Zukunft noch eher immer mehr Aufgaben der Grundbetreuung von Kindern und Jugendlichen zu erfüllen hat und deshalb eine sehr gründliche Kenntnis der Kinderheilkunde benötigt. Dies bezieht sich sowohl auf seine prophylaktische wie auch therapeutische Tätigkeit.

Das Buch richtet sich in erster Linie an die Allgemein- oder Familienärzte in der DDR und enthält die dortigen gesetzlichen Regelungen, Verordnungen und Möglichkeiten. Die Stoffauswahl mußte im Interesse des Umfangs den Möglichkeiten und Ansprüchen des Allgemeinarztes angepaßt beschränkt erfolgen, und so stehen Diagnostik und Therapie im Vordergrund, während auf theoretische Erörterungen verzichtet wurde.

Das Buch besteht aus 11 Kapiteln. Nach einer kurzen Einführung und Besprechung der Grundlagen der Pädologie und Physiologie, werden die Bedeutung der Anamnese betont und sodann die physikalische Untersuchung des Kindes, deren Eigenheiten, die Grundzüge der Arzneiverordnung, diagnostische und therapeutische Techniken beschrieben.

Das größte Kapitel 7 ist der speziellen Diagnostik und Therapie der wichtigsten Krankheiten des Kindes- und Jugendalters gewidmet. Ein Kapitel befaßt sich mit dem Problem der chronisch kranken oder behinderten Kinder und ein anderes mit den Notfällen. Die letzten Kapitel behandeln die Fragen der Vorsorge und Gesundheitserziehung und den gesetzlichen Bestimmungen.

Im Anhang findet man noch Nomogramme und Kurven zur Berechnung verschiedener Körpermaße, ein Medikamentverzeichnis, ein Diagnoseverzeichnis, Infekt- und Anfallskalendar.

Das Buch faßt zahlreiche sich in der Praxis angebende Probleme zusammen, u. a. Grenzen und Kompetenz, Verantwortung, Kooperation mit dem Pädiater, Überweisung an den Pädiater usw.

Neben der notwendigen Basisinformation ist das Buch auch zum schnellen Nachschlagen für den Familienarzt, den Allgemeinmediziner gedacht.

K SCHMIDT

Pädiatrie für Krippenerzieherinnen Herausgegeben von E SCHMIDT-KOLMER. 128 Seiten mit 30 Abbildungen und 13 Tabellen. Verlag Volk und Gesudheit, Berlin 1985. Preis DM 17,—

Das Buch ist ein Lehrbuch für die Fachschulausbildung von Krippenerzieherinnen. Die von bekannten Kinderärzten verfaßten 13 Kapitel beweisen gut die Vorteile der getrennten Ausbildung von Krankenhaus- und Krippenpersonal. Die Autoren haben es gut ausgewählt, wieviel und welche Kenntnisse über Krankheiten die sich mit der empfindlichsten Altersklasse beschäftigenden Säuglings- und Kleinkinderzieher benötigen. Was aber über die normale Entwicklung und Pflege der gesunden Säuglinge und Kleinkinder mitgeteilt wird, erscheint ungenügend. Fachleute werden nicht nur von der Kargheit dieser Teile, sondern auch von ihren Inhalt überrascht sein, da hier viele Vorgehen und Methoden empfohlen werden, deren Verbannung von der Säuglings- und Kleinkinderpflege in den meisten Ländern schon gelungen ist. Einzelheiten, wodurch die Pflegearbeit aus einem einfachen hygienisch-technischen Vorgehen zu wirklichen Bestandteilen und Mittel der Erziehung werden, sind nur im allgemeinen, oder gar nicht erwähnt.

Judith FALK

M WUNDERLICH: *Direkte fetale Elektrokardiographie* 86 Seiten mit 53 Bildern und 3 Tabellen, J. A. Barth Verlag, Leipzig 1985. Preis M 19,40

Die kleine Monographie faßt die wichtigsten Kenntnisse über die direkte fetale Elektrokardiographie zusammen. Der Autor befaßt sich kurz mit der indirekten fetalen

Elektrokardiographie, und die anderen auf der direkten Elektrokardiographie beruhenden intrauterinen diagnostischen Verfahren werden lediglich erwähnt.

Nach der Schilderung des fetalen Herzens und Kreislaufes und deren Besonderheiten, wird die Methodik der direkten Elektrokardiographie und das normale fetale EKG erörtert und auch auf die Möglichkeit von Fehlinterpretationen hingewiesen. Desweiteren wird die Herzachsenschätzung bzw. deren Bedeutung bei Nabelschnurkomplikationen oder bei der Diagnose von kongenitalen Vitien behandelt. Den Störungen der Reizbildung und Erregungsleitung ist ein umfangreiches Kapitel gewidmet. Bei der Einteilung der Rhythmusstörungen hat sich der Autor an das Schema von Mücke und Bartel gehalten. Hinsichtlich der Beurteilung der verschiedenen Dysrhythmien wird betont, daß einige harmlos sein können (Sinusextrasystole), bei der Mehrheit der Rhythmusstörungen jedoch Hypoxie oder ein kongenitales Vitium im Hintergrund steht. Ganz kurz wird dann die Möglichkeiten der intranatalen Therapie von Rhythmusstörungen besprochen. Von praktischer Bedeutung ist die Erörterung der charackteristischen EKG Zeichen des sterbenden Feten, sowie das Kapitel über Einfluß von Medikamenten auf das fetale Herz. — Abschließend finden wir einen Gedankengang über die Perspektiven der fetalen Elektrokardiographie.

Das Buch kann auf das Interesse von Geburtshelfer und Neonatologen rechnen, doch werden es Kardiologen, Pädiater und Fachassistenten an Intensivtherapie-Abteilungen mit Nutzen lesen.

J Kiszel

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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Acta Paediatrica Hungarica 27 (4), pp. 283—288 (1986)

Sodium and potassium concentrations of red blood cells and plasma in children with nephrotic syndrome, uraemia and pyelonephritis

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The sodium and potassium concentrations of the red blood cells and the plasma in 38 children with pyelonephritis (19 acute, 10 chronic and 9 healed), 5 children with uraemia, and 20 children with nephrotic syndrome were compared with those of control children. The red blood cell sodium concentration was lower in patients with acute pyelonephritis, uraemia, and steroid-treated nephrotic syndrome, and higher in those with chronic pyelonephritis and nephrotic syndrome not treated with steroids. Except in uraemic cases, these alterations were not accompanied by plasma sodium and potassium changes. The results might be explained by pathological Na^+ and K^+ transport processes in the red cell membrane. The possible role of extracellular fluid volume changes, sodium loss and water retention are discussed.

The Na^+ and K^+ composition of the human red blood cells (RBCs) are close to those of other cells, and the RBC is a good model for the study of intracellular electrolytes and membrane transport processes [1, 18, 19]. The Na^+ and K^+ concentration of the RBCs have previously been investigated mainly from the aspect of cardiac glycosides or arterial hypertension [7, 14, 26, 27].

We have studied the Na^+ and K^+ concentrations of the RBCs and plasma in children with pyelonephritis, uraemia and nephrotic syndrome, with a view to establish whether there was a connection between the type of the disease and the electrolyte differences.

Patients and Methods

The RBC and plasma concentrations of Na^+ and K^+ were determined in 63 children with renal diseases and in 16 healthy control children, by the method described by Fortes Mayer and Starkey [5]. All patients were undergoing treatment in our Department; they comprised 19 with acute pyelonephritis, 10 with chronic pyelonephritis, 9 with healed pyelonephritis, 5 with uraemia and 20 with nephrotic syndrome. There was no difference in age distribution between the patients and the controls. Besides the massive pyuria and bacteriuria observed in urine obtained by bladder puncture, the criteria of pyelonephritis were the clinical symptoms and the decrease in renal concentrating capacity. Cases were regarded as chronic if leucocyturia and bacteriuria had persisted for longer than one month in spite of directed antibiotic treatment. In addition, urinary con-

centrating capacity remained depressed and I. V. urography revealed symptoms of chronic pyelonephritis. In 3 of the nephrotic syndrome patients, a moderate degree of clearance reduction was observed; the others did not display azotaemia. During the time of the examination, the nephrotic syndrome patients received uniform doses per weight of lasix, KCl and spironolactone, with prednisolone or chlorambucil treatment when necessary. 21 observations were performed on 15 patients before or without prednisolone treatment, and 23 observations were performed on 17 patients during prednisolone treatment. Moderate hypertension (average 21.5/12 kPa) was observed in 2 of the nephrotic syndrome patients and 3 of the uraemic patients. They participated in methyldopa and prazosine treatment. A pathological acid-base finding was observed in 2 uraemic patients with a moderate degree of compensated metabolic acidosis kept in equilibrium by the administration of 1 g $NaHCO_3$ daily. The serum urea nitrogen level in the uraemic patients was 22—27 mmol/l; one of them underwent haemodialysis treatment.

Results are presented in Tables I and II. Statistical evaluation was performed with Student's t test.

Results

Table I presents RBC and plasma Na^+ and K^+ concentrations in healthy children and in children with acute, chronic or healed pyelonephritis and with uraemia. A significantly lower RBC Na^+ level was observed in acute pyelonephritis, and a significantly higher one in chronic pyelonephritis. The observed electrolyte values for the healed pyelonephritis cases corresponded to those of the controls. In the uraemic patients, higher plasma K^+ and lower RBC Na^+ concentrations were found. Because of the low number of cases, sigfinicance was not calculated.

Table I

Plasma and red blood cell Na^+ and K^+ concentration in healthy children and in children with acute, chronic or healed pyelonephritis and with uraemia

	n	Plasma Na^+ $\bar{x}$	mmol/l SD	Plasma K^+ $\bar{x}$	mmol/l SD	Red blood cells Na^+ $\bar{x}$	mmol/l SD	Red blood cells K^+ $\bar{x}$	mmol/l SD
Healthy children	16	140	±2	4.0	±0.3	6.5	±0.6	94.4	±3.1
Acute pyelonephritis	19	139	±2	4.1	±0.4	4.8	±0.8**	93.6	±3.1
Chronic pyelonephritis	10	140	±2	4.0	±0.5	7.6	±1.0*	96.4	±3.4
Healed pyelonephritis	9	139	±4	4.1	±0.5	6.4	±0.4	96.0	±4.0
Uraemia	5	137	±6	4.6	±0.5	4.9	±1.2	92.5	±3.8

* $p < 0.01$
** $p < 0.001$

Table II lists the results of the nephrotic syndrome patients. Without steroid treatment, the RBC Na^+ concentration was significantly higher, while the plasma Na^+ concentration was significantly lower than the levels of the controls. In response to steroid treatment, the RBC Na^+ level fell to a significantly lower value as compared to the controls, while the plasma Na^+ increased to normal.

TABLE II

Plasma and red blood cell Na^+ and K^+ concentrations in nephrotic syndrome patients

	n	Plasma Na^+ $\bar{x}$	mmol/l SD	K^+ $\bar{x}$	mmol/l SD	Red blood cells Na^+ $\bar{x}$	mmol/l SD	K^+ $\bar{x}$	mmol/l SD
Before or without prednisolone treatment	21	137	±3*	4.2	±0.9	8.1	±1.4**	93.7	±4.7
During prednisolone treatment	23	140	±2	4.0	±0.3	5.1	±1.1**	93.3	±4.2

* $p < 0.01$
** $p < 0.001$

DISCUSSION

The Na^+ and K^+ concentrations of the control RBCs and plasma were in accordance with recent data in the literature [3, 5].

The lower RBC Na^+ concentration observed in acute pyelonephritis was of interest, as in other investigations higher RBC Na^+ levels were found in diseases involving acute inflammation of the airways. The cause of the latter phenomenon is presumably the inhibitory effect of inflammation on the activity of the Na—K pump. Furthermore in chronic pyelonephritis an impaired urinary concentrating mechanism is leading to polyuria and extracellular water loss. The compensatory water efflux from the cells into plasma causes a relative higher Na^+ concentration in RBC. These changes in Na—K pump activity and extracellular fluid volume are possibly corresponding to the alteration of RBC Na^+ level in chronic pyelonephritis. A possible reason for the lower RBC Na^+ level in acute pyelonephritis is that in pyelonephritis PGE synthesis by the kidney is enhanced [23]. PGE probably inhibits tubular Na^+ and water reabsorption. The process acts in the direction of hyponatraemia, which is counteracted by the Na^+ leaving the RBCs. In healed pyelonephritis the RBC Na^+ level is normal.

Both elevated and depressed RBC Na^+ levels have been described in uraemic diseases [4, 11, 24, 25, 28]. The inhibitory effect of uraemic toxins on the function of the Na—K pump is assumed to be the cause of the elevation in RBC Na^+ level [11, 13]. This was disputed by Funder and Wieth [6], who considered the role of acid-base changes. Cumberbatch and Morgan [2] attributed the lower RBC Na^+ level to the decreased permeability of the membranes. As to the RBC electrolyte changes, Sigström [21] distinguished between slowly and rapidly progressing uraemia. Another possible explanation is that in patients with chronic uraemia a renal sodium loss and water retention exist simultaneously, causing a lower level of sodium in the extracellular fluid. This was counteracted by Na^+ efflux into plasma, and water

transport into RBC. In 4 of our 5 uraemic patients, the RBC Na^+ concentration was depressed, while in some cases higher plasma K^+ values were found.

In the patients with nephrotic syndrome, high RBC Na^+ and low plasma Na^+ levels were observed before or without steroid treatment. Similar values were measured after therapy had been stopped. The cause of the low plasma Na^+ level may have been the systemic diuretic treatment. There may be a number of reasons for the increase in the RBC Na^+ concentration. A role is presumably played in this by lasix treatment, which blocks the Na^+, K^+-ATPase activity. We believe that another important causal factor are the hypercholesterinaemia, hyperlipidaemia and hypertriglyceridaemia typical of the nephrotic syndrome. The pathological lipoprotein composition of plasma leads to a change in the lipid composition of the RBC membrane. Accordingly, there is a change in the fluidity of the membrane, which has a substantial influence on the Na^+, K^+-ATPase activity and the part-phenomena of Na^+ and K^+ transport [10,17]. Jackson and Morgan [10] induced an increase in the RBC Na^+ level by the administration of cholesterol in vitro.

In response to steroid treatment, RBC Na^+ concentration fell significantly as compared to the control level. The effects of steroid treatment on the Na^+, K^+-ATPase activity have already been described [9, 20]. The decrease in RBC Na^+ concentration as a result of steroid therapy has been discussed previously in some patients with various diseases [12]. We did not observe any change in the RBC K^+ concentration; the continuous administration of diuretics may have had a part in this. The plasma Na^+ level was found to be lower before or without steroid treatment, and to normalize when steroids were given. As a consequence of the plasma Na^+ increase and RBC Na^+ decrease in response to steroid treatment, it may be assumed that steroid treatment gives rise to the altered position of the extra-intracellular electrolyte equilibrium by enhancement of the activity of the RBC Na—K pump.

The effect of steroid treatment in decreasing the RBC Na^+ concentration may be connected with the beneficial therapeutic action of the steroids. In work on fibroblast cell cultures Mendoza [15] found that the uptake of Na^+ plays an important role in the regulation of cell proliferation. Since it has been demonstrated that virtually all cell membranes possess Na^+, K^+-ATPase activity [1, 19, 20], the steroids inhibit cell proliferation by decreasing the intracellular Na^+. Further studies are needed to investigate the possible role of natriuretic hormone [8, 22] in the altered membrane transport of sodium during the changes of extracellular fluid volume and in nephrotic patients with and without steroid therapy.

We have attempted to classify the nephrotic syndrome patients in accordance with the basic process. They were divided into steroid-sensitive and steroid-resistant cases. No essen-

tial difference was, however, found between the electrolyte values for the two groups, either before or during prednisolone treatment.

Total-body isotope examinations have revealed that there may be appreciable total-body electrolyte differences, even when the plasma electrolyte composition is normal [16]. Our results demonstrate that the Na^+ and K^+ compositions of the RBCs may vary in different ways than those of the plasma in certain renal diseases.

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Acta Paediatrica Hungarica 27 (4), pp. 289—293 (1986)

Prognostic factors in acute lymphoid leukaemia of childhood. I. Cytogenetic studies

Judit Szollár, T Révész, Éva Szakmáry, Gabriella Kardos, Ilona Zimonyi, D Schuler

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The results of chromosonal analysis of bone-marrow cells of 30 children with untreated acute lymphoid leukaemia are reported. On the basis of the modal chromosome number found in the cell clone showing the most frequent aberration, the patients could be classified into hypodiploid, pseudodiploid, hyperploid and normal groups. Pseudodiploidy predicted a poor prognosis while the survival rate of patients with normal or hyperploid chromosome counts was favourable.

Since the advent of banding techniques chromosome analysis has increasingly been used for characterisation of malignant cell proliferation. Among the various types of acute leukaemia it was the acute myeloid type (AML) in which correlation between morphological properties and cytogenetic characteristics has been described: e.g. 8; 21 translocation was fairly frequent in type M_2 15; 17 translocation in M_3 (promyelocytic) leukaemia [6]. The importance of cytogenetic studies was further reinforced by the fact that certain translocations involve cellular oncogens or protooncogens and they may have a causative role in activation of the proliferative process. For instance, in 8; 14 translocation characteristic of Burkitt's lymphoma, the protooncogen c-myc is transferred, in Philadelphia positive CML the oncogen c-abl is transferred from chromosome 9 to 22 [5, 12]. In acute lymphoid leukaemia (ALL) the initial attempts to find such correlations have been less successful. In this condition the caryogram is often indistinct, making exact localisation of breaks and translocations nearly impossible. In recent years improved techniques and increasing interest have resulted in a growing importance of cytogenetic studies in ALL [13].

Our attention was turned first to the chromosome analysis of bone-marrow cells of patients afflicted by AML. In recent years we have regularly investigated the spontaneously dividing cells of the bone-marrow specimen obtained for diagnostic purposes. Like other investigators, we have found certain correlations between chromosome aberrations and the type of leukaemia on the one hand and the outcome of the disease on the other hand. Here we offer some results of these observations.

Material and Methods

Patients. 30 patients affected by ALL, admitted to either of our departments during the years 1981—1983, participated in the study. Some of their data are summed up in Table I. The diagnosis was set up by bone-marrow aspiration or biopsy. Criteria of complete remission were as follows: complete clinical recovery, normal peripheral blood picture, at least moderately cell-rich bone-marrow, less than 5% lymphoblasts in the bone-marrow. All bone-marrow or extramedullary exacerbations of the leukaemic process were regarded as a relapse.

Table I

Some haematological parameters of ALL patients

Modal chromosome count in the cell clone with the most frequent chromosomal aberration	Number of patients	Mean age, years	Mean Hb level, mmol/m	Mean initial leucocyte count, G/l	FAB type	Cell surface marker
35—45	5	3	5.3	24	L1 = 2 L2 = 3	T = 2
46—pathological						
a. t(9; 22)	4	5	3.9	14	L1 = 1 L2 = 3	T = 1
b. t(2; 3)	1	4	5.6	8.8	L2	—
c. t(8; 14)	3	10	6.1	3.8	L3 = 3	B = 3
47—60	6	3	5.2	32	L1 = 4 L2 = 2	—
46—normal	11	5	5.3	7.2	L1 = 9 L2 = 2	cALLa + = 7

Cytogenetic methods. Spontaneous division of bone-marrow cells obtained for diagnostic purposes was examined either in direct preparations or after an incubation for some hours. The technique of Rowley and Potter [8] was applied. Karyotyping was carried out in microphotograms obtained after modified ASG-trypsin banding [10]. In evaluation we observed the recommendations of the International System for Human Cytogenetic Nomenclature [7]. The presence of a clonal aberration was stated whenever it occurred in at least two diploid, pseudodiploid or hyperdiploid cells or at least three hypoploid cell divisions.

In order to judge the clinical importance of the cytogenetic finding the aberrations were classified according to the modal chromosome number of the clone showing the most frequent chromosome aberration. 35—45 was classified as hypodiploid, group two contained the pseudodiploid lines carrying translocations, 47—60 was regarded as hyperdiploidy and the fourth group comprised cases with a normal 46 caryotype.

Results

In 30 cases, 65% of all ALL cases diagnosed during the three years, we were able to obtain appropriately banded metaphases of good quality, 10—30 in each patient. Comparison of the patients' clinical, haematological and cytogenetic data resulted in

TABLE II

Relationship between cytogenetic finding and clinical course in ALL

Modal chromosome count in the cell clone with the most frequent chromosomal aberration	Number of patients	Remission rate	Duration of remission, months	Survival time, months	Death rate	
35—45	5	3/5	13	15	3/5	
46—abnormal						
a. t(9; 22)	4	4/4	10	13	2/4	6/8
b. t(2; 3)	1	1/1	10	11	1/1	
c. t(8; 14)	3	1/3	1	1	3/3	
47—60	6	6/6	13	14	2/6	
46—normal	11	9/11	7	8	2/11	

some characteristic relationships (Table II). The mean age of patients exhibiting 8; 14 translocation was higher than that of the three other groups and all three patients' cells exhibited L_3 morphology. Surface marker studies revealed in all three patients cells of type B. The majority of cases with a normal 46 karyotype showed type L_1 and a positive reaction with cALLa antigen.

In evaluating the clinical course, usual survival curves could not be constructed because of the shortness of follow-up periods. The poorest remission rate and clinical course were seen in B-cell leukaemia exhibiting 8: 14 translocation. All three patients died soon after onset of the disease. The best clinical results were achieved in the group with 46 chromosomes and in the group characterised by hyperploidy (Table II). The overwhelming majority of these patients 15 among 17, attained remission and 13 of them are still in remission at the time of preparing this manuscript. Two of the four patients with 9: 22 translocation relapsed and died. The girl with 2: 3 translocation died of alveolar proteinosis during complete remission. Similarly, infection was the cause of death in one patient with hypoploidy being in remission at the time of death. Hyperploidy was encountered in 6 patients; 2 of them died, 4 are still in complete remission. The best remission rate was seen in this group.

DISCUSSION

Improved chromosome banding technique allowed us to perform cytogenetic analysis of bone-marrow blast cells in 30 children with recently diagnosed ALL admitted in the period 1981 to 1983. The 65% success rate approximated the international mean but lagged behind the results achieved by the great centers with extensive experience. Yunis et al. [15] obtained evaluable karyotypes by bone-marrow cultures and methotrexate banding in 90—95% of all cases examined. Their

results have not yet been confirmed by many investigators, but their achievement is still remarkable for the high success rate and resolving power. The possibility of identifying several thousands of bands foretells a new era of even more refined analysis of breaks.

Our patients were classified according to the modal chromosome count of the clone exhibiting the most frequent chromosome aberration. Five patients showed hypoploidy. According to the morphological FAB classification [2] two of these patients were classified as L_1 and three as L_3; in two patients surface marker tests revealed T-cell properties (Table I). In spite of the short follow-up, two patients have already relapsed and another patient died during complete remission.

There is no unanimous opinion about the chances of remission in the literature. Some authors regard hypoploidy as a sign of poor prognosis [4], in others' opinion these patients face an outcome more favourable than the average [3, 9, 13]. In our experience the rate of hypoploidy is influenced by the technique of preparation, therefore only clonal aberrations must be used for prognostic analysis.

Nearly all investigators agree in that the worst prognosis is encountered in the group with 46 plus translocation, i.e. pseudodiploidy [11, 13, 14]. Our findings confirm this opinion, as these patients posed the most severe clinical problems, although their age or blast mass alone would not have classified them into a group of poor prognosis. Their remission rate did not significantly differ from that of the other groups; still, these patients had the lowest survival rate. Especially cases with B-type cells and 8; 14 translocation showed a poor response to therapy: only one patient attained transitory remission and all three patients died soon after diagnosis (Table II). This type of translocation results in rapid, agressive proliferation and promotes the development of chemotherapy-resistent cell clones. Recent observations, however, suggest that therapy with new types of cytostatic drugs capable of destroying cells with a short cell cycle can be successful. Two Philadelphia positive (9: 22 translocation) cases out of four have died but the two survivors have been in remission for 10 and 19 months. The death of the child with 2: 3 translocation in complete remission could be ascribed to chance.

The best results were achieved in the groups with hyperploidy and normal karyotype. Fifteen out of seventeen patients belonging to either group attained full remission and 13 of them are still in remission. This is in agreement with other observations of investigators [9, 13].

Chromosome analysis of the bone-marrow at the time of diagnosis is not only helpful in characterising the leukaemic process but also presents important prognostic information. Our findings have corroborated previous observations in that certain types of chromosome aberration, especially pseudodiploidy predict relative re-

sistance to usual therapy. By help of Cox's multivariance analysis the participants of the Third International Workshop on Chromosomes in Leukaemia stated that the prognostic value of chromosomal aberrations is independent of the prognostic factors, like age, sex and initial leucocyte count [13].

Further advance in preparation techniques will increase the value of cytogenetic findings in the diagnosis and therapy planning of leukaemia. By the rapid progress in molecular genetics it can be hoped that certain DNA technologies will soon be used in the exact diagnosis of leukaemia; e.g. rearrangement of the immunoglobulin chain genes in B-cell lymphoma and leukaemia already proves the presence of monoclonal cell proliferation at an early stage [1].

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Prognostic factors in acute lymphoid leukaemia of childhood. II. Cell surface markers

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Monoclonal sera have been used to determine the surface phaenotype of leukaemic cells during the last three years. Bone-marrow specimens of 57 children with recently diagnosed acute lymphoid leukaemia were examined; four cases were classified as T-cell leukaemia, 2 cases as B-cell leukaemia, in 37 cases cALLa was positive and fourteen children were classified as O-cell type, based on the absence of markers. Analysis of symptom-free survival revealed a very poor prognosis in B-cell leukaemia; there was no significant difference between the remaining groups. Within the cALLa positive cases L_1 exhibited a markedly more favourable prognosis than L_2.

Recognition of the membrane properties of the two principal cell types, T and B has opened a new approach to refined classification of lymphoid leukaemia. A great deal of lectins, viral and bacterial antigens have been found to bind differently to various types of leukaemic cells; thereby they can be used for characterisation of cell populations [6]. Detection of the common acute lymphoid leukaemia antigen (cALLa) had a large impact on the classification of childhood leukaemia since this is the antigen of differentiation, indicating the degree of maturation at which differentiation of the leukaemic lymphocytes has been arrested or at which malignant proliferation has started [7, 9]. Studies on the cell surface properties not only entail better biological characterisation of the blast cells but also are of prognostic value. As early as in the late seventies were published the first observations indicating that T and B cells carry a poor prognosis and that cases with cells exhibiting cALLa positivity have a better chance for survival [3, 4, 19]. The initially used methods using E rosette formation, demonstration of the presence of immunoglobulins at the cell surface or heterologous anti-cALLa sera have been gradually replaced by monoclonal antigens. The great advantage of the latter is their high-grade specificity and availability in nearly unlimited quantities.

Techniques using monoclonal antigens have led to exact typing of leukaemic cells and thereby to modernization of diagnostics. In this paper we report on our preliminary results.

Material and Methods

Patients. Data of 57 patients diagnosed since 1981 were examined (Table I). There were 35 boys and 22 girls, the mean of the groups lay between 3 and 6 years. According to the surface properties the patients were classified into four groups: the lymphoblasts of the bone-marrow or peripheral blood reacted either with T-cell antisera or B-cell antisera or antisera against the common ALL antigen or with none of these sera; the latter cells were termed O-cells.

The initial clinical and laboratory findings of the four groups were compared by histograms constructed as described earlier. There were no significant intergroup differences in leukocyte count and rate of hepatosplenomegaly, phenomena reflecting initial blast mass. Mediastinal lymph-node enlargement was encountered more frequently in cases affected by T-cells than in the remaining groups.

Therapy was uniform, corresponding to the recommendations of the National Working Group for Therapy of Childhood Leukaemia. Morphological classification was carried out according to the FAB principles. The patients' remission time was compared by the log-rank test [16].

Cell surface marker tests. Blast cells were obtained from the bone-marrow aspirate

Table I

Initial clinical data of ALL patients

Number of cases	cALLa positive 37	O-cell 14	T-cell 4	B-cell 2	P
Initial leukocyte count, higher than 30/lower than 30 G/l	29/8	10/4	3/1	1/1	0.8
Hepatomegaly	4	3	0	1	0.2
Splenomegaly	10	4	1	1	0.9
Mediastinal tumour	1	2	2	0	0.01
Hb over 6.2/below 6.2 mmol/l	31/6	13/1	4/0	2/0	0.6
Median age, years	4	4	3	6	
Boys/girls	20/17	9/5	4/0	2/0	

Table II

Monoclonal antibody (MoAB) panel used for phenotyping leukaemic cells

MoAB	Specificity	Reference
J5	common ALL antigen	21
VIL—A1	common ALL antigen	11
VID—1	Type Ia	15
VIB—C5	B-cells and precursors, cALL cells, neuroblastoma cells	12
VIB—E3		
VIP—1	OKT—9 equivalent transferrine receptor	12
VIP—2b	OKT—10 equivalent activated T-cells, blast cells, etc.	12
Leu—1	pan-T-cells	14
VT—12		
Leu—2a	T-suppressor cells	14
VIT—8		
Leu—3a	T-helper cells	14
VIT—4		

TABLE III

MoAB labelling pattern of the principal ALL phenotypes

MoEA	Type of leukaemia			
	cALL	"O"-cell	T-cell	B-cell
J5	+	−	−	±
VIL—A1				
VIB—C5	+	−	−	+
VIB—E3				
HLA—Dr	+	±	±	+
VID—1				
VIP—1	−	−	−	−
VIP—2b	+	±	±	−
VIT—6	−	−	±	−
VIT—12	−	−	+	−
Leu—1				
Leu—3a	+	−	±	−
VIT—4				
Leu—2a	−	−	±	−
VIT—8				
TdT	+	±	±	−
SMIg	−	−	−	+
ER	−	−	+	−

(in three cases from peripheral blood) obtained at the first examination, the blasts were separated by Ficoll gradient centrifugation. The serum panel used for determination is shown in Table II. The cells were treated first with the corresponding monoclonal antigen, this was followed by the $Fab_{(2)}$ fragment of fluorescein-labelled anti-mouse Ig serum of rabbits. The test was evaluated by fluorescent microscopy, 200 cells were examined in each specimen.

Evaluation, phenotype groups. T-cell leukaemia was diagnosed if more than 50% of the blast cells were positive for E-rosette formation and could be labelled with monoclonal anti-T sera. For B-cells, the criteria were demonstration of surface immunoglobulin (Sm Ig) or labelling with anti-B-cell sera. In the cALLa positive group the cells were positive for Ia(HLA-DR) and dTt in addition to positivity with the specific anti-cALLa serum. Cells showing no binding with cALLa antiserum were termed O-cells. In some cases there was only Ia labelling, in these patients acute myeloid leukaemia was excluded by cytochemistry and absence of positive reactions with antimyeloid serum.

RESULTS

Out of the 57 patients 37 had "simple" cALLa positive leukaemia, there were four cases with T-cell, 2 cases with B-cell and 14 with O-cell leukaemia (Table I). The groups did not differ in respect of initial peripheral leukocyte count, the rate of hepatosplenomegaly was nearly identical in the four groups. Mediastinal tumour was present in 2 out of 4 patients with T-cell leukaemia; this was in spite of the small number of cases, significantly higher than in the three other groups ($p = 0.01$). There was no difference in initial haemoglobin levels, age and sex-ratio among the groups.

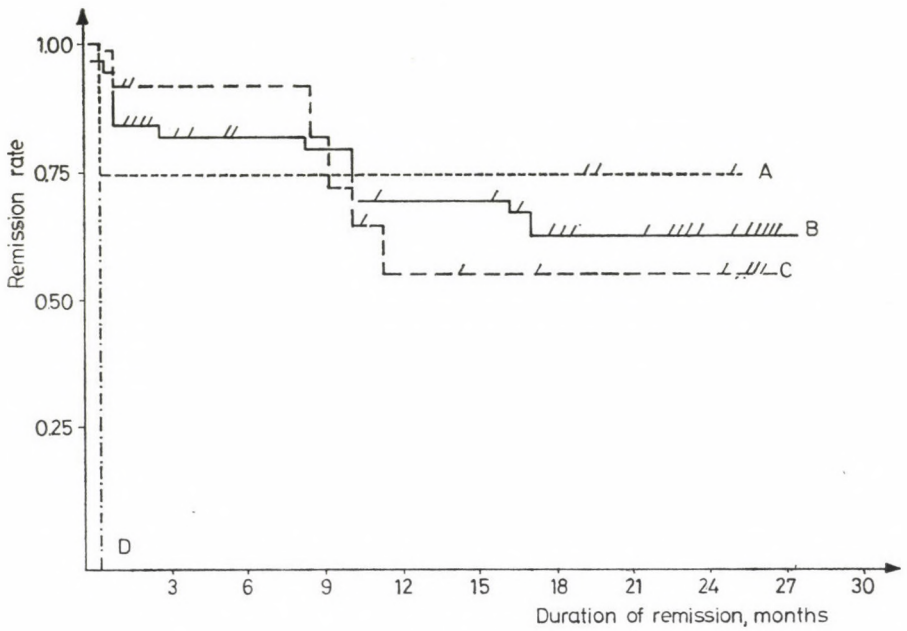
1.00
0.75
0.50
0.25
Remission rate
A
B
C
D
3
6
9
12
15
18
21
24
27
30
Duration of remission, months

Fig. 1.

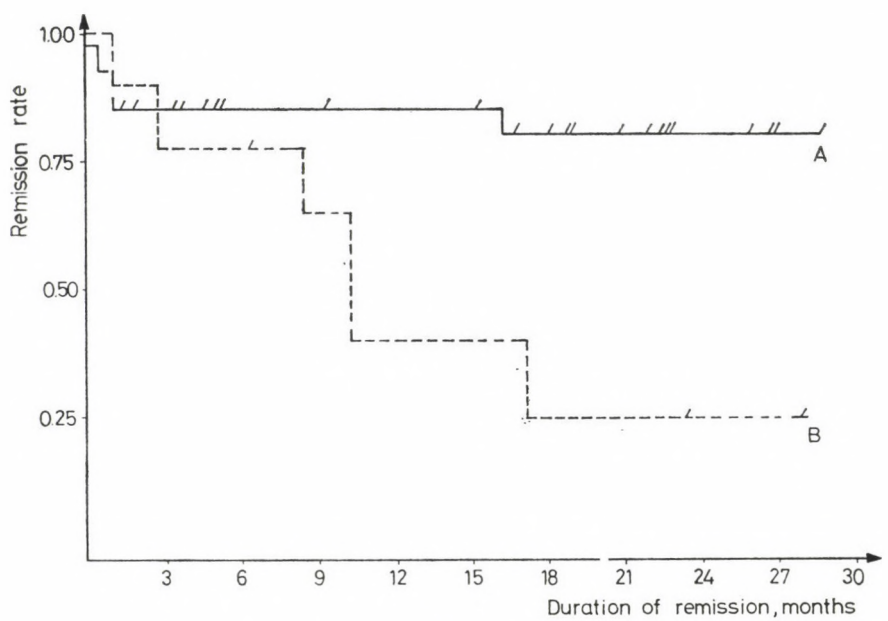
1.00
0.75
0.50
0.25
Remission rate
A
B
3
6
9
12
15
18
21
24
27
30
Duration of remission, months

Fig. 2.

Analysis of the symptom-free survival rate showed that continuous remission could be achieved in three out of four patients afflicted by T-cell leukaemia while both children with B-cell leukaemia died within a few months after onset of the disease. The remission curves of the cALLa positive and O-cell group showed an intermediate position and did not attain 50% at the time of compilation of the manuscript (Figure 1). The significant difference between the two groups was due exclusively to the loss of the two B-cell patients. Within the cALLa positive group the effect of cell morphology on survival rate was also examined; a much better symptom-free survival rate was encountered with type L_1 than with L_2 (Figure 2).

Discussion

We have used monoclonal antibodies for diagnostic purposes since 1981. At the time of diagnosis, 37 patients out of 57 had "simple" cALLa positive leukaemia, i.e. this developmental antigen was present on the surface of the blast cells. Their proportion within the whole material was lower than 70%, the mean percentage published in the literature [8, 9, 13]. At the same time, the proportion of O-cell leukaemia cases (14 per 57) in our material was higher than expected on the basis of the published 10—15%. The discrepancy may be ascribed to the small number of patients. It may also be suspected that in the first cases poor binding of the applied antibodies resulted in false-negative results with cALLa. Examination of a large series will help us to clarify the real situation.

The prognostic value of surface marker tests was examined by their effect on the symptom-free survival rate (Figure 1). Quite surprisingly, the highest curve was found in T-cell leukaemia, where only one out of four children relapsed during follow-up. There is some controversy in the literature about the predicting role of the T-cell property. While some authors judge it unequivocally to predict a poor prognosis [4, 19], others and ourselves think that after correction for initial leukocyte counts and introduction of appropriate intensive treatment its ominous character disappears [8, 17]. Another unexpected finding was the comparatively good remission curve of cases with O-cell leukaemia, attaining that of cALLa positive cases.

False-negative cases, present spuriously in the O-cell group, might be one explanation. Both patients with B-cell leukaemia were lost soon after onset, within two and three months; the leukaemic process advanced inexorably. Now we already realise that our treatment, corresponding to methods recommended internationally in those days, was not capable of stopping this particularly rapid and aggressive type of leukaemia. It seems that new protocols, based on entirely different principles, will bring about dramatic improvement in the prognosis of this type of hitherto extremely sombre outlook.

Since the advent of marker tests several authors have questioned the prognostic value of morphological features. We have therefore attempted to find out whether a difference could be found between the remission curves of L_1 and L_2 patients within the cALLa positive group. Patients with L_1-leukaemia exhibited a significantly better survival rate. This has once again confirmed our earlier statement on the importance of morphological types [10]. We shall settle the problem by multivariance analysis of all imaginable prognostic factors in a large series.

In addition to their diagnostic value, monoclonal antibodies play an increasing role in the treatment of leukaemia. At present they are principally used in the preparative actions before autologous and allogeneic bone-marrow transplantation. While in autologous transplantation, removal of residual leukaemic cells is aimed at, in the case of allogeneic transplantation elimination of the host's T-cells is desirable in order to prevent graft versus host reactions. In fact, there have been attempts to utilise monoclonal antibodies in vivo. Unfortunately, the initial results are not too promising, since their effect is weak and transient. One of the reasons of this failure is the fact that these antibodies have no or only weak activating effect on the human complement system, thereby the desired blast-killing is not achieved. Another problem is antigen modulation, i.e. the antigen disappears from or is modulated on, the surface of surviving cells treated with the antibody, and this prevents further binding of antibodies to the cell surface. The use of new-type, non-modulating antibodies or their coupling with toxins may open new prospects to leukaemia therapy of this kind.

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Nutritional evaluation of adolescents: usefulness of anthropometric indicators in the diagnosis of obesity

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The results of body measurements and several anthropometric indices in the detection of obesity of 351 adolescents aged 15 to 19 years attending the preuniversities in Havana are presented. Body weight and height values were between the 50th and 75th percentiles of the Cuban national norms while those of the triceps and subscapular skinfolds were between the 90th and 97th percentiles. The high correlation between the two relative body weight indices and the sum of the three skinfolds evidenced the value of these indices in field studies. The study showed that determining the incidence of obesity remained a problem as it depended on the anthropometric indicators and the cutoff points that were used and which resulted in values ranging from 9.6% to 53.7%.

Adolescence is a stage of growth and development accompanied by complex morphological and physiological changes in which nutrition plays an important role. Physical activity also has a profound influence on body dimensions and is an important factor in the accumulation of excess adipose tissue when energy output is less than energy input. The comparison of body dimensions using national references in order to assess the nutritional status of a given population, is more appropriate than when compared to international references as the former reflects the interaction between genetic and environmental factors.

The measurement of fat folds, especially the triceps skinfold, has been used repeatedly in nutritional studies; values considered "normal" suggest an adequate energy intake [3, 9]. Other authors [8, 11], however, reported that the subscapular skinfold was a better indicator of nutritional status. The use of the triceps skinfold is not adequate because it is supposedly under genetic control while the subscapular skinfold is more responsive to nutritional factors and consistently shows a higher correlation with weight [7]. The need to relate body measurements or an index of relative weight to medical problems has been the purpose of many investigations [2, 18]. Various indices of relative body weight have been used in the past years among which the body mass index proposed by Quetelet in 1832 continues to be used in epidemiological studies [14, 15, 16]. Comparison of actual weight with that expected for age and height,

using national or international references, has also been used in these studies. In this paper body measurements and the use of several anthropometric indices in the detection of obesity in a group of adolescent boys and girls are presented.

Materials and Methods

From two preuniversities in the City of Havana 438 students were randomly selected. Of these, 132 boys and 219 girls aged 15 to 19 years volunteered for the study. Weight, height, triceps, subscapular and suprailiac skinfolds were measured following the techniques described by the International Biological Program [25]. The Harpenden caliper was used and the measurements recorded to the nearest 0.1 mm on the left side of the body. The Cuban national norms [13] were used as reference for height, weight and skinfolds, and to calculate an index of relative weight by comparing actual weight with that expected for age and height. The percentage of body weight (%W) was calculated as follows [22].

$$\frac{\text{Actual weight (Kg) of subject at present age}}{\text{Actual length (cm) of subject at present age}} = A$$

$$\frac{\text{50th P expected weight (Kg) for corresponding age}}{\text{50th P expected length (cm) for corresponding age}} = B$$

$A/B \times 100$ = Index for surveyed subject.

A percentage equal or greater than 120 indicates obesity, 110—119 overweight, 90—109 normal weight and less than 90 underweight.

The body mass index ($W: H^2$) was also used as another index of relative weight. The 97th percentile of the national norms for skinfolds was defined as obesity, as well as the value proposed by Seltzer and Mayer [21] for the triceps skinfold.

Log transformation of weight and skinfolds were used for comparison and simple linear correlations. Student's *t*-test was used for comparison between sexes and the chi square test to determine significant difference for the indices used to define obesity. The level of statistical significance was chosen at p of less than 0.05.

Results

Body weight, height, and skinfold measurements in boys and girls are presented in Tables I and II. As expected the boys were significantly taller and heavier than the girls ($P < 0.05$) and in both sexes these values were between the 50th and 75th percentiles of the Cuban national norms, whereas the triceps and subscapular skinfolds were between the 90th and 97th percentiles. The subscapular skinfold showed a tendency to increase with age in both sexes.

Figure 1 presents the distribution of heights, weights and three skinfolds compared to the antional norms with a greater skewness to the right of all the body measurements, particularly the suprailiac skinfold.

The linear coefficient correlations between height, weight and the sum of the three skinfolds with the relative weight indices are shown in Table III. The body mass index showed a low correlation with height while the

TABLE I

Height and weight (mean ± S.D.)

	Boys				Girls			
Age	No.	Height (cm)	Weight[1]	(Kg)	No.	Height (cm)	Weight[1]	(Kg)
15.0—15.9	37	168.6* ±7.1	1.748* ±0.062	(56.0)	53	159.5 ±6.4	1.716 ±0.078	(52.0)
16.0—16.9	46	170.1* ±6.7	1.774* ±0.067	(59.5)	93	160.5 ±6.1	1.737 ±0.060	(54.6)
17.0—17.9	36	172.5* ±5.7	1.793* ±0.053	(62.1)	48	158.0 ±5.9	1.727 ±0.062	(53.4)
18.0—19.9	13	172.4* ±6.7	1.782* ±0.052	(60.2)	25	160.5 ±5.3	1.730 ±0.042	(53.7)

[1] Log_{10} transformed data
The numbers in parentheses indicate the antilogarithm of weight.
* Values significantly greater than the mean for girls $p < 0.05$.

TABLE II

Skinfold thicknesses (mean ± S.D.)

Age	No.	Triceps[1]	Subscapular[1]	Suprailiac[1]	Total[1] Skinfold
15.0—15.9	37	0.993 ±0.152 (9.8)	0.983 ±0.231 (9.6)	1.125 ±0.218 (13.3)	1.575 ±0.159 (37.6)
16.0—16.9	46	0.996 ±0.162 (9.9)	0.992 ±0.144 (9.8)	1.157 ±0.228 (14.3)	1.605 ±0.161 (40.3)
17.0—17.9	36	0.988 ±0.146 (9.7)	1.026 ±0.112 (10.5)	1.206 ±0.201 (16.1)	1.626 ±0.138 (42.2)
18.0—19.9	13	0.976 ±0.145 (9.5)	1.058 ±0.141 (11.4)	1.102 ±0.217 (12.6)	1.589 ±0.145 (38.8)

[1] Log_{10} transformed data
The numbers in parentheses indicate the antilogarithm of skinfold.

Age	No.	Triceps[1]	Subscapular[1]	Suprailiac[1]	Total[1] Skinfold
15.0—15.9	53	1.259 ±0.158 (18.1)	1.168 ±0.157 (14.7)	1.419 ±0.148 (26.2)	1.843 ±0.129 (69.7)
16.0—16.9	93	1.291 ±0.131 (19.5)	1.233 ±0.126 (17.1)	1.439 ±0.172 (27.5)	1.877 ±0.138 (75.4)
17.0—17.9	48	1.267 ±0.092 (18.5)	1.205 ±0.135 (16.0)	1.416 ±0.161 (26.1)	1.859 ±0.109 (72.3)
18.0—19.9	25	1.273 ±0.915 (18.7)	1.212 ±0.011 (16.3)	1.432 ±0.151 (27.0)	1.863 ±0.105 (72.91)

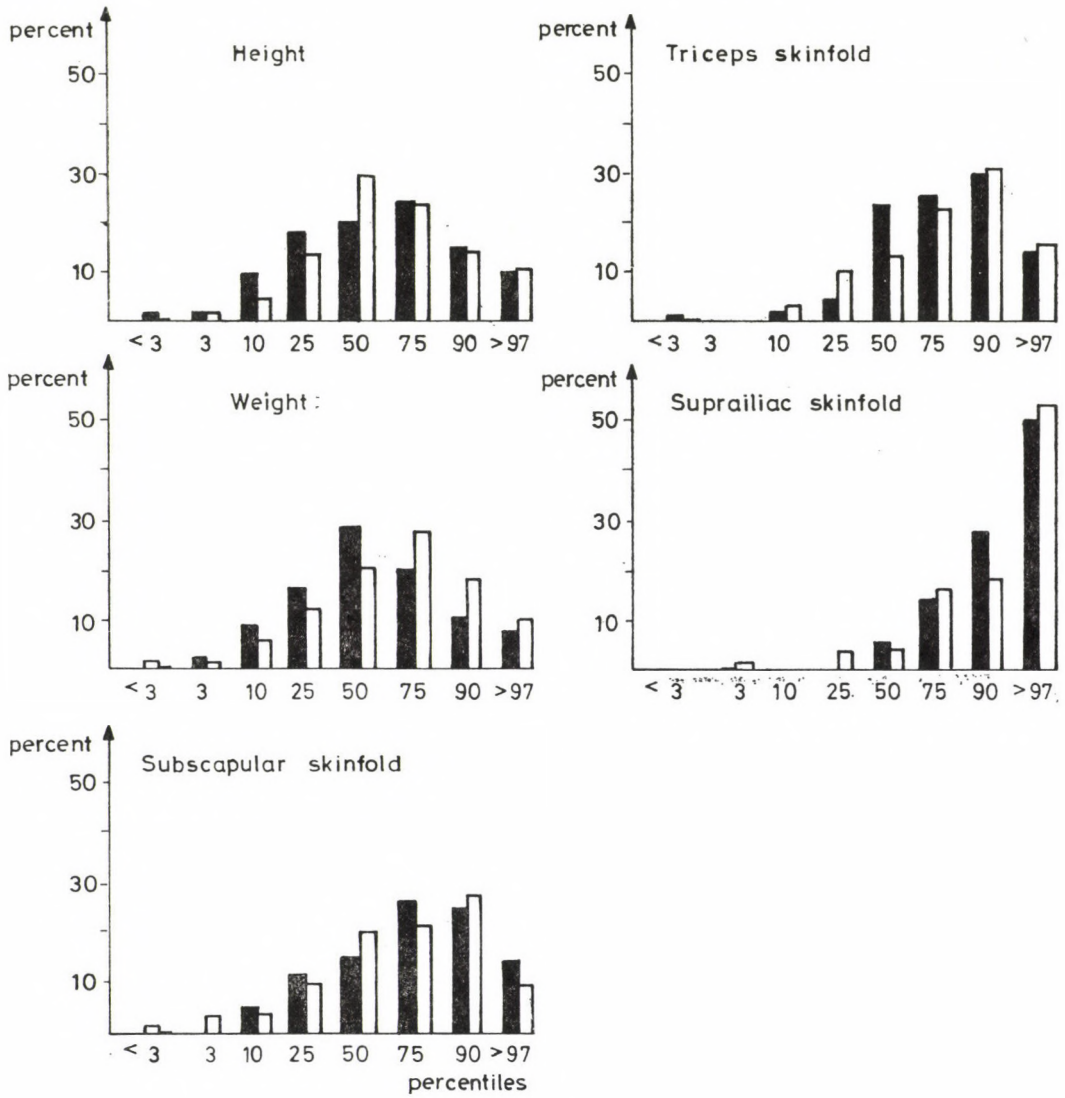

FIG. 1.

percentage of body weight was significantly high ($p < 0.05$), which indicates a degree of dependency with respect to height. The rest of the correlation coefficients was high ($p < < 0.05$).

Table IV provides the linear coefficient correlations describing the relationship between weight, three skinfold thicknesses, and the sum of two and three skinfolds. The correlation coefficients between weight and subscapular skinfolds were the highest while the sum of two and three skin-

TABLE III

Correlation coefficients for height, weight(1) and total skinfolds(1) vs relative weight indices

	%W		W : H²	
	Boys	Girls	Boys	Girls
Height	0.29*	0.29*	0.05	0.04
Weight	0.85*	0.85*	0.79*	0.85*
Summed fat folds	0.67*	0.70*	0.68*	0.73*

(1) Log_{10} transformed data
* $p < 0.05$

TABLE IV

Correlation coefficients between weight (a) and skinfolds (a)

Skinfold	Boys	Girls
Triceps	0.47	0.61
Subscapular	0.64	0.65
Suprailiac	0.58	0.54
Triceps + subscapular	0.59	0.70
Triceps + subscapular + suprailiac	0.63	0.54

(a) Log_{10} transformed data

TABLE V

Comparison of incidence of obesity using several indices

	Boys		Girls	
	No.	per cent	No.	per cent
$\%\overline{W} \geq 120$	40	30.3 a	45	20.5 a
Suprailiac skinfold (1)	67	50.7 b	118	53.7 b
Triceps skinfold (1)	22	16.7 c	37	16.9 c
Triceps skinfold (2)	19	14.4 c	36	16.5 c
Subscapular skinfold (1)	12	9.6 d	32	14.5 c

Different letters differ significantly $p < 0.05$
(1) 97th percentile based on Cuban national norms.
(2) Based on Seltzer and Mayer (21)

folds did not contribute to a higher correlation with weight than just the subscapular skinfold.

Table V presents the incidence of overweight using different criteria. The percentages of relative weight and suprailiac skinfold evidenced the highest incidence of obesity and were statistically different from each other and from the other skinfold percentages. The incidence of obesity between boys and girls derived from the various indices was not significantly different.

Discussion

Body weight and height values were between the 50th and 75th percentiles of the Cuban national norms and similar to those reported in another school in the City of Havana [23]. The three skinfold's values were also in the higher percentiles. The national norm's include urban and rural children from all the provinces some of which are less developed. This probably explains the higher values, although the percentage of boys and girls with skinfold thicknesses, especially the suprailiac in the 97th percentile, suggests an excess amount of adipose tissue. The triceps and subscapular values are slightly higher than those reported by Jenicek and Demerjian in Franco-canadian adolescents [10] and by the Health Examination Survey [11]. The triceps skinfolds were similar to those reported by Clarke et al. [1] in high-school children from Vermont. The technique involved when measuring the suprailiac skinfold is difficult [11] and should only be undertaken in specific studies. Nevertheless we were interested in this skinfold because of the tendency to deposit excess adipose tissue in this area. Two decades ago, Laska-Mierzejwska [17] reported in a study done in the schools of the City of Havana that the abdominal skinfold was large in both sexes regardless of race. We are faced with the problem of deciding which skinfold or combination of skinfolds would best assess the nutritional status of various population groups.

Garn et al. [7] found that the subscapular skinfold was more adequate in this assessment because it uniformly showed higher correlations with weight in Michigan children and adolescents aged 3 to 19 years. The sum of the subscapular plus triceps skinfold did not improve the predictive efficiency. In our study the results were similar not only with the sum of two folds but with the sum of three folds (triceps, subscapular plus suprailiac) as well. The problem of which anthropometric indicator should be used in the assessment of obesity became apparent (Table V). The incidence of obesity as determined by percentage of relative weight and the three skinfolds ranged from 9.6%—53.7%. The highest percentages corresponded to the suprailiac skinfold, which were significantly different from the other indices. The use of one skinfold by itself, does not take into account the individual differences in fat distribution as well as those due to age, sex and ethnic factors.

The exceptionally large accumulation of adipose tissue in the suprailiac area warrants more further studies of the possible factors that contribute to it. The other question is whether or not these adolescents are obese. Evidently the value of a single skinfold is not sufficient for the diagnosis [6]. Probably more than one skinfold should be taken which would be more representative of the fat distribution at different ages, and which would also take into account sex and ethnic factors. Jolliffe [12] advocated the sum of three skinfold thicknesses

giving values of 60 mm and over in men, and 75 mm and over in women as indicative of obesity. It seemed that more precision could be obtained if a selection of skinfolds were made on the basis of correlations not with weight but with total body fat, as determined by ^{40}K or other techniques [8, 24]. Actually this is what is done when regression equations are derived from anthropometry. Roche [20] in a review concerning the measurement of skinfold thickness and the difficulties posed by the selection of sites, the validity of the data obtained, and its transformation to estimated areas of adipose tissues, concluded that there was still much to be done, including the answer as to the normal level of fatness in terms of function and survival.

The results of our study have confirmed these difficulties. The suprailiac fold seemed to overestimate the incidence of obesity, whereas the triceps and subscapular skinfolds may have underestimated it. The results of the linear correlations between the relative body weight indices (percentage of weight for height and body mass index) and the sum of the three skinfolds were high, 0.67 and 0.70 respectively, which indicates their usefulness as a measure of obesity. Womersley and Durnin [25] especially recommended the W: H^2 which in our study showed a high correlation with weight and the sum of the three skinfold thicknesses, but a low correlation with height; this was not the case for the percentage of weight for height index which showed a positive correlation with height. Still, the use of the W : H^2 index as an indicator to be applied in population studies of different ethnic origins assumes that the relation between weight and height remains invariable. This probably is not completely true as these proportions vary with age, sex and with ethnic origin. The latter has great significance in the American populations due to the high percentage of racial mixture. Jordan [13] reported on a greater length of the lower extremities and a shorter length of the trunk in the Cuban population as compared to the English population of the same height. Also, the biacromial and bitrochanteric diameters are smaller in the Cuban than in the English population. This would mean that the difference in weight for each body segment would entail a change in the proportion between height and total body weight which probably should be taken into account when values for the diagnosis of obesity are to be established with different populations. The above facts point to the necessity of values for the body mass index which are specific for specific populations if epidemiological studies of obesity are to be performed with this index.

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Mast cell degranulation after a single dose of gliadin in the jejunum of patients with coeliac disease

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The exact pathomechanism of jejunal damage caused by gliadin in coeliac disease has not been clarified. 0.3 g of gluten per kg bodyweight was administered to 14 children affected by coeliac disease, being on gluten-free diet, five hours before jejunal biopsy. There was no change in the number of intraepithelial lymphocytes and eosinophils while cellular infiltration of the lamina propria exhibited a marked increase and the number of mast-cells per tissue unit significantly decreased as compared with controls. It is concluded that mast-cells may play an important role in the mechanism of gluten induced jejunal damage.

The central event in coeliac disease is a jejunal damage elicited by gliadin in sensitive individuals. While on gliadin containing diet they develop villous atrophy, epithelial flattening, increased synthesis of epithelial cells and increased cellular infiltration of the mucosa. Since the exact pathomechanism of gliadin sensitive enteropathy is not clear, the large number of hypotheses is not surprising. Recently, the eventual role of mast-cells has been put forward [6].

Mast cells, originating from the mesenchyma, play an outstanding part in tissue growth and regeneration and they participate in inflammatory and immunological processes as well. They are found in great numbers in organs exposed to the external environment. They contain granules which markedly influence adjacent tissues if released from the cell. A decrease of the mast-cell number has been observed in the jejunum of patients affected by coeliac disease if continuously exposed to gliadin [4, 5], and increased tissue levels of histamine have been observed under such circumstances [2].

This has prompted us to investigate the effect of a single dose of gliadin on the number of jejunal mast-cells in children with coeliac disease keeping a gliadin free diet and undergoing routine rebiopsy.

Material and Methods

Study group. This comprised 14 children ranging in age from 7 to 15 (mean, 10) years in whom persistence of gliadin sensitivity in clinical remission induced by gliadin-free diet had been tested by reintroduction of gluten, without performing a second biopsy (this was the case before the publication of ESPGAN criteria [7]) and all patients had shown exacerbation of coeliac disease symptoms after the test. The second biopsy prescribed by the ESPGAN

principles was now performed in order to test the effect of gliadin-free diet. The children had been keeping the diet for 2 to 6 years.

Control group with coeliac disease. 13 patients with confirmed coeliac disease were selected on the basis of histological findings observed at their second jejunal biopsy, comparable with the findings of the study group in respect of main parameters, villus/crypt height ratio and intraepithelial lymphocyte count. Their mean age was 5.4 years, with a range from 3 to 12 years.

Healthy control group. 11 children admitted for evaluation of stunted growth in whom jejunal biopsy demonstrated normal findings. Their mean age was 6.6 years ranging from 1 to 13 years.

The children of the study groups received 0.3 g/kg bodyweight gluten (Aleuronat, Blattmann Co). Jejunal biopsy was performed by a paediatric Watson capsule 5 hours later. The specimen was taken from the duodeno-jejunal junction, fixed in formol and embedded in paraffin. 5μm sections were stained with methylene blue and examined by microscopy, using an ocular lens provided by a rectangular grid, at 640-fold magnification. Mast-cells and eosinophils were counted in 1 mm^2 areas. Cells sited in both the villi and between the crypts were counted. The number of intraepithelial lymphocytes per 100 epithelial cells was calculated, and the ratio of villus and crypt height was also measured.

Results

Figure 1 shows the number of intraepithelial lymphocytes. There was no difference in this respect between the study group and the control group of patients affected by coeliac disease. Similarly, there was no difference in the villus/crypt height ratio (mean

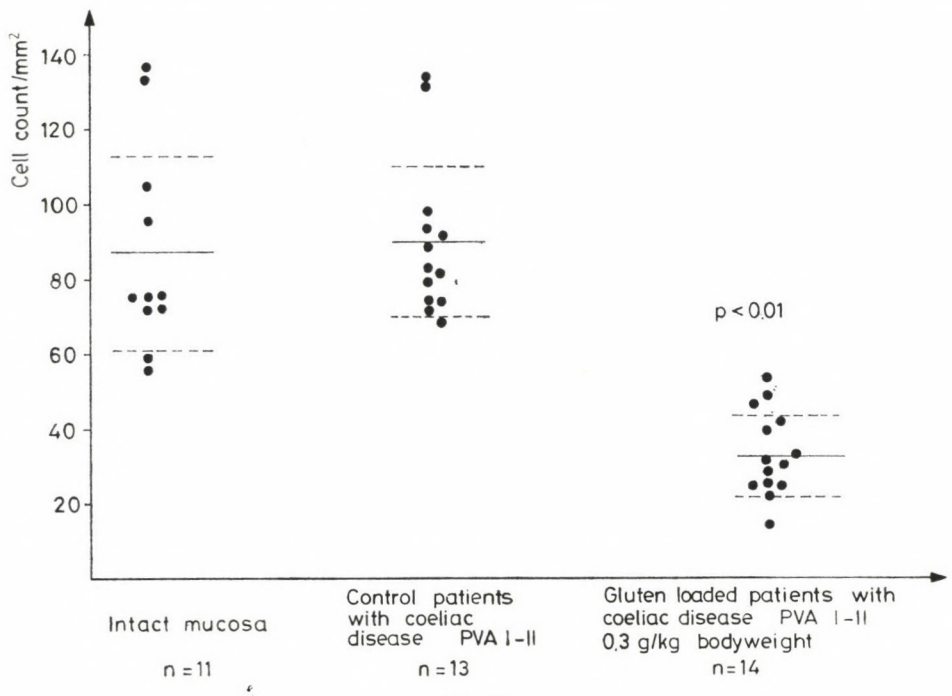

Fig. 1.

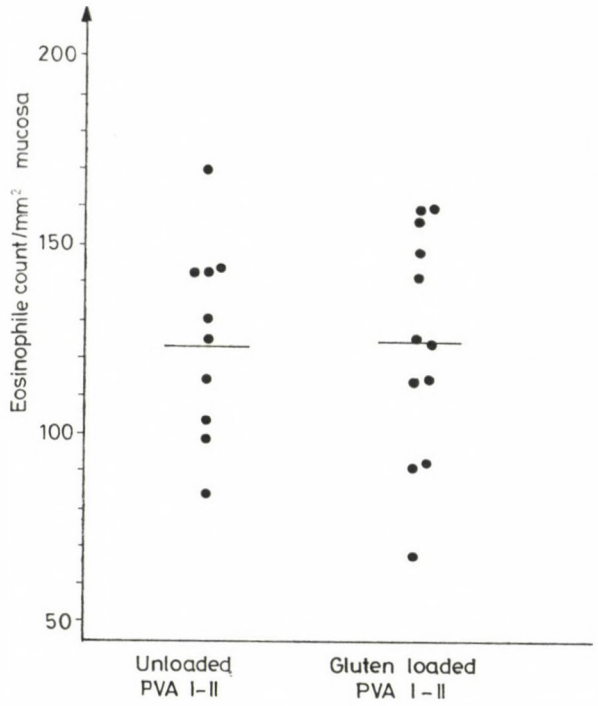
200
150
100
50
Eosinophile count/mm² mucosa
Unloaded
PVA I-II
Gluten loaded
PVA I-II

FIG. 2.

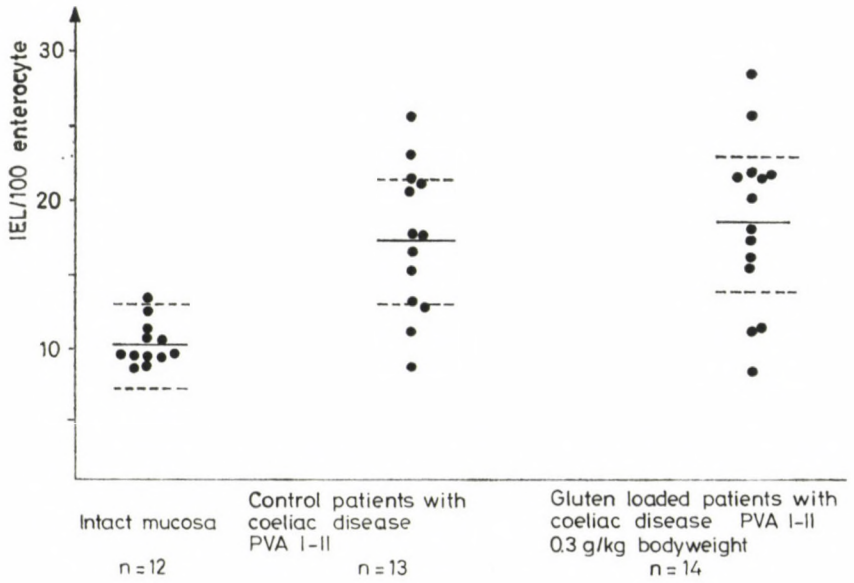
30
20
10
IEL/100 enterocyte
Intact mucosa
n = 12
Control patients with coeliac disease PVA I-II
n = 13
Gluten loaded patients with coeliac disease PVA I-II 0.3 g/kg bodyweight
n = 14

FIG. 3.

ratio = 2.45 in coeliac controls and 2.6 in the study group).

There was, however, a difference of cell count per 1 mm² lamina propria. In the coeliac control group this value was 1.23 times while in the study group 1.92 times higher than in the healthy controls. This means that in sensitive patients the single gliadin load provoked an increase in cellular infiltration of the lamina propria (Fig. 2).

In addition, significant changes in the mast-cell counts were encountered (Fig. 3). This figure was identical in healthy and unloaded children with coeliac disease (mean, 89.7 ± 20 SD and 87 ± 26 SD, respectively) while 5 hours after gliadin loading markedly lower values were obtained (mean, 32.4 ± 10.5 SD); this represents a significant difference ($p < 0.01$).

The findings are summarized in Table I.

TABLE I

All values are expressed as means ± SD

	n	IEL per cent	Height ratio villus/crypt	Cell count per mm²	Eosinophil count/mm²	Mast-cell per mm²
Healthy controls	11	10.4 ± 3.2	3.4	1800	131 ± 27.6	89.7 ± 20
Control patients with coeliac disease	13	17.2 ± 4	2.45	1990 ± 499	125 ± 24.4	87 ± 26
Gluten loaded patients with coeliac disease	14	18.5 ± 4.3	2.6	3460 ± 843	124.6 ± 29	32.4 ± 10.5
p. between coeliac loaded patients and controls		N.S.	N.S.	$p < 0.01$	N.S.	$p < 0.01$

IEL: intraepithelial lymphocyte
All values are expressed as means ± SD

DISCUSSION

Serial biopsies performed in patients with gluten sensitive enteropathy after a single load of gliadin showed pronounced changes in the jejunal mucosa within a few hours [1, 3]. These observations have made us to perform a single biopsy five hours after the ingestion of gluten.

The dose and nature of the gliadin fraction used in loading is not indifferent. Anand et al. [1] applied a large dose (40 g) of fraction B while Marsh et al. [6] used a relatively small dose of digested gluten (100—1500 mg). In this study gluten, containing all gliadin fractions, was used in a dose of 0.3 g/kg body weight, recommended for combined gliadin-xylose loading tests [9] and the degree of pathological changes is probably influenced by the dose of gliadin. Dose-dependence was encountered in the intraepithelial lymphocyte count 12—48 hours after loading [6], but in this study no similar change was demonstrated 5 hours after ingestion of the provoking agent.

A characteristic change induced by

gluten loading is an increased cellular infiltration of the lamina propria [1, 3]. In this study the increase was attributable to an increase in the number of lymphocytes and polymorphonuclear granulocytes but not of eosinophils. Kósnai et al. [5] observed a significant increase in eosinophil count in untreated children with gluten sensitive enteropathy; this has been confirmed in the present study. In order to determine the exact time of onset of an increase in tissue eosinophilia, serial biopsies would have been necessary but we did not perform biopsies before loading for ethical reasons. Similarly, serial biopsy after loading does not seem justified in children. Moreover, serial biopsies performed by the blind method may furnish misleading results since it may happen that a subsequent specimen is taken from the zone of reaction of the preceding biopsy. For these reasons we preferred to compare the findings of the study group with those obtained in unloaded children with treated coeliac disease exhibiting comparable histology.

Marsh [6] suggests that oedema of the lamina propria, damaged basal membrane, epithelial detachment and necrosis ("intraepithelial bleb"), fibrinogen deposition due to increased vascular permeability and erythrocyte aggregation all point to the role of mast-cells in the pathomechanism of gliadin induced jejunal damage. Dollberg et al [4] showed mast-cell degranulation following gluten loading while Challacombe and Dawkins [2] found increased tissue histamine levels. It is well known that the bulk of histamine in the intestinal wall is present in the mast-cells [8]. Kósnai et al [5] have shown that the number of mast-cells is significantly lower in untreated patients than in children with treated coeliac disease. Strobel et al [11], however, obtained results contradicting those of Dollberg et al. [4] and Kósnai et al [5]: they demonstrated increased mast-cell counts in the jejunum of untreated patients with gluten sensitive enteropathy. Strobel et al [11] explained this difference by the fixation technique being different in these studies [10] and in addition there are more immature mast-cells with a low proteoglycan content if the condition is untreated and fixation and staining properties of these young mast-cells may be quite different. The high histamine level in untreated patients is more compatible with an increased number of mast-cells. In our opinion, there may be an additional factor: mast-cells exhibit a refractory period after degranulation and during this period the granules are resistant to staining. It is not beyond imagination that some authors happened to fix their specimens during the refractory period while others did that thereafter.

In this study, fixation of the specimen was carried out 5 hours after loading, i.e. certainly during the refractory phase following degranulation. The role of mast-cells has been confirmed once again since their number — more correctly, the number of mast-cells susceptible to staining — appeared to be markedly reduced

after the single dose of gliadin. In our study we applied a traditional fixation and staining method subjecting the specimens to staining at the same time; materials from all groups were fixed by the same method. At the moment we cannot decide whether degranulation is performed via IgE mediation or by non-immune reactions. This would need additional studies.

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Thyroid hormones and thyroglobulin autoantibodies in insulin dependent diabetes mellitus

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Serum T_4, FT_4, T_3, and TSH were measured in a group of children with insulin dependent diabetes mellitus and a control group. In the insulin dependent diabetes mellitus group, serum T_3 concentration was significantly lower than the control values. Serum T_4, FT_4 and TSH level did not differ. The difference in serum T_3 concentration was significant between diabetic children with good or poor control.

Thyroglobulin antibodies were investigated in diabetic children by Serono's "hTg antibodies" kit. Thyroglobulin antibodies were present in 14.5%. TSH concentration did not differ in antibody positive and negative cases, but one child with diabetes had evidence of moderately impaired thyroid reserve.

During the last few years a considerable amount of evidence has been obtained for abnormal thyroid function in insulin dependent diabetes mellitus (IDDM) [1,4,7,10,12,13]. Abnormalities in circulating thyroid hormone levels, referred to as the low T_3 syndrome, have been described in uncontrolled and poorly controlled patients with IDDM. In addition, diabetes as an autoimmune endocrinopathy is frequently associated with presence of thyroid autoantibodies in serum [6, 11, 14].

We have attempted to (i) compare the serum concentration of T_4, FT_4, T_3 and TSH in a group of children with IDDM and in healthy controls; (ii) investigate the possible relationship between thyroid hormone abnormalities and the degree of metabolic control of the diabetic state; (iii) determine the frequency of thyroglobulin antibodies detected in sera of IDDM patients; (iv) compare thyroid function in IDDM patients with or without thyroid antibodies.

Material and Methods

Thirty diabetic children and adolescents (13 boys and 17 girls) and 11 healthy controls (5 boys and 6 girls) participated in the study. Mean age $\pm$ SD of diabetic patients and healthy controls were not significantly different (10.6 ± 4.3 years vs. 10.8 ± 2.7 years). The duration of diabetes at the time of the study was 1 to 11 years (mean $\pm$ SD $= 4.3 \pm 3.8$). The diabetic subjects were treated by two daily injections of Monotard MC and Actrapid MC insulin. Insulin dosage was prescribed according to the glucose quantity in three urine fractions collected during 8 hour periods, and blood glucose concentration measured after breakfast with the glucose

oxidase method. The groups of poorly and well controlled diabetic patients were divided according to their urinary glucose concentrations [8]. Children with well controlled diabetes (11 boys and 13 girls) during three months preceding study did not have a urinary glucose concentration exceeding 3%. Patients with poorly controlled diabetes (2 boys and 4 girls) during the preceding three months repeatedly exhibited glucosuria exceeding 3%.

The serum concentrations of T_3 and T_4 were determined by commercial RIA kits. Serum FT_4 was measured by RIA method of Radiochemical Centre (Amersham), TSH was investigated by RIA-mat-TSH kit of Byk Mallinckrodt. The thyroglobulin antibody titres were determined by "hTg antibodies" kit of Serono. The hTg test was performed in 48 diabetic children (20 boys and 28 girls). Statistical analysis was performed by Student's *t* test.

Results

The serum levels of thyroid hormones and TSH in the diabetic patients and in healthy control ones are shown in Table I. Serum T_4 and FT_4 concentrations were almost identical in the two groups. The T_3 level was significantly lower in diabetic subjects than in controls, but T_3 concentrations of IDDM patients were in the normal range. TSH levels did not differ in the two groups.

Statistically significant difference in thyroid hormones and TSH was not found between the hormone concentrations of boys and girls, neither in the diabetic nor in the control group.

The influence of metabolic control is demonstrated in Table II. There was a considerable difference in serum T_3 concentrations of well and poorly controlled IDDM patients. It was remarkable that the degree of significance was different between the compared groups.

The frequency of thyroglobulin antibodies in 48 children with IDDM is shown in Table III, together with the different antibody titres: 7 diabetic children (3 boys and 4 girls) had thyroglobulin antibodies.

The serum TSH concentrations measured in antibody positive, antibody negative, and in control groups are shown in Figure 1, and individual serum TSH concentrations in antibody positive and control groups are also demonstrated. TSH concentra-

Table I

Serum concentration of thyroid hormones and TSH in diabetic patients and in healthy controls (mean ± SD)

Groups	T_4 nmol/l	FT_4 pmol/l	T_3 nmol/l	TSH mE/l
Control (n = 11)	131.6 ± 21.2	19.30 ± 3.98	2.69 ± 0.44	1.8 ± 0.7
IDDM (n = 30)	116.4 ± 18.4	18.79* ± 3.60	2.16 ± 0.52	1.9 ± 1.1
t-test (p)	NS	NS	<0.01	NS

NS = non-significant; * = n = 12

TABLE II

Serum T_3 concentration (mean ± SD) in healthy controls and in diabetic patients according to diabetic metabolic control

Groups	(n)	T_3 (nmol/l)	t test (p)
Control	(11)	2.69 ± 0.44	<0.01
IDDM (total)	(30)	2.16 ± 0.52	<0.05
Good metabolic status	(24)	2.30 ± 0.44	<0.001
Poor	(6)	1.64 ± 0.42	<0.01

TABLE III

hTg antibody positivity in 38 children with IDDM

Antibody titre	n	Frequency per cent
1 : 1 000	2	4.17
1 : 10 000	4	8.33
1 : 20 000	1	2.05
Antibody positivity:	7	14.58

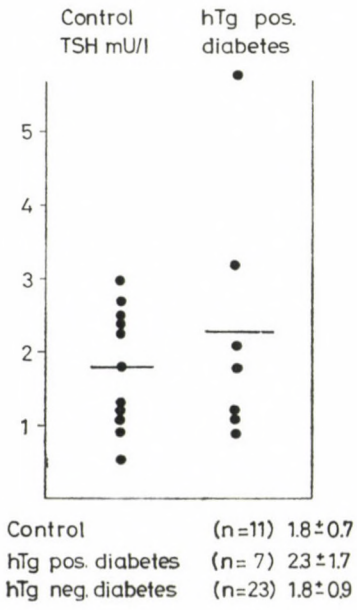

FIG. 1. Serum TSH concentration in healthy controls and in antibody positive and negative diabetic patients

tion did not differ in the compared groups. The TSH level, however, in one of our patients, was 5.8 mU/l. Her thyroglobulin antibody titre was 1 : 20 000 while thyroid hormone concentration showed a normal range.

Discussion

Recently, alterations of circulating thyroid hormones have been demonstrated in IDDM by several authors [1, 4, 7, 10, 12, 13] who found the following characteristics: low serum concentration of T_3, increased rT_3, normal to low T_4, and normal TSH. It has been assumed that these changes were related to glucose utilisation [3], and a good diabetic control has been found to restore it [3, 13]. A recent report on the effects of diabetes mellitus and insulin treatment upon serum thyroid hormone parameter concluded, however, that thyroid hormone concentrations were not influenced by variations of serum glucose [1].

In our study the mean T_3 levels were lower in diabetic children than in healthy controls of the same age, suggesting that the "low T_3 syndrome" [9] existed in our material, too. The T_4 and FT_4 concentrations did not differ, so the low T_3 level may have been a consequence of an impaired peripheral T_3 production from T_4 [10]. The T_3 concentrations strongly depended in diabetic children on the quality of metabolic control. Our findings confirmed the results recently obtained by Dorchy et al. [4].

The relationship between T_3 and metabolic state in IDDM indicates an association between the severity of thyroid abnormalities and diminished glucose metabolism. Since glucose utilisation is related to the degree of insulin deficiency, normal serum T_3 seems to be an index of optimum insulin replacement and the T_3 level can be used as an indirect parameter of metabolic control in IDDM [3]. On the other hand, it is worth considering that thyroid function in diabetic children should be assessed by the measurement of serum T_4 and FT_4 [3, 4].

Patients with IDDM commonly have co-existent autoimmune thyroiditis characterized by the presence of circulating thyroid antibodies [6, 11, 14] and biochemical evidence of impaired thyroid reserve [5]. Serum TSH concentration is accepted as a useful index of an impaired thyroid reserve in symptom-free autoimmune thyroiditis [2]. For this reason, in our study thyroid antibodies and thyroid functions were investigated simultaneously.

The prevalence of thyroglobulin antibodies was similar to that previously reported in children [4, 11]. Thyroid hormones and TSH concentration did not differ in antibody positive and negative cases, but in our study one antibody positive child with IDDM had evidence of moderately impaired thyroid reserve. So, we suggest to determine TSH concentration from time to time in antibody positive cases of IDDM.

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Uptake of beta-hydroxybutyrate, acetoacetate and glucose by the forearm of the newborn infant

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Forearm muscle metabolism was studied in twelve appropriate for gestational age premature infants suffering from respiratory distress. Arterial blood was obtained by puncture of the radial artery as clinically indicated for measuring oxygen tension, and venous blood samples were taken from the same arm's deep brachial vein. This arrangement allowed to study the arterial–deep venous differences of beta-hydroxybutyrate, acetoacetate and glucose in simultaneously taken blood samples. Net muscular uptakes of beta-hydroxybutyrate and glucose were observed, however, in four studies a virtual net production of acetoacetate was found. The arterial–deep venous concentration differences of both ketone bodies correlated positively with their arterial concentration within the 20 to 120 nanomol/ml range. Such a correlation was not observed in glucose utilization. It is concluded that forearm muscles in the neonate take up ketone bodies and this is in part concentration regulated.

Ketone body production is usually regarded as an exclusively hepatic process, ketone bodies being continuously produced by the liver and utilized by extrahepatic tissues [7, 11, 22]. The enzymatic possibilities of utilizing ketone bodies are common among animal and human tissues [3, 6, 8, 9]. The enzyme responsible for the NADH linked reversible reduction of acetoacetate to beta-hydroxybutyrate (beta-hydroxybutyrate dehydrogenase, E.C. 1.1.1.30.) is present in most tissues, tightly bound to the mitochondria [6]. In contrast to the adult organism, the fetal liver apparently behaves like extrahepatic tissues in the fetus because it can oxidize ketones [17].

Fetal tissues have been shown to utilize ketone bodies as oxidative fuels, thereby sparing glucose, and in brain and liver, rates of ketone oxidation are proportional to their concentration [1, 5, 18]. Studies of ketone body utilization by skeletal muscle in man have involved measurements of arteriovenous differences across the forearm or leg [2, 4, 21]. In obese humans starved for three days the ketone body uptake by the forearm muscle represents 50% of the oxygen uptake [12], thus ketone bodies may be an important substrate for skeletal muscle during caloric deprivation.

In contrast to the large number of experiments on adults and animals, little information is available on the ketone body utilizing capacity in neonatal muscle [15]. The aim of the pres-

ent work was to study the uptake of ketones and glucose in the neonatal forearm.

Materials and Methods

Twelve appropriate for gestational age premature infants admitted to the perinatal intensive care unit because of respiratory distress were selected for the study. All infants were considered to be without major medical problems and were in stable clinical condition. Mean birth weight was 1748 g (range, 1430 to 2100 g), each infant was in the first ten hours of life. Following the first clinical examination, a butterfly needle (Minifly, Alois Duschek GmbH, Vienna, Austria) was inserted as deeply as possible into a profound brachial vein for giving parenteral fluid and taking venous blood samples. Arterial blood was obtained by puncture of the radial artery for monitoring oxygen tension and acid-base parameters. Approximately 0.5 ml from the simultaneously collected blood was separated into heparinized test tubes, immediately centrifuged, and the plasma was stored at −20 °C until analysis.

Plasma beta-hydroxybutyrate and acetoacetate level was measured by standard enzymatic methods [10, 23], glucose was estimated by commercially available combined enzymatic kits (Boehringer, Mannheim, FRG). The equations of regression lines were calculated by the least squares method.

Results

A total of twelve studies was performed on ketone body and glucose concentrations. The study comprised results from eleven observations on beta-hydroxybutyrate and eight on acetoacetate. In one instance despite of the high arterial level of beta-hydroxybutyrate (744.63 nanomol/ml) the arterio-venous difference remained unexpectedly low (10.15 nanomol/ml). Four paired blood samples showed a net acetoacetate production, these data were excluded from statistical analysis.

The relationship between arterial beta-hydroxybutyrate concentrations and arterial-venous differences are shown in Fig. 1. There was a close linear correlation ($r = 0.556$) between the arterial beta-hydroxybutyrate supply and uptake in the 20 to 120 nanomol/ml range. The equation of regression line was $y = 0.2429 \times$

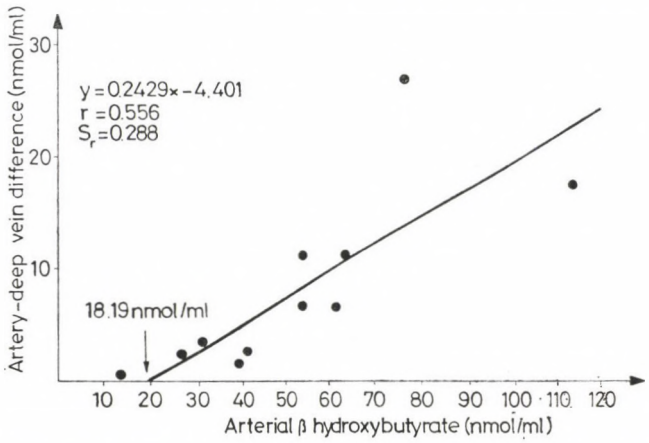

Fig. 1. Arterial concentration of beta-hydroxybutyrate versus its arteriovenous difference (n = 11)

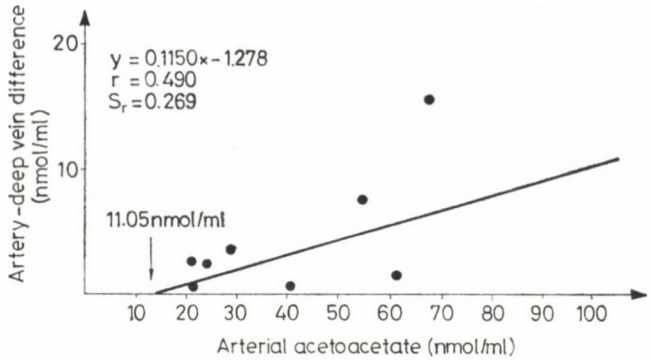

FIG. 2. Arterial concentration of acetoacetate versus its arteriovenous difference in neonatal forearm (n = 8)

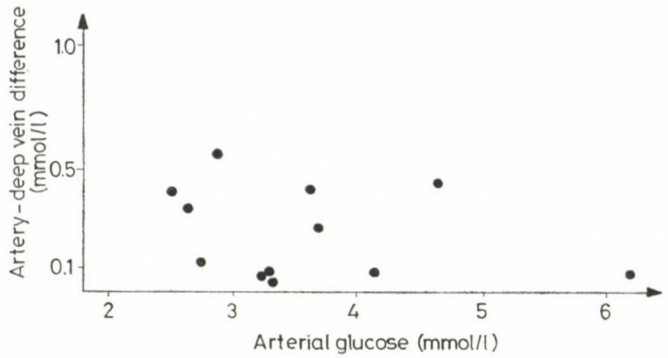

FIG. 3. Arterial concentration of glucose versus its arteriovenous difference (n = 12)

× —4.401. Results of eight observations on acetoacetate uptake by neonatal forearm are shown in Fig. 2. As it is seen, there was a linear correlation between the arterial availability and uptake, the correlation was less striking than in the case of beta-hydroxybutyrate (r = 0.490; the equation was y = 0.115X—1.278). The arterial glucose concentration had no effect on the arterial–deep venous difference, as it is shown in Fig. 3.

DISCUSSION

Due to their low concentration in neonates under normal conditions, beta-hydroxybutyrate and acetoacetate are not major substrates for energy production. Caloric deprivation in neonates and older children is, however, associated with higher ketone levels providing a considerable amount of alternative fuels for metabolism of tissues[19]. In the present study comparatively low beta-

hydroxybutyrate and acetoacetate concentrations were found in the newborns, probably due to the depressed activity of the enzyme carnitine palmitoyl acyltransferase within the first three days of life which makes the neonate unable to produce ketone levels as high as are found in older children [19, 20].

The uptake of both ketone bodies in the present study was found to be a concentration dependent process, the correlation between the arterial ketone levels and the arterial–venous difference was more close in the case of beta-hydroxybutyrate. Similar results were reported on cerebral uptake in adult humans [4, 14]. It seems of interest that in one newborn infant, despite the high arterial beta-hydroxybutyrate level, its uptake from the blood circulation was moderate, suggesting the possibility that its elimination from the blood is saturable at high concentrations.

It is noteworthy that in four studies a virtual net acetoacetate production was observed. In view of this finding the synthetic pathways of acetoacetate should be considered. One possible mechanism is the NAD—NADH interconversion linked reduction of beta-hydroxybutyrate, which reaction depends on the mitochondrial NAD/NADH ratio [6, 15]. This way may represent a "shuttle" function of the ketone bodies in respect of the movement of the reducing equivalents between different tissues and organs depending on their mitochondrial redox state. Another theoretical mechanism of acetoacetate production might be the "de novo" synthesis, for example from free fatty acids, as it has been proposed on the basis of forearm studies in adults [4].

Glucose uptake in the present study was not concentration regulated; corresponding to present knowledge, glucose uptake of skeletal muscle is strongly insulin dependent [13, 16].

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Increased birth prevalence of isolated hypospadias in Hungary

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The birth prevalence of simple (isolated) hypospadias increased significantly in Hungary up to 1978. Since then, although fluctuating, it has remained at this higher level.

The Hungarian Congenital Malformation Registry (HCMR) indicated that the birth prevalence of hypospadias was increasing up to 1978, since then, although fluctuating it has remained at this high level (Table I). The increasing birth prevalence of hypospadias has been observed in other countries, too, e.g. in Norway [1], Sweden [11] and the United Kingdom [16, 13].

Evaluation of an increase in the birth prevalence of congenital anomalies is a difficult task. First, it is important to consider whether an unusual rise in birth prevalence might not be a random phenomenon only because in epidemiology "unexpected events are expected". Second, the possibility of technical biases, e.g. changes in definition and classification, diagnosis, ascertainment and notification, confounding variables including demographic traits of the study population, etc., have to be excluded. Third, it is necessary to attempt to establish adequate hypotheses explaining time trends and to test them.

The purpose of this paper is to evaluate critically the increased birth prevalence of hypospadias observed in Hungary.

Materials and Methods

First, the data recorded in the HCMR, 1970—1983 for hypospadias were evaluated.

Second, within the frame work of "A Joint International Study on the Epidemiology of Hypospadias" [12] one cohort of the HCMR material, index patients born in 1975, was selected for a follow-up study in 1983 to determine the rate of misdiagnosis and completeness of notification. This cohort was selected because index patients born in 1975 were after the usual term of surgery by the time of the study, and they had not been included in our previous study [4]. The HCMR involves 334 index patients born in 1975 with the four digit code of 752.6 entitled "Hypospadias and epispadias" (Fig. 1). This item includes only simple (isolated) abnormalities in the HCMR, multiple congenital abnormalities including hypospadias are recorded in different codes beginning with 759. As it ap-

TABLE I

Data-base of hypospadias in Hungary, 1970—1983

Year	Total birth	Total male livebirth	Total congenital		Simple (isolated) hypospadias				Complex hypospadias	Together		Multiple hypospadias	Total		
			No.	‰	No.	‰	ML‰	‰		No.	‰		No.	‰	%
1970	153.339	78.366	3711	24.20	85	0.55	1.08	2.29	5	90	0.59	11	101	0.66	2.72
1971	152.159	77.611	4843	31.83	172	1.13	2.22	3.55	3	175	1.15	17	192	1.26	3.96
1972	154.688	79.309	5349	34.58	173	1.12	2.18	3.23	3	176	1.14	20	196	1.27	3.66
1973	157.623	80.657	5288	33.55	217	1.38	2.69	4.10	3	220	1.40	25	245	1.55	4.63
1974	187.957	95.887	6750	35.91	252	1.34	2.63	3.73	15	267	1.42	19	286	1.52	4.23
1975	195.847	99.907	7441	37.99	334	1.71	3.34	4.49	12	346	1.77	29	375	1.91	5.04
1976	186.916	95.350	7572	40.51	365	1.95	3.83	4.82	16	381	2.03	29	410	2.19	5.41
1977	179.152	91.063	6585	36.76	377	2.10	4.14	5.73	14	391	2.18	25	416	2.32	6.32
1978	169.524	86.455	7277	42.93	402	2.37	4.65	5.52	14	416	2.45	34	450	2.65	6.18
1979	161.677	82.172	6905	42.75	329	2.03	4.00	4.76	6	335	2.07	28	363	2.25	5.26
1980	149.829	76.115	6912	46.13	331	2.21	4.35	4.79	8	339	2.26	20	359	2.40	5.19
1981	144.062	72.920	6223	43.20	310	2.15	4.25	4.98	10	320	2.22	16	336	2.33	5.40
1982	134.579	68.778	6161	45.78	322	2.39	4.68	5.23	8	330	2.45	14	344	2.56	5.58
1983*	128.160	65.082	5440	42.45	279	2.18	4.28	5.13	7	286	2.23	12	298	2.33	5.48

‰ per 1000 total births
ML male livebirth
* preliminary figures

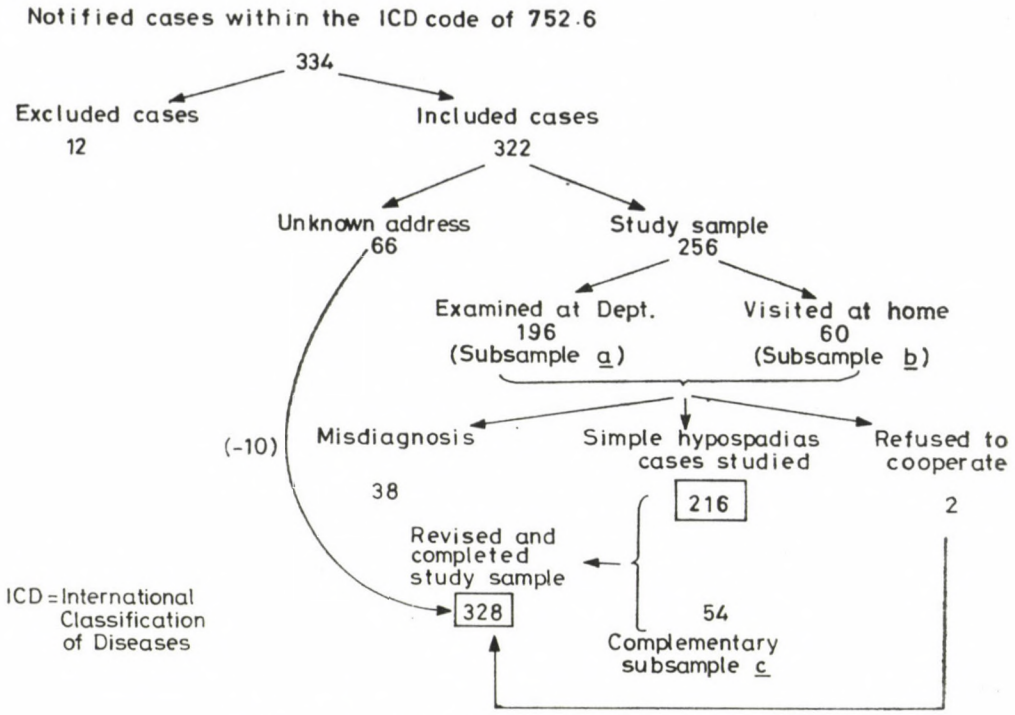

FIG. 1. Data base of study cohort born in 1975

peared at the check-up, six index patients (1.7%) were recorded twice. (They were notified with somewhat different personal data and first the computer could not identify them.) Two index patients with notified hypospadias turned out to be girls. Four children had epispadias. These 12 cases were excluded. The remaining 322 index patients were evaluated in two steps. First, we invited by letter the index patients with their parents for a personal examination into the Department of Paediatric Urology, Heim Pál Hospital for Sick Children, Budapest. Due to changed address, the letters to 66 index patients (20.5%) were returned to us. We assumed that there was no relationship between unknown addresses and the variables of hypospadias (i.e. there was no selection bias), and these cases were neglected. Out of the remaining 256 cases of the study sample, 196 index patients (76.6%) visited J. T. together with their first degree relatives. This is subsample *a*.

As a second step, the 60 index patients (subsample *b*) who did not appear at examination, were visited by E. C. that their hypospadias should be checked-up. We took advantage of the opportunity of meeting the parents of hypospadiacs, and epidemiological data were obtained by the help of the printed questionnaire by personal interview. The epidemiological data of subsamples *a* and *b* did not significantly differ, thus they were evaluated together. The parents of two index patients refused to cooperate, both boys had glandular hypospadias.

In a third phase of the cohort study an alphabetic list of names of index patients examined personally was sent to the head of all (eight) Hungarian paediatric surgery departments asking them to complete this list with the non-notified index pa-

tients born with hypospadias in 1975. All departments replied. This is the complementary subsample *c*.

Results and Discussion

Statistical evaluation of the increase of hypospadias

Three groups of hypospadias were separated (Table I).

(i) *Simple* (single or isolated) hypospadias: this term excludes cases of hypospadias associated with any other genital and extragenital congenital anomalies, but includes the direct consequences of the malformation: meatal stenosis, congenital torsion, chordae, bifid scrotum.

(ii) *Complex* hypospadias was defined as hypospadias associated with the following genital anomalies of males: undescended testicle(s), hydrocele, congenital inguinal hernia(s) and malformations of the external male genitalia. In general, complex hypospadias is the manifestation of GAM, i.e., *g*enital *a*nomalies of *m*ale [7].

(iii) *Multiple* hypospadias, i.e., multiple congenital abnormalities comprising hypospadias and other types of extragenital congenital abnormalities.

Using the Cohran test, the increase in the birth prevalence of simple hypospadias was shown to be significant ($p < 0.01$) in Hungary during the year 1970—1983. Neither complex nor multiple hypospadias had, however, shown a significant increase, their annual changes could be explained by random fluctuation (except the maximum of multiple cases in 1978). As far as we know the recent Hungarian birth prevalence values of simple hypospadias, particularly in 1978 and 1982, were the highest among the published figures based on populations [10, 12]. The male-specific live-birth prevalence of simple hypospadias was as high as 4.65 and 4.68, respectively. Only relatively small or hospital based incidences were higher than the recent Hungarian figures, e.g. 5.4 in New York based on 2,793 male births [15], 7.6 in Minnesota based on 4,474 male births [9] and 8.2 in Rochester, Minnesota, based on 13,776 male births [17]. All these allowed to conclude that the increase of birth prevalence of simple hypospadias in Hungary, 1970—1983, was significant statistically.

Technical biases and confounding variables

The following task was to estimate the impact, more precisely the proportion of technical biases and confounding variables in the increase of simple hypospadias.

(i) *Definition* and *classification* of hypospadias have not been modified in the last 14 years.

(ii) A change in *diagnostic skills* and attention, e.g., the detection of slight hypospadias, mainly minor forms of glandular type, could not be excluded. We exam-

ined personally 254 index patients (Fig. 1). Three index patients were notified as simple hypospadias but they also had other congenital anomalies (omphalocele, congenital pyloric stenosis and congenital dislocation of the hip, respectively). Thirty-five boys had no hypospadias by personal examination. Oedema of the penis at birth may lead to incorrect diagnosis of simple hypospadias [14]; they were reported with one exception (glandular) as hypospadias without the type being mentioned. These 38 misdiagnosed cases were excluded from the study sample. One of the further seven index patients had a minor anomaly (haemangioma in the pectoral region), the other six had functional disorders (Friedreich ataxia with a positive family history; mental retardation and hypacusis; mental retardation, deafness and squint; myopia; renal tubular acidosis), these cases were not excluded. Thus, out of 254 examined index patients, the diagnosis of simple hypospadias was confirmed in 216 cases, i.e., the *validity of diagnosis* was 85.0% (Fig. 1).

The type distribution of hypospadias was different in cases born in 1970—1972 and 1975 (Table II), the difference being most pronounced between our previous study sample (based on notified and operated non-notified cases) and the present study sample (based on notified cases) ($\chi^2_2 = 14.4$; $p < 0.01$). Percentage figures of mild hypospadias were 51%, 56% and 65% in the previous sample, the present complementary subsample *c* and the present revised study sample, respectively. Proportions of mild, medium and severe simple hypospadiacs were 66%, 29% and 5% in the present completed and revised study sample, with a probably decreasing relative order of ascertainment bias.

(iii) *Ascertainment* and *notification* of simple hypospadias have increased considerably mirroring the increase of notification of total congenital anomalies (Table I). Still, the increase in notification of simple hypospadias exceeded the general trend. On the one hand the proportion of simple hypospadias increased from 3.6% to 5.2% of the total congenital anomalies between 1971 and 1982. On the other hand, the increase was 44% in the total congenital anomalies while 112% in simple hypospadias in the period studied. (Index patients born in 1970 were obviously under-ascertained; while figures of 1983 are preliminary, therefore they were not considered in evaluation of the data.)

Further 68 cases with simple hypospadias in complementary subsample *c* were ascertained

TABLE II

Distribution of types of simple hypospadiasis in previous and present study samples

Category	Type	Definition	Previous study sample No.	%		Present study sample revied No.	%		Complementary subsample c No.	%		Present completed and revised study sample No.	%	
Mild	Glandular	the opening is distal to the sulcus coronarius	69	23.5		77	35.3		8	11.8		85	29.7	
	Coronal	the opening is within the sulcus coronarius	80	27.2		73	33.5		30	44.1		103	36.0	
Medium	Penile	the opening is proximal to the sulcus coronarius	134	45.6		59	27.1		25	36.8		84	29.4	
Severe	Penoscrotal	the opening is in the immediate vicinity of the penoscrotal junction	0	0.0		4	1.8		4	5.9		8	2.8	
	Scrotal	the opening is in the scrotal region	0	0.0	3.7	1	0.5	4.1	1	1.4	6.9	2	0.7	4.8
	Perineoscrotal	the opening is between the two halves of, or behind the cleft scrotum	11	3.7		4	1.8		0	0.0		4	1.4	
	Total		294	100.0		218	100.0		68	1000		286	100.0	

within the cohort born in 1975 from the records of paediatric surgery departments, and 14 cases were notified to the Registry but owing to an incorrect address they were not examined. Thus, we had 54 "new" cases. Taking into consideration these cases, too, the total number of ascertained simple hypospadias patients would be 376. This approach showed a 85.6% completeness of notification in simple hypospadias. Still, on the one hand it was necessary to subtract the 38 misdiagnosed cases from the above total of 376. On the other hand, it seemed wise to exclude further ten cases from the 66 index patients with unknown address owing to the 15% probability of misdiagnosis. Thus, the total number of index patients with simple hypospadias born in 1975 could be supposed to be 328, which represented a 1.67 birth prevalence in Hungary in 1975. It was only 2% lower than the recorded rate in the HCMR, because the rate of misdiagnosis and incompleteness of notification nearly completely balanced out each other. If it were a general pattern, it would augment the relevance of recorded birth prevalences, but data of one single year did not allow this conclusion.

Simple hypospadias was evaluated only in males in this study and the majority of our cases (327/328 = 99.7%) occurred in livebirths, thus a male-specific livebirth prevalence of 3.27 per 1000 male livebirths in 1975 could be calculated.

There are significant differences in birth prevalence data of simple hypospadias among the twenty territorial units of Hungary. The maximum (5.3) and the minimum (2.2) indicated a wide range. An obvious territorial trend, the so-called cline, however, could not be detected and the fluctuation mainly indicated the territorial differences in notification.

The problem of underreporting of congenital anomalies is well-known and usually high percentage figures (e.g., 44.3%) were reported [8].

(iv) Changes in *confounding variables.* Maternal age and particularly parity have a slight effect on the birth prevalence of simple hypospadias [5, 12]. There was no considerable shift in maternal age and parity distribution comparing the figures of the seventies with the data of the eighties in Hungary.

The conclusion is that the changes in diagnosis, ascertainment and notification may explain a considerable proportion of the increased prevalence of simple hypospadias in Hungary but probably not the entire rise. This statement is based on two arguments. First, owing to the incomplete notification, true birth prevalence figures

have been higher than the recorded ones in the past years. Second, recorded figures of 1978 and 1982 exceeded the true birth prevalence of simple hypospadias determined in 1973. Simple hypospadias was screened by neonatologists prepared for the study in a representative sample involving 10,203 livebirths in Hungary in 1973, and a rate of 2.2 per 1000 total births was found [5]. The recorded birth prevalence was 1.4 in 1973 (Table I). Some recent recorded birth prevalence figures exceeded the level of true birth prevalence of simple hypospadias found in 1973.

Finally, the aetiological factors should be studied. It is known that there are racial, probably genetic, differences in the rate of simple hypospadias [3]. The Hungarian population is, however, racially fairly homogeneous being exclusively of European origin. [The effect of environmental factors will be summarised in another paper [1].) The main conclusion of the aetiological approach was that the increasing frequency of couples with a history of infertility among parents in general may explain the recent increased birth prevalence of simple hypospadias in Hungary.

The ratio of fertile and subfertile couples depends on the reproductive activity of the fertile ones. Therefore, the proportion of children born of subfertile couples was low in the developed countries some decades ago because of the relatively high reproduction rate of fertile couples. Intensive birth control of fertile couples on the one hand and the increasingly effective treatment of subfertility on the other hand, increased the proportion of children of subfertile couples within the newborn population. Owing to the progress in the treatment of subfertility, the previous proportion of childless couples (about 12%) has decreased significantly (below 6%) in Hungary. Thus, the increasing proportion of children of subfertile couples may explain the increased birth prevalence of simple hypospadias (Fig. 2).

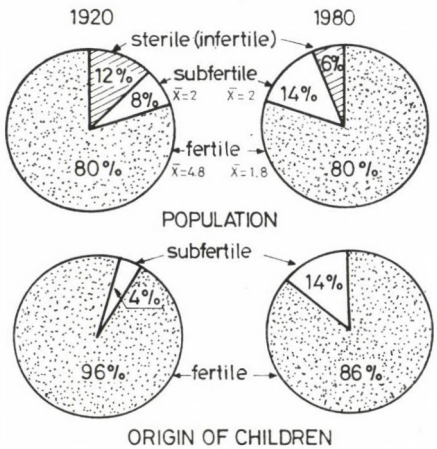

FIG. 2. Estimated proportion of fertility and origin of children

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Book reviews

New Results in Clinical and Biological Research Including Pediatric Oncology

New Results in Clinical and Biological Research Including Pediatric Oncology. Edited by R. NETH, R. C. GALLO, M. G. GREAVES, G. JANKA. 517 pages and 178 figures and 127 tables. Springer, Berlin–Heidelberg–New York 1985. Price: DM 198,–

This excellent book comprises the papers read in Wilsede, GFR, in June, 1984. Two Frederick Stohlman memorial reviews introduce the four main sections. The first memorial paper was offered by Professor Mitchison, the well-known figure of tumour immunology, on the reactivity of helper and suppressor cells with some macromolecules of the host organism itself. The second one was delivered by Duesberg on the mutual relationship and specificity of protooncogens and oncogens and their role played in oncogenesis.

The first section deals with diagnosis and therapy of leukaemia. Several papers describe new methods in bone-marrow transplantation (in vitro treatment of the graft) and recent results achieved by bone-marrow transplantation in various types and stages of leukaemia. Further issues are very high and very low dose cytosine arabinoside therapy, various forms of recent protocols of chemotherapy, prognostic factors, drug resistance, and methods to overcome infections of leukaemic patients.

Malignant transformation is the main topic of the second section; oncogenic compounds, chromosome aberrations and viruses are dealt with. Although the part of chromosomal aberrations played in the initiation of malignant cell proliferation has not been clearly established, their specificity and their relationship to protooncogens is of utmost importance in studies on oncogenesis.

Cellular biology is the title of the subsequent section, discussing the conditions and mechanisms of normal cell proliferation and differentiation, giving insight into the problem of differentiation arrest in the course of malignant proliferation and the importance of growth factors.

The fourth section sums up the papers on immunology; here receptors, antigens and cellular defence are reviewed. In addition to results and attempts of therapy, the volume presents an excellent review of basic research on leukaemia, in a concise style comprehensible for experts and clinicians having no own experience in virology and gene recombination techniques. Each paper is followed by references, the alphabetical index appears very helpful.

D SCHULER

SCHNEIDER, D.-R.: *Geschädigte Säuglinge und Kleinkinder.* 166 Seiten mit 21 Abbildungen, 20 Tabellen und 7 Schemata, Georg Thieme, Leipzig 1985. Preis DM 45,–

Das Buch, Ergebnis einer multidisziplinären Zusammenarbeit, ist eine ausführliche Anleitung zur Betreuung und Förderung geschädigter Säuglinge und Klein-

kinder in Kinderkrippen. Der Autor, der Leiter der Rehabilitationspädagogischen Förderungseinrichtung Erfurt, versteht unter Früherziehung einen Prozeß zur Minderung oder Beseitigung der Behinderung durch rechtzeitiges Einsetzen rehabilitationspädagogischer Maßnahmen, sofort nach dem Auftreten meßbarer Entwicklungsrückstände, als auch Verhütung einer Lernbehinderung bei pathologischen-anatomischen Besonderheiten als Folge schädigender prä-, peri- und/oder postnataler Noxen auf das in der Entwicklung befindliche Zentralnervensystem. Die Früherziehung wird als ganztägiger rehabilitationspädagogischer Prozeß auf der Grundlage einer medizinisch-rehabilitationspädagogischer Zusammenarbeit realisiert. Der Eckpfeiler der Früherziehung ist die kollektive Erziehung: die Früherziehung wird in der Gruppe und durch die Kindergruppe verwirklicht. Individuelle Förderung kann nicht ausgeschlossen werden, steht aber nicht im Vordergrund der Arbeit in den Früherziehungsgruppen. Im Rahmen der Forschung des Autors hat sich gezeigt, daß heterogene Gruppen bessere Erfolge erzielen, als homogene Gruppen. Die praktische Arbeit erfolgt mit der Gesamtgruppe, entsprechend dem individuellen Entwicklungsbild. So wird die geplante Handlungsfolge zur Tätigkeitsfolge für alle Kinder der Gruppe, es entwickeln sich deutlich soziale Beziehungen der Kinder untereinander, und die gruppendynamische Entwicklung schlägt sich auf das Individuum nieder.

Mit der Anwendung des im Buch vorgelegten Förderprogramm wurden zwischen 1976 und 1980 213 geschädigte Säuglinge und Kleinkinder rehabilitiert. Das durchschnittliche Aufnahmealter war im 24. Lebensmonat, mit einem Entwicklungsalter von 15 Monate und einem Entwicklungsquotienten um 0,61. Nach 15monatiger Früherziehung konnte im Lebensalter von 39 Monaten und Entwicklungsalter um 30 Monate ein Entwicklungsquotient von durchschnittlich 0,78 erreicht werden.

J Falk

Gynäkologie im Kindes- und Jugendalter. Herausgegeben von M. Heinz. 162 Seiten mit 63 teils farbigen Abbildungen und 36 Tabellen. Georg Thieme, Leipzig 1985. Preis DM 42,—

Nach dem in den Jahren 1972 und 1974 erschienen Buch von Heinz und Hoyme ist das vorliegende Werk eine weitere in der DDR publizierte Facharbeit über dieses spezialisierte Gebiet der Gynäkologie, was an sich selbst auf das Beleben der kindergynäkologischen Tätigkeit hindeutet. Als Anhänger der »Prager Schule« hat die Herausgeberin seit 1968 eine bahnbrechende Arbeit in ihrer Heimat geleistet und ihre vielseitigen Erfahrungen in dieser Arbeit zusammengefaßt, so daß Gynäkologen und Pädiater und auf den Grenzgebieten tätigen Urologen und Chirurgen bei ihren wichtigsten und brennenden Problemen einen Leitfaden erhalten.

Das mit Beiträgen von sechs kompetenten Mitarbeitern verfaßte Buch gliedert sich in 15 Kapitel und verfügt über ein ausführliches weiterführendes Literaturverzeichnis. Der praxisbezogene Aufbau ist logisch und übersichtlich, der Text ist klar. Bei den einzelnen Krankheitsbildern wird die Therapie, ergänzt mit den in der DDR anwendbaren Medikamenten und deren Dosen angeführt.

Als besonders wertvoll seien die Kapitel über die Störungen der Geschlechtsentwicklung (Heinz) und über die Störungen der Pubertät und Adoleszenz (Göretzlehner) hervorzuheben.

Wenn man das Buch durchblättert, muß man sich über die von dem allgemeinen Gebrauch abweichende Definition des Begriffes Pubertät hinwegsetzen: Göretzlehner versteht unter Pubertät den Zeitraum zwischen Auftreten der Schambehaarung und der ersten Regelblutung (Seite 85), während Heinz unter Pubertät die präpubertale Phase beginnend mit der Entwicklung der sekundären Geschlechtsmerkmale bis zum 13—14. Lebensjahr versteht; auch in dem Kapitel über die psychologischen Probleme (Meyer—Probst) erscheint die Be-

stimmung der Pubertät etwas unsicher. — In der Fachliteratur werden heute zur Pubertät die nach der Menarche folgenden 1—2 Jahre miteinbezogen. Es wäre nicht richtig von Präpubertät zu sprechen, wenn sich diese in Zeit und Erscheinung nicht von der Pubertät differenzieren ließe. Bei Mädchen ist die Menarche eine einfach deutbare Reifungserscheinung, so daß zur Zeit der Pubertät die Menarche und auch die Ereignisse der ersten zwei gynäkologischen Jahre eine Rolle spielen.

In dem Kapitel über Entzündungen und Infektionen vermißt man leider einige neue Aspekte hinsichtlich der viralen und sexual übertragenen bakteriellen Infektionen (STD); so werden z. B. bei den Herpesinfektionen die Vidarabinbehandlung und andere therapeutische Möglichkeiten oder die in letzter Teit beschriebenen schweren Folgen der Mycoplasma- und Chlamydiainfektionen nicht erwähnt.

Diese diskutable Fragen und Bemerkungen sollen jedoch den Wert des für die Praxis so nützlichen Buches nicht mindern.

Judit ÖRLEY

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CMEA Chemotherapy Symposium 1984

Edited by
S. ECKHARDT and S. KERPEL-FRONIUS

In English. 1985. 644 pages. 17×25 cm
Hardcover. $45.00/DM 126,—/£32.50

This symposium was dedicated to commemorate the 10th anniversary of the CMEA cancer research agreement in the frame of which Bulgaria, Czechoslovakia, Cuba, the GDR, Hungary, Poland and the USSR actively cooperate in joint chemotherapy programs. The research efforts of the last 5 years were surveyed, which were directed towards the development of new anticancer agents or improved application of the existing ones within the participating countries. In addition to this monographic section, papers on the newest results of recent and ongoing projects were read at the symposium by specialists from the above countries as well as from Yugoslavia. The main subjects dealt with are new animal model systems, screening data, structure activity relationship, mechanism of action, pharmacokinetic properties and finally the clinical evaluation of new cytostatic agents, or new combinations. For proper interpretation, the activities of new drugs were compared to widely known compounds in the test systems used. The clinical data were presented according to the WHO recommendations. In this way the editors hope to make accessible important observations, using internationally accepted criteria, many of which were not published previously or only in the native language of the contributors.

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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.
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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.

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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.

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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.

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Initial dose: 2 times 2 POTESEPTYL tablets
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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.

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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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CARIES PREVENTION BY DOMESTIC SALT FLUORIDATION

By K. Tóth

In English. 249 pages, 15 figures. 14 × 21 cm.
Hardcover $18.00/DM 54,—/£14.50
ISBN 963 05 3476 2

In Hungary a series of clinical trials started in 1966 with the aim of testing the caries-preventive effect of fluoride added to domestic salt in different concentrations. Experiments were carried out paralell with three different salt-F concentrations and as a result caries fell by more than 50 per cent. Investigations confirmed furthermore that fluoridesupplemented salt has the same effect on deciduous teeth as fluoridated water.

In this book also the physiological aspects of salt fluoridation like daily optimal intake, tolerable and harmful amounts, drinking habits are dealt with. Finally the recent results reached in the field of caries prevention are assessed.

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Abbreviations should be spelled out when first used in the text. *Drugs* should be referred to by their WHO code designation (Recommended International Nonproprietary Name); the use of proprietary names is unacceptable. The *International System of Units* (SI) should be used for all measurements except blood pressure.

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