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CONTENTS

Amador M, González ME, Córdova L, Pérez N: Diagnosing and misdiagnosing malnutrition 391
Amador M, Rodriguez C, González ME, Bacallao J: Assessing obesity with body weight and height 381
Balla G, Makay A, Pollák Z, Matkovics B, Lakatos L, Karmazsin L: Damaging effect of free radicals liberated during the reduction of oxygen: its influencing by drugs 319
Barta L, Pena M, Tichy M, Regöly-Mérei A. Koltai G, Bedő M: The effect of caffeine and physical exercise on blood lactate levels of obese children 343
Borbolla L: Abnormal dematoglyphics in absence of thumb 417
Book reviews 121, 255, 375, 483
Cserháti E, Mezei G, Kelemen J, Póder G, Gegesi A: The prognosis of severe bronchial asthma in childhood on the basis of late reexaminations 473
Czeizel A: Current issues in the practice of genetic counselling 201
Czinner A, Barta L, Tichy M: Effect of exercise on the lipid parameters in the fasting state and under glucose load 403
Ertl T, Sulyok E, Németh M, Tényi I, Csaba F, Varga F.: Hormonal control of sodium content in human milk 309
Fekete M, Harangi E: Cerebrospinal fluid concentration in preterm and full term newborn infants 1
Fekete M, Soltész Gy, Horváth M: The cerebrospinal fluid of newborn infants with perinatal hypoxia 179
Gencik A, Genciková A, Pálová A: Familial occurrence of partial trisomy 4q and probable monosomy 5p due to 4q/5p translocation 291
Járai L: Intrauterine weight retardation and the concomitant length retardation: a statistical analysis of anthropometric data 9
Kajtár P, Molnár S, Várbíró M: Fatal infectious mononucleosis. Report of a case with review of the literature 17
Karmazsin L: Professor L. Kulin 80 years 253
Katona F, Halász M, Berényi M, Szabolcs P, Izsák K: Subdural effusion in the first six months of life 219
Kecskés L, Juricskay Zs, Tatai Zs, Szécsényi M: Dynamics of adrenal steroidogenesis in childhood: steroid excretion in prepubertal and pubertal girls 151
Kiss, P, Osztovics M: Ring chromosome 15 409
Klujber L, Szűcs R, Baranyai Zs, Gabulya J, Soltész Gy: Glycosaminoglycan and hydroxproline excretion in diabetic children 27
Kosztolányi G: A diagnostic survey of patients referred for chromosome analysis ... 35
Kovalovszki L, Villányi Z, Pataki I, Veszelovszky I, Nagy ZB: Isolation of aerobic bacteria from the placenta 357
Lakatos L, Hatvani I, Oroszlán G, Karmazsin L, Matkovics B: D-penicillamine in the prevention of retrolental fibroplasia 327
László A, Kaiser G, Simon M: HLA antigens in bullous epidemolysis, congenital ichthyosis and ectodermal dysplasia 447
Lukács FV, Breckner M, Lázár E, Sziráki K, Sulyok C: Über die Pathogenese des Bronze-Baby-Syndroms: Koproporhyrinentleerung hyperbilirubinämischer Neugeborenen 211

Marosvári I, Köves S: Haemodialysis treatment of acute renal failure in hypertonic dehydration 145
Méhes K, Bajnóczky K: Normal chromosomes in liveborn neonates weighing less than 1000 g 41
Molnár D, Kardos M, Soltész G, Baranyai S: Intravenouse glucos tolerance test in childhood obesity 127
Molnár D, Soltész G: Metabolic and hormonal effects of fasting in obese children 45
Morón E C, Révész T, Schuler D: Lymphocyte markers in childhood ALL: the lack of correlation with prognosis 171
Oroszlán G, Lakatos L, Karmazsin L: Neonatal oxygen toxicity and its prevention: S-penicillamine offers benefits without harmful side-effects 459
Osztovics M, Jójárt G: 11q-syndrome 239
Osztovics M, Kiss P: Trisomy 5q15—q31 due to maternal insertion, in s(6; 5) (q21; q15q31) 231
Osztovics M, Kiss P: Down syndrome: correspondence of clinical diagnosis and karyotype 261
Osztovics M, Tóth S, Wilhelm O: Unusual chromosome aberrations in 3 children with Down syndrome 283
Pazonyi I, Kun A, Czeizel A: Congenital postural deformity association 431
Pintér A: Major anomalies of the genitourinary tract in the neonate 51
Pólus K: Skin haemangioma: treatment by cryosurgery 167
Rubecz I: Energy metabolism, substrate utilization, metabolite and hormone levels in infants fed various parenteral solutions 59
Roppert F, Sulyok E, Varga F, Csaba I F, Petz A: Thyrotropin and prolactin response to ambient temperature in newborn infants 189
Schultz K, Weisenbach J: Cardiomegaly in hypoglycaemic small-for-gestational-age infants 69
Soltész G, Molnár D, Kardos M, Baranyai Zs: Effect of glucagon infusion on some plasma metabolites and hormones in obese children 137
Soltész G, Molnár D, Klujber L, Klujber L, Kardos M: Relationship between metabolic control and plasma lipoprotein level in diabetic children 75
Soltész G, Molnár D, Mestyán J: Effect of intravenous alanine on blood glucose, glucagen and growth hormone levels in diabetic children 195
Storcz J, Mestyán J: Long-term prognosis of asphyctic neonates from an intensive care unit: Intrauterine retarded infants at high risk of cerebral palsy 361
Storm W: Use of thrombocytopenia for the early identification of sepsis in critically ill newborns 349
Szabó B, Jezerniczky J, Kávai M, Nagy A, Nagy B: Immunological findings in primary malabsorption 337
Tóth Z, Vachter J, Szeifert G, Nemes Z, Csécsei K, Török O, Harsányi A, Papp Z: Antenatally diagnosed thanatophoric dysplasia 423
Véghelyi P V: Professor J Mestyán 60 years 119
Walcz E, Seri I, Kuti D, Révész T, Schuler D: Developmental changes in lymphocyte surface markers 299
Whyte P K, Sinclair J C, Bayley H S, Campbell D, Singer J: Energy cost of growth of premature infants 85
Wilhelm O, Osztovics M: 9p deletion syndrome 247
Young M: Protein turnover rate in early life 99

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Cerebrospinal fluid protein concentration in preterm and full term newborn infants

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Cerebrospinal fluid protein concentration was studied in 296 newborn infants suffering from various perinatal pathological conditions. A statistically significant negative correlation was found between CSF protein and gestational age, birth weight and postnatal age. It is suggested that CSF protein concentration is primarily related with the functional maturity of the blood-brain-CSF barrier for protein, and is not basically influenced by various diseases of the newborn infants.

In the human newborn the clinical symptoms of the various disorders of postnatal adaptation are fairly unspecific, and even more unspecific are those affecting the CNS. In consequence, uncertainties often appear in the correct evaluation of laboratory findings.

Concentration of protein in the CNS of the newborn infant has been studied by many authors [1—9]. There is general agreement among all of them that the CSF protein level is higher in full term and even more so in preterm newborn infants than the values observed later in infancy and childhood. At closer scrutiny, however, the mean and individual CSF protein values measured in different groups of neonates show wide variations and differences, causing difficulties in interpretation in individual cases. The diversities may originate from sources like (*a*) the definition of prematurity by birth weight and not by gestational age; (*b*) the relatively small groups of patients studied; (*c*) differences in the laboratory methods used by the authors; (*d*) the impossibility of obtaining CSF from healthy newborn infants, in view of ethical reasons, consequently the lack of normal values; and, lastly, (*e*) the lacking details in most of the studies of the clinical data and history of the patients involved in the trial.

In the present study an attempt has been made to examine the CSF protein concentration in preterm and full term newborn infants sufferring from various perinatal pathological conditions, with special reference to the relationship between CSF protein content and birth weight, gestational age, postnatal age and serum protein concentration.

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday.

Supported by the Scientific Research Council, Hungarian Ministry of Health (3—23—0502—04—1'M).

Patients and Methods

During the period of 1st January, 1976, to 31th December, 1980, all preterm (gestational age <37 weeks) and full term (gestational age ≥ 37 weeks) newborn infants admitted to our Special Care Baby Unit were subjected to study, provided that the lumbar puncture was indicated on clinical grounds within the first three weeks of age. The CSF samples were cultured and examined for cell count, glucose and protein concentration. Protein was estimated by turbidimetry [10]. Patients with blood stained CSF were excluded from the study as also those with bacterial or abacterial meningitis or encephalitis.

A total of 296 newborn infants were studied, of whom 168 and 128 were preterm and full term, respectively. Distribution of the population by birth weight and gestational age is shown in Table I. 52/168 (30.9%) of the preterm and 36/128 (28.1%) of the full term neonates were born from pathological pregnancy, while in the rest the pregnancy was uneventful. 22.6% of the preterm (38/168) and 19.5% of the full term babies (22/128) were delivered either by Caesarean section or forceps; 31 of the preterm (18.4%) and 26 of the full term (20.3%) infants needed resuscitation at birth.

Distribution of the 296 patients according to maturity, sex, clinical diagnosis and final outcome is shown in Table II. Group I comprised patients suffering from acute respiratory disorders such as hyaline membrane disease and the variants of type-II respiratory distress syndrome. Group II contained neonates with congenital malformatious and Group III thoes with perinatal infections. Group IV consisted of neonates admitted because of various disorders of postnatal adaptation, none of which was associated with increased CSF protein concentration. In Table II it is seen that 168/296 (56,7%) were preterm and the rest (43.3%) full term infants. 60.6% (40 cases) of the deaths were due to acute respiratory disorders associated with subependymal and/or intraventricular haemorrhage in 13 patients, as revealed by necropsy. Furthermore, 21.2% (14 patients), 15.1% (10 patients) and 3.0% (2 patients) of the deaths were due to congenital malformations, perinatal infections and miscellaneous conditions, respectively.

In 100 of the newborn infants a blood sample was taken within 3 days before or after the lumbar puncture. Serum protein concentration was measured by the biuret reaction. The mean ($\pm$S.D.) birth weight and gestational age of infants in this group was 2558 ± 720 g and 36.6 ± 3.2 weeks, respectively. Mean CSF protein concentration was compared in the various birth weight and gestational age groups and correlation analysis was performed between parameter-pairs like CSF protein — birth weight, CSF protein — gestational age and CSF protein — postnatal age. Also, an attempt was made to compare the CSF protein content of patients who died with respiratory distress and those who survived it, with that of infants sufferring from conditions not causing hypoxia and oxygen dependency.

For statistical analysis, standard mathematical tests were used.

Table I

Distribution of the 296 newborn infants according to birth weight and gestational age

≤1250	1251–1500	1501–1750	1751–2000	2001–2500	2501–3000	>3000 g
18	35	24	39	62	61	57

<30	30—32	33—35	36—37	≥38 weeks
21	42	81	55	97

TABLE II

Distribution of 296 newborn infants according to maturity, sex, clinical diagnosis and final outcome

	No. (%)	I				II				III				IV			
		died		survived		died		survived		died		survived		died		survived	
Full term																	
males	88 (100)	6	(6.8)	23	(26.1)	8	(9.0)	10	(11.3)	1	(1.1)	17	(19.3)	1	(1.1)	22	(25.0)
females	40 (100)	1	(2.5)	7	(17.5)	4	(10.0)	2	(5.0)	1	(2.5)	7	(17.5)	0	(0.0)	18	(45.0)
Preterm																	
males	102 (100)	19	(18.6)	40	(39.2)	2	(1.9)	2	(1.9)	4	(3.9)	14	(13.7)	1	(0.9)	20	(19.6)
females	66 (100)	14	(21.2)	16	(24.2)	0	(0.0)	2	(3.0)	4	(6.0)	6	(9.0)	0	(0.0)	24	(36.3)

I = acute respiratory disorders; II = congenital malformations; III = perinatal infections, meningitis and encephalitis excluded; IV = miscellaneous. (Figures in parentheses indicate intra-group percentage distribution).

Results

Table III shows CSF protein levels measured in various birth weight groups of the patients. It is seen that with increasing birth weight CSF protein decreases gradually, but remains above 70 mg/dl even in babies with a birth weight of >3000 g CSF protein concentration was significantly ($p < 0.05$) higher in newborns with birth weights of 1750 g or less than in those with birth weights of more than 1750 g. In the patients, a strongly significant negative correlation ($r = -0.2527$, $p < 0.001$) was found between birth weight and CSF protein concentration ($\bar{y} = -0.01\bar{x} + 119.78$).

CSF protein concentration measured in the different gestational age groups is seen in Table IV. Similarly to birth weight, the mean protein level fell steadily with advancing gestational age, but not to below 70 mg/dl. Newborn infants of gestational age of 33 weeks or more had a significantly ($p < 0.01$ and $p < 0.001$) lower CSF protein concentration than those of less than 30 weeks gestational age. Gestational age correlated with CSF protein content negatively and significantly ($r = -0.2779$, $p < 0.001$, $\bar{y} = -2.64\bar{x} + 178.13$).

Studying the effect of postnatal age on the CSF protein level, a highly significant reverse correlation ($r = -0.2896$, $p < 0.001$, $\bar{y} = -2.42\bar{x} + 98.90$) of the two parameters could be detected.

Comparison of mean (±S.E.) CSF protein concentration of preterm and

Table III

CSF protein concentration and birth weight

Birth weight, g	≤1250	1251–1500	1501–1750	1751–2000	2001–2500	2501–3000	>3000
No.	18	35	24	39	62	61	57
mean (mg/dl)	112	98	111	83	77	71	75
S. D.	36	44	58	45	41	38	50
S. E.	8	8	12	7	5	5	7
Range	34–162	31–186	33–200	22–210	22–212	26–186	22–210

Table IV

CSF protein concentration and gestational age

Gestational age, weeks	<30	30–32	33–35	36–37	≥38
No.	21	42	81	55	97
mean (mg/dl)	118	98	86	76	73
S. D.	44	38	49	40	47
S. E.	9	6	5	5	5
Range	34–200	31–186	31–210	23–212	22–210

full term infants suffering from various diseases is shown in Fig. 1. CSF protein level was significantly ($p < 0.02$) higher in preterm infants with acute respiratory disorders than in those without hypoxia and oxygen dependency. In contrast, in full term infants, CSF protein concentration was closely similar in patients with severe hypoxia and in those who did not suffer from hypoxia (67.5 ± 13.3 and 65.8 ± 9.5 mg/dl). Therefore, CSF protein content does not seem to have a prognostic significance in neonatal pathology and, most probably, the type and/or severity of the neonatal conditions is not related to CSF protein concentration.

In the 100 newborn infants with serum protein measurement, a signif-

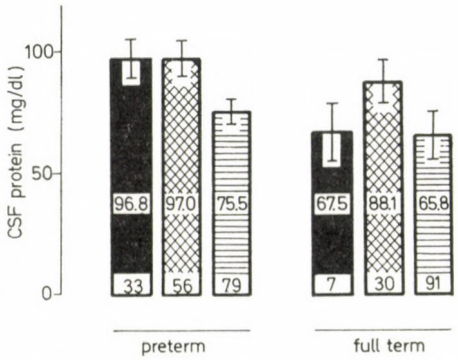

FIG. 1. Mean ($\pm$ S. E.) CSF protein concentration in preterm and full term newborn infants ■ = died with acute respiratory disorder; ▨ = suffered from acute respiratory disorder; ▤ = patients without hypoxia or asphyxia and oxygen dependency. (Figures in the middle and bottom of bars indicate mean CSF protein level and the number of patients, respectively.)

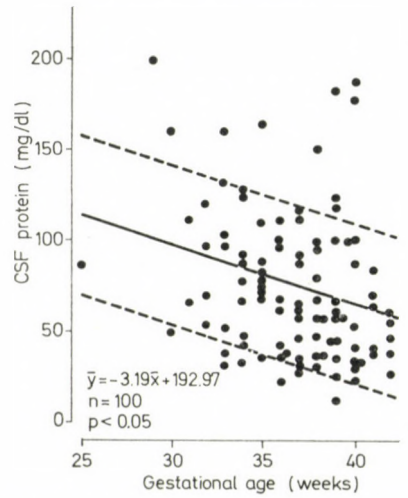

FIG. 2. Gestational age and CSF protein concentration

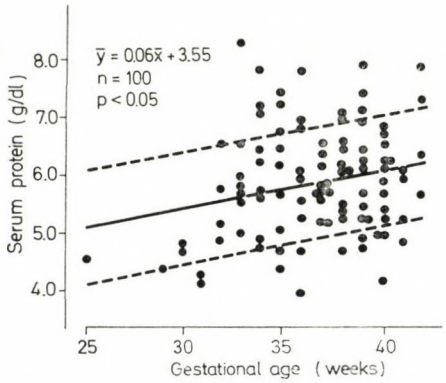

FIG. 3. Gestational age and serum protein concentration

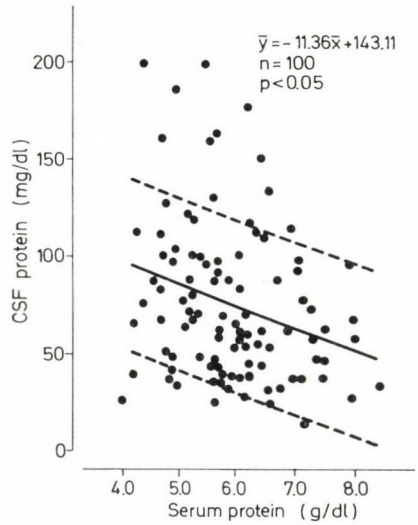

FIG. 4. Serum protein and CSF protein concentration

icantly negative correlation ($r = -0.2379$, $p < 0.05$) was observed between gestational age and CSF protein level (Fig. 2), whilst a positive relation ($r = 0.2210$, $p < 0.05$) was found between gestational age and serum protein concentration (Fig. 3), and a significant reverse correlation ($r = -0.2474$, $p < 0.05$) between serum and CSF protein concentration (Fig. 4).

DISCUSSION

The present results have confirmed the previous observations [1–9] that the CSF protein level is remarkably higher in newborn age than in infancy and childhood. Nonetheless, the wide range of individual values (Tables III and IV) make it questionable whether the CSF protein concentration has a use in diagnosis and prognosis in new-

born infants. The same conclusion can be drawn from the data shown in Fig. 1. We found very similar mean CSF protein concentrations in full term infants suffering from severe acute respiratory disorders with fatal outcome and in those who had not experienced hypoxic or asphyctic insults. In preterm babies the mean CSF protein level was significantly lower in patients without hypoxia and oxygen dependency than in those who had been exposed to them, but the wide individual variations would make it unrealistic to presume a direct relations between CSF protein content and neonatal conditions or prognosis.

The cause of the increased neonatal CSF protein concentration is debated, but the increased permeability of the blood-brain-CSF barrier must certainly have a predominant role in it. In principle, the increased permeability might be due to some disease but may well be a phenomenon related to the functional maturity of the newborn infant. We suggest the latter possibility, since a statistically significant correlation was observed between CSF protein concentration and gestational age, birth weight, and postnatal age.

The positive correlation between gestational age and serum protein level (Fig. 3) and the negative correlation between gestational age and CSF protein concentration (Fig. 2) support the view that both the serum and protein levels depend on the maturity of the newborn infant, but in an inverse way. At the same time the inverse relation between serum and CSF protein (Fig. 4) indicates that the blood-brain-CSF protein barrier already functions in pathological preterm and full term newborn infants, and it seems logical to suppose that the function of the protein barrier is related to maturity and is not affected by pathological conditions.

Finally, two points should be kept in mind when drawing conclusions from the results of this study. First it is to be emphasized that all CSF samples were obtained from sick newborn infants, and no attempt could of course be made to establish normal values. Second, it is certain that qualitative analyses of protein in serum and CSF would provide a better understanding of the mechanisms regulating the CSF protein content in newborn infants.

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Intrauterine weight retardation and the concomitant length retardation: A statistical analysis of anthropometric data

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In 237 low birth weight for gestational age term infants (birth weight $\leq$ 10th percentile; gestational age $\leq$ 37 wk) percentage deviations of birth weight and crown-heel length for gestational age were correlated (*a*) in a series of linear regressions of three subsequent groups formed according to the magnitude of weight retardation (*b*) in the pooled material by non-linear regression. Linear regressions yielded equivocal results as to the existence of concomitant length retardation (no correlation in group 1, significant correlation in group 2 and 3). The significant non-linear correlation ($i = 0.66$; $p < 0.001$) and the exponential type regression curve (Y) % length deficit = 2.45 $1.04^{X'}$ % weight deficit) gave an explanation of the controversial findings. If mild to moderate weight retardation is included in studies comparing weight and length, a greater variability and, possibly, a conclusion of non-existence of concomitant retardation will result while, if severe or extreme weight retardation are screened out using stricter criteria (e. g. 5th, 3rd percentiles, —2 SD) one would find more infants with significant length retardation and the conclusion would be the opposite, i. e. an obligatory length retardation following malnutrition.

In anthropometric-statistical studies the results should never be generalized and extrapolated below, or beyond the cut-off points chosen.

Intrauterine weight retardation is usually, but not necessarily, accompanied by a certain degree of length retardation [3, 8, 12, 14, 20]. If weight gain and growth of body length in utero are equally impaired, the result is a proportionally (symmetrically) retarded newborn infant or one with a Type I IUGR (intra-uterine-growth-retardation). If growth in weight is more or solely affected, disproportionate (asymmetrical) retardation or Type II IUGR occurs [3, 12, 13, 14, 18, 20, 24]. These types are thought to have a different aetiology and reflect, besides genetic factors, the severity and duration of intrauterine malnutrition [3, 5, 6, 12, 18, 20].

Although anthropometry proved a valuable tool for screening out intrauterine growth retardation and detecting different forms of malnutrition [2, 3, 8, 9, 10, 12, 13, 14, 22, 23, 24], there are still some controversial views regarding its usefulness especially in differentiating between symmetrically and asymmetrically retarded (underweight and wasted) infants [1, 11, 15, 16, 17, 21]. These studies suggest that in intrauterine retardation/malnutri-

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday.

tion skeletal growth is always impaired, i.e. low-birth-weight-for-gestational age never goes without short-crown-heel-length-for-gestational age.

This has led us to reinvestigate the problem by a statistical-mathematical approach, i.e. by contrasting percentage deviations of weight and length-for-age, in order to find an answer to the questions (*i*) is it obligatory for all weight retarded neonates to have a retardation in crown-heel length as well and, if so, (*ii*) is there any regularity between the magnitude of intrauterine weight retardation and that of the concomitant length retardation?

Material and Methods

The study population comprised 237 low-birth-weight-for-gestational age term ($\leq$37 weeks) neonates. All of them had birth weights below the 10th percentile according to our local weight-for-gestational age chart; infants of diabetic mothers, those with major malformations, chromosomal anomalies and inborn errors of metabolism were excluded.

For assessing the magnitude of intrauterine retardation percentage deviations from the mean values of birth weight and crown-heel length for gestational age were calculated for each individual infant according to our local standard [2]; separate charts were used for boys and girls. Since all weight deviations and all but 11 length deviations (no deviation in 3, excess length in 9 cases) were negative, these will be referred to as weight deficit (wt. def.) and length deficit (lth. def.) The 9 excess length percentages were regarded as negative deficits and given a minus sign in computations.

According to the magnitude of weight deficit, three groups were formed (Table I): 1. *mildly to moderately* retarded (wt. def. 16–30%); 2. *severely retarded* (wt. def. 31–45%); 3. *extremely retarded* (wt. def. >46%). The use of percentage deviations and the advantage of this type of classification has been described in detail [3, 9, 12]. The data for each group and the total material (mean gest. age, weight, length, wt. def., lth. def.) are given in Table I.

Linear regression analyses of length deficit (Y) on weight deficit (X) were performed in each group separately and, with their data pooled, in the total material (Table II, Fig. 1). The samples were tested for linearity and homogeneity by analysis of variance and co-variance; Snedecor's F

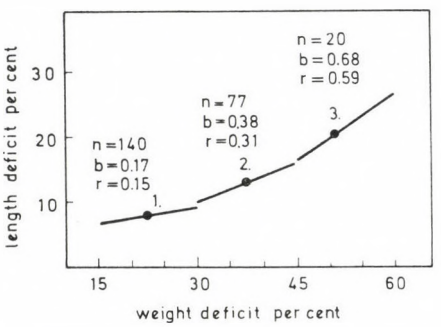

Fig. 1. Linear regression lines of the individual groups (1 mild to moderate, 2 severe, 3 extreme, weight retardation); length deficit plotted against weight deficit on a linear scale; dots represent the mean Y, X, values. Note that the individual lines resemble an exponential type curve, and the mean values might also be connected to lie on a curve

TABLE I

Relevant data of 237 low birth weight for gestational age newborn infants divided into three groups according to the magnitude of weight retardation

Group (wt. def.)	No. of cases	Gest. age, week	Birth weight, g	C-H length, cm	Weight deficit, per cent	Length deficit, per cent
1	140	39.7±	2544.5±	49.3±	22.2±	7.7±
(16–30%)		0.15	16.1	0.23	0.33	0.38
2	77	39.6±	2010.0±	46.5±	37.8±	12.7±
(31–45%)		0.20	22.9	0.33	0.45	0.74
3	20	40.1±	1676.5±	43.5±	50.2±	19.7±
(> 46%)		0.32	46.6	0.71	1.21	1.40
Total	237	39.7±	2297.6±	47.9±	29.7±	10.4±
		0.22	23.7	0.22	0.67	0.39

Group 1: mildly to moderately, Group 2: severely, Group 3: extremely, retarded in birth weight for gestational age. Mean ± SE.

TABLE II

Linear regression of length deficit for gestational age on weight deficit for gestational age according to severity of weight retardation

Group (wt. def.)	No. of pairs	Regr. const. a	Regr. coeff. b	Correl. coeff. r	p
1 (16–30%)	140	+3.90	+0.17	+0.15	n. s.
2 (31–45%)	77	−1.53	+0.38	+0.31	< 0.01
3 (≥ 46%)	20	−14.68	+0.68	+0.59	< 0.01
Total	237	−0.54	+0.37	+0.63	< 0.001

Group 1: mild to moderate, Group 2: severe, Group 3: extreme, weight retardation. Note that the slope of Y on X becomes steeper, and the correlation closer with increasing weight deficit (see also Fig. 1).

was applied to test significance. To establish a possible non-linear relationship a logarithmic transformation of Y_i values was carried out and a linear regression of log Y on X computed according to the equation: $Y = \log a + (\log b)\, X$; after decoding, the regression equation was expressed in the final (exponential) form: $Y = ab^X$ [19].

RESULTS

The relevant data of the three groups formed on the basis of increasing weight deficit are listed in Table I. Mean gestational ages, 39.7 ± 0.15, 39.6 ± 0.20, and 40.1 ± 0.32 weeks, did not differ significantly from each

other and from that of the total material (39.7 ± 0.22). Mean birth weights, 2544.4 ± 16.1, 2010.0 ± 22.9, and 1676.5 ± 46.6 g, were in accordance with the grade of retardation in each group. Mean crown-heel lengths, 49.3 ± 0.23, 46.5 ± 0.33, and $43.5 \pm \pm 0.71$ cm, decreased parallel with decreasing average weights. Mean weight deficits for age were 22.2 ± 0.33, 37.8 ± 0.45, and $50.2 \pm 1.21\%$, whereas average deficits in crown-heel length, 7.7 ± 0.38, 12.7 ± 0.54, and $19.7 \pm 1.40\%$, were less in magnitude [3, 9, 12] but similar in trend.

The influence of weight deficit on length deficit was then tested by a *series of linear regressions:* (*i*) in the total (pooled) material of 237 pairs; (*ii*) in three subsequent sections divided according to the magnitude of weight retardation (Table II, Fig. 1). In the total material, a highly significant correlation was found between weight and length deficit for gestational age ($Y = 0.37\ X - 0.37$; $r = = 0.63$; $p < 0.001$). A separate analysis of the different groups, however, yielded equivocal results: there was no correlation ($r = 0.15$; $p > 0.1$) in group 1, whereas group 2 ($r = 0.31$; $p < 0.01$), and 3 ($r = 0.59$; $p < 0.01$) showed a significant correlation. The regression equations also differed; the regression coefficients were 0.17, 0.38, and 0.59 in group 1, 2 and 3, respectively. Thus the only conclusion to be drawn is that the slope of the equation as measured by the regressive coefficient "b" becomes steeper and the correlation as measured "r" closer with the increase of the weight deficit.

Figure 1 shows the individual regression lines and the mean Y_1X_1, Y_2X_2, Y_3X_3 values plotted against each other. Two questions arise, *viz.* (*i*) is the curve rather than a straight line connecting the mean values and (*ii*) are the lines part of one common linear regression?

Analysis of variance showed that a non-linear connection exists and 43% of the total variance of Y (length deficit) is caused by non-linear effects. Further analysis by co-variance supported this finding and proved that (*i*) samples differ significantly from linear regression ($F = 4.40$; F^1_{232}; $p < 0.05$); (*ii*) the slopes of regression lines differ significantly ($F = 3.12$; Fg^2_{230}; $p < 0.05$); and (*iii*) no common linear regression can be applied ($F = = 3.02$; F^4_{230}; $p < 0.05$).

According to this, calculation of a common linear regression (Table II), however significant, cannot be justified or, at the most, one could say that a non-linear regression would better and more exactly characterize the connection between weight deficit and length deficit in this material. A best-fit curve was, therefore, sought for to describe the possible non-linear relationship. A simple graphic test (Fig. 2) suggested an exponential connection, thus a regression of log Y on X was computed (see methods) in the total material of 237 pairs.

Individual length deficits plotted against weight deficits on a linear scale, and the best-fit regression curve obtained are shown in Fig. 3. The regression equation describing the best-fit curve: $Y(\%$ lth. def.$) =$

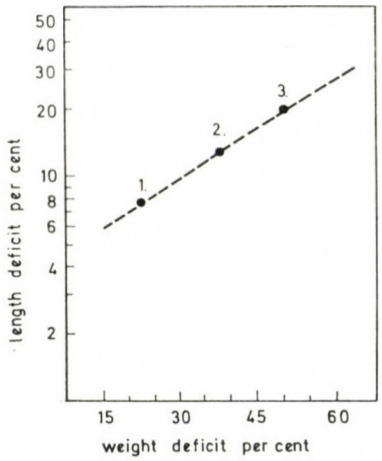

FIG. 2. Average values of length retardation plotted against weight retardation on semilogarithmic scale. Note that the mean values are situated along a straight line suggesting a logarithmic (exponential) connection

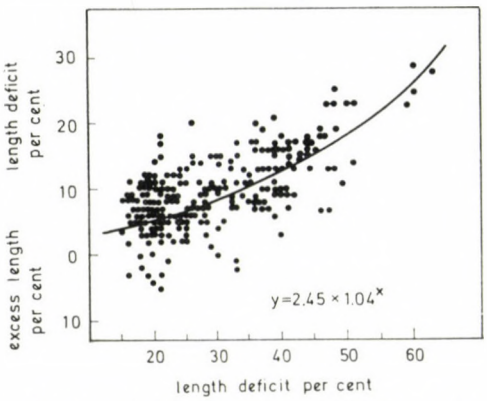

FIG. 3. Individual length deficits plotted against weight deficits on a linear scale; the best fit curve was drawn according to the equation $Y = 2.45 \times 1.04^{X}$

$= 2.45 \times 1.04^{x/\%\ \text{wt. def.}}$ sfi. proves to be exponential; the non-linear correlation index was highly significant ($i = 0.66$; $p < 0.001$).

DISCUSSION

There is an inherent association between body weight and length [3, 8, 12] which reflects the growth potential of the individual [12, 19], and will be more or less influenced by gestational age [3, 8, 9, 10, 12, 22]. It lies at hand to ascribe any correlation between percentage deviations of weight and length for age to an indirect effect of absolute weight and length, as well as to gestational age.

The fact that growth in length slows down and practically commences at 37–38 weeks of gestation

[3], and also that the weight/length ratio does not change in the last weeks of gestation [3, 8, 12, 13, 14] would exclude the latter possibility, since the present material consists only of term gest. age $\leq$ 37 wk) infants. In addition, the effect of gestational age on length deficit did not prove in a multiple correlation (partial correlation coeff. : gest. age/lth. def. = -0.12; wt. def./lth. def. = $= +0.82$).

Linear regression of absolute length on absolute weight in weight deficit groups as well as in the total material did not show a similar pattern in slope and correlation to weight and length deficit (i.e. increasing with increasing weight retardation). Neither did variance and co-variance analyses supply evidence against linearity and homogeneity regarding the correlation of these absolute parameters. Thus, the linear relationship of birth weight and crown-heel length could not possibly result in an exponential type of correlation of percentage deficits, as shown in this material.

There must be other factors, possibly nutritional (e.g. onset, duration, and severity of malnutrition), responsible for this exponential type of correlation between intrauterine weight and length retardation.

It was not the aim of this study to deal with the aetiology, pathogenesis and clinics of intrauterine retardation/malnutrition, questions which are discussed extensively in the literature [1, 2, 3, 4, 5, 6, 9, 12, 13, 14, 15, 16, 18, 20, 24]. The problems we tried to tackle are primarily methodological [3, 7, 10, 12, 20, 22, 23], that is if weight retardation will be followed by a more or less regular and obligatory length retardation, than the contrasting of weight and length for screening out stunted and wasted infants may not be a useful and valid method and would therefore be superfluous.

Our study pointed to the problem [7, 12] of the cut-off points and sample size and range. If one has a curvilinear relationship like that shown in Fig. 3, it is evident that by cutting it into sections, the more the better, several linear regressions can be obtained (Fig. 2). When the upper and lower cut off points are approaching each other, the original curvilinear shape will be diminished and a misleading conclusion might be drawn as to the linearity, and to the significance of the correlation of the parameters in question. This happens in our example as well as in other anthropometric studies contrasting weight and length (or derived parameters). In addition, in exponential type curves it might be decisive which part will be cut out: in our material the first flat section represented by group 1 (mild to moderate weight retardation) resulted in a non-significant linear correlation, whereas the second and third sections cut out from the steeper parts of the curve (groups 2 and 3) yielded stronger and significant correlations.

The conclusion we want to draw is that the more severe the weight retardation, the more expressed the

length retardation and, as we have already pointed out, [3] its frequency will be augmented. In other words, if one choses a lower cut-off point, i.e. milder criteria, for screening out intrauterine weight retarded infants, the variation in length retardation will be greater and thus the freuqency of a wasted type of malnutrition higher; and if one chooses stricter criteria (e.g. 5th or 3rd percentile, or -2 SD) the probability of severe concomitant length retardation and the frequency of stunted (symmetrically retarded) infants will be greater [3].

It is therefore important that in anthropometric studies the cut-off points (limits, range) should always be stated, as well as properly chosen for fulfilling the preset criteria and requirements of the particular study in question [3, 7, 12]. It is obvious that in statistical-anthropometric studies the correlations established for a particular section should never be generalized and/or extrapolated below and beyond the limits chosen.

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Fatal infectious mononucleosis. Report of a case with a review of the literature

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Fatal infectious mononucleosis in a child with a possible defect of T-cell suppressor function is reported. Due to an unbalanced immunoregulatory mechanism, a malignant lymphoma-like polyclonal, diffuse lymphoproliferation occurred. The possible oncogenic potential of Epstein-Barr virus in immunodeficient states is discussed.

Epstein-Barr virus (EBV), a herpes-like DNA virus, the causative agent of infectious mononucleosis (IM), has a unique lymphotropic property infecting only bone marrow derived B lymphocytes in human and some primate hosts. It does not cause cell death, but persists in the cells in a latent form and stimulates the EBV-genome-containing B-cells to proliferate. In the course of primary infection, antibodies are produced against particular viral antigens and a striking cellular response is evoked on the part of the T-cells. It is assumed that many of the symptoms accompanying IM are a direct reflection of the intense immunologic interactions which occur and that the cytotoxic T-cell response serves to limit the continued proliferation of infected B-cells [2, 8, 13, 14, 22, 26, 27, 30]. This host control mechanism is not well understood; the T-cell mediated immunity may be of great importance in making IM a self-limited disease. This immunoproliferative disorder may be considered the benign counterpart of malignant lymphoma. Nevertheless, fatal and life-threatening complications may arise, such as an obstruction of airways, haemolytic anaemia, thrombocytopenia, agranulocytosis or aplastic anaemia [2, 5, 17, 22, 26]. Infiltration by lymphocytes often causes dysfunction of liver, heart, kidney and the CNS [7, 9, 12, 22] and, rarely, neoplastic conversion of IM into malignant lymphoma is seen in patients who are immunodeficient [3, 18, 19, 20, 21], but even with good host immunity, EBV may have an invasive course [5].

REPORT OF A CASE

R. A., a boy born at term had been referred to us on the first day of his life because of Rh incompability and

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday

hyperbilirubinaemia. After an exchange transfusion he had recovered rapidly. At the age of 3 years he had had purulent otitis with protracted fever, then severe anaemia, moderate leukopenia and thrombocytopenia and hepatosplenomegaly. A haemoblastosis had been excluded by the peripheral blood and bone marrow findings; serum IgG had been normal, IgM and IgA elevated. In this period he had had varicella with a normal course after antibiotic and iron therapy and bilateral antrotomy. In the next year, in connection with an upper respiratory tract infection he had again become anaemic with moderate hepatosplenomegaly and cervical lymphadenopathy. Cervical lymph node biopsy had shown reactive changes, the bone marrow had been normal, and the specific antibodies to EBV antigen, negative. After tonsillectomy the child had been entirely asymptomatic, developed well, and his physical and haematological findings had been normal.

In August, 1980, at 4.5 years of age, a febrile illness occurred with pharyngitis and cervical lymphadenopathy, liver enlargement and a spleen palpable 4 cm below the costal margin. A 10 day antibiotic treatment had no effect. ESR was 42 mm/h, haemoglobin 6.4 mmol/l, haematocrit 32%, WBC 3.2×10^9/l, platelets 110×10^9/l, differential counts: 56% polymorphonuclears, 4% monocytes, 32% lymphocytes and 8% atypical mononuclear cells. Cultures of blood, urine and throat were negative. Chest X-rays showed moderate hilar adenopathy and heavier bronchovascular markings. A virus infection, probably IM, was diagnosed. The Paul-Bunnel reaction was negative, but the serologic tests for EBV specific antibodies were consistent with a recent EBV infection (Table I). Cytomegalovirus or toxoplasma infection were excluded

TABLE I

Serological results

Date		18. 01. 1979	29. 08. 1980	02. 09. 1980	09. 09. 1980
Toxoplasma		ND	ND	ND	1 : 10
CMV		1 : 64	ND	ND	1 : 40
HSV		1 : 4	ND	ND	1 : 160
HZV		ND	ND	ND	1 : 10
Paul-Bunnell reaction		neg.	neg.	neg.	neg.
EB-VCA	IgM	neg.	1 : 40	1 : 160	1 : 320
	IgG	neg.	1 : 80	1 : 40	1 : 1280
EBNA		neg.	neg.	neg.	neg.

ND: not done, CMV: cytomegalovirus, HSV: Herpes simplex virus, HZV: Herpes-zoster-varicella virus, EB-VCA: Epstein-Barr virus capsid antigen, EBNA: EBV nuclear antigen.

by the serological results. Antibiotic therapy had no effect. The high fever with daily spikes persisted, the lymph nodes, liver and spleen size, anaemia, leukocytopenia and thrombocytopenia increased progressively.

Bone marrow biopsy showed beside normal myelopoiesis a slightly hyperplastic erythropoiesis. Some large mononuclear cells had an irregular loosely structured nucleus and a faintly basophilic cytoplasm. The number of plasma cells was slightly increased and some deformed normoblasts appeared. There was no sign suggestive of leukaemia or lymphoma (Fig. 1).

The lymph node architecture was effaced, with partly recognizable primary follicles and some sinuses. An extreme paracortical immunoblast-plasma cell reaction, which at sites seemed focal, was characteristic. Amongst the many immature immunoblasts with prominent nucleolus, some multinucleated forms similar to Sternberg-Reed cells occurred (Fig. 2). Mitotic cells were not abundant, but many cells showed marked pyknosis. The highly immature and extensive immunoblast-plasma cell proliferation suggested a malignancy, but the repeatedly performed immunoperoxidase reaction for immunogloublin demonstrated that the plasma cells as well as the immature plasmoblasts-immunoblasts were of polyclonal nature indicative of a reactive process (Fig. 3).

Immunological studies revealed increased levels of serum immunoglobulins *viz.* IgG, 17.6 g/l; IgA, 2.96 g/l; IgM, 3.0 g/l. There was marked hypocomplementaemia, and a significantly increased cold agglutinin titre (1 : 512). Examination of the E and EAC rosettes revealed a normal T-cell number with increased number of active T-cells and of B-cells in peripheral blood. The lymphocytes showed augmented blastic transformation on phytohaemagglutinin (PHA) stimulation. The diagnosis of IM seemed to be justified by the serologic, histologic and immunological findings.

The patient's febrile condition remained unchanged, the leukocyte count ($1.2-1\times10^9$/l) and platelet count ($20-40\times10^9$/l) remained low despite of substitution. In spite of negative bacterial cultures, combined antibiotic treatment and later prednisolone were given, but the deteriorating condition could not be influenced, the cervical lymph nodes, the liver and spleen grew further. SGOT increased from 29 to 92 U/l, SGPT from 11 to 81 U/l. The prothrombin level was 23%. IgA increased to 7 g/l, IgM to 5.2 g/l. X-rays showed heart dilatation, and the ECG a myocardial lesion. On the 20th day gastrointestinal bleeding and jaundice with moderate direct bilirubinaemia ensued. Repeated bone marrow biopsy showed the same histology as before, only the mononuclear cells had moderately increased in number. The gastrointestinal haemorrhage continued despite of blood replacement, and with signs of hepatic dysfunction heavy proteinuria and mild azotaemia developed. The patient died on the 21th hospital day.

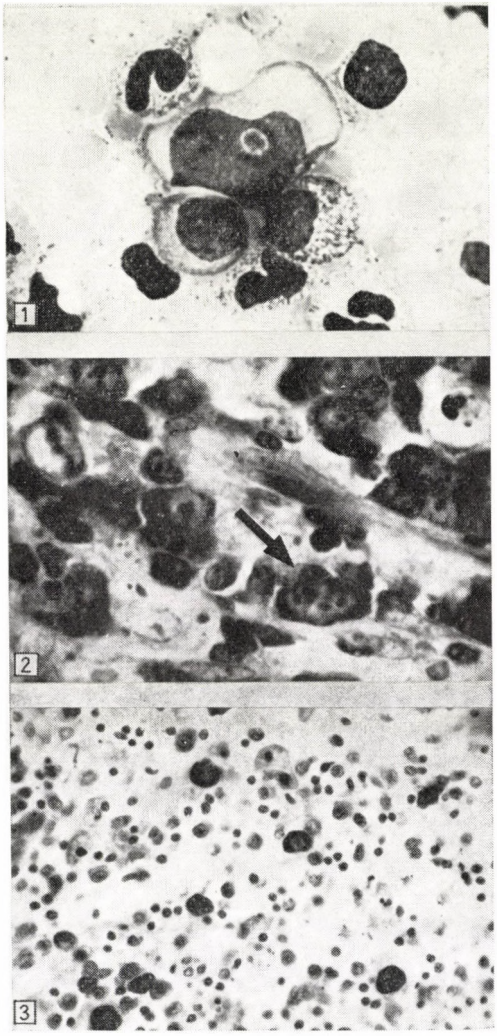

FIG. 1. Bone marrow biopsy. Beside mature myeloid cells a plasma cell and a large mononuclear cell with wide cytoplasm and prominent nucleolus can be seen. Imprint preparation, May-Grünwald-Giemsa staining, ×1000

FIG. 2. Marked immunoblast-plasma cell infiltration with Sternberg — Reed-like cell (arrow). Lymph node, Giemsa staining, ×1000

FIG. 3. Immature immunoblasts — plasma cells show a positive immunoperoxidase reaction for IgM — lambda light chains. Lymph node, ×400

Necropsy revealed generalized lymphadenopathy, hepatosplenomegaly, cerebral and pulmonary oedema, myocardial and renal fatty degeneration, and fatty infiltration of the liver. The intestinal mucosa was hyperaemic and partly haemorrhagic, the Peyer plaques were protruding and oedematous.

The architecture of lymph nodes was partly distorted with an extreme

immunoblast-plasma cell proliferation and Sternberg-Reed like cells and several macrophages. The immunoperoxidase reaction showed the proliferation to be unequivocally polyclonal, the IgG positive cells predominated, but IgA and IgM positive cells were also seen. Concerning the light chains of Ig-s, cells containing both kappa and lambda light chains were seen. The kappa/lambda ratio was 0.66. Similar infiltrations were noted in the intestinal tract, in the thymus, the spleen and the lungs. In the myocardium, perivascular oedema and slight lymphocyte-plasma cell infiltration and in the liver, periportal immunoblast-plasma cell infiltration and focal necrosis were found.

Discussion

During the two and a half year follow-up of the patient the suspicion of malignant lymphoma or leukaemia had been raised many times, but could never be proved. He reacted with lymphadenopathy, hepatosplenomegaly and pancytopenia to common upper respiratory tract infections. Still, he thrived well, the disquieting symptoms were transient and, in fact, absent in the last year. His serum immunoglobulin levels were normal and his lymphoid tissues revealed no pathologic reaction. The varicella ran a normal course, therefore the possibility of an immunodeficiency was not considered. In this respect, the family history was also negative.

At the beginning of the last illness the signs were suggestive of IM, but the possibility of malignant lymphoma was also considered. On the basis of specific serologic tests there was no doubt of a recent EBV infection, i.e. IM [2, 8, 27], in spite of the negative Paul-Bunnell test which is characteristic at this age [22, 29]. The IM turned out to be a rapidly progressing lymphoma-like fatal illness similar to the X-linked lymphoproliferative immunodeficiency syndrome [3, 18, 19, 20, 21] or to the sporadic cases of overtly or latently compromised immunocompetence [5, 23, 24, 28, 31]. The findings in 11 such lymphoma-like fatal cases of IM are shown in Table II.

Though the T-cell function was not thoroughly evaluated, a deficient cellular immunity could not be excluded. In the presence of a good EBV specific antibody response, the IM showed a grave course. The absolute number of lymphocytes was low and investigations of the cellular immunity revealed a hyperreactivity in PHA induced blastic transformation, a normal proportion of E-rosette forming and an increased proportion of B-cells in peripheral blood.

In contrast, an acute IM with balanced, well-controlled immune responses is characterized by increased absolute and relative numbers of T lymphocytes, a low proportion of circulating B-cells and a hyporeactivity to PHA stimulation [6, 13, 26]. These findings suggest an insufficient suppressor function of T-cells resulting in an unlimited reactive lymphoproliferation [11].

TABLE II

Lymphoma-like fatal cases of EBV-induced infectious mononucleosis reported in the literature

Authors Cases, age/sex	Serologic tests: Heterophil antibodies	Serologic tests: Spec. EBV	Immunological examinations	Histologic and necropsy findings
Bar et al [3]				
16y/M	pos.	neg.	spontaneous blastic transformation, no increase to PHA stimulation	Diffuse involvement of all lymphoid tissues by infiltration by immature cells, some differentiation to plasma cell.
17y/M	pos.	—	—	Infiltration in bowels, liver, brain. Disseminated lymphoproliferation with infiltration and actual tumour formation in liver, spleen, lymph nodes, intestinal tract, lungs, brain and meninges.
22y/M	pos.	—	—	Lymphoreticular infiltration with atypical lymphocytes, lymphoblasts and occasional plasma cells. Blurred architecture of lymph nodes. Infiltrations in liver, spleen and gastrointestinal tract.
Purtilo et al [18]				
23m/M	pos.	—	serum IgG : 12g/l IgA : 6.2 g/l IgM : log/l	Lymph nodes: markedly expanded germinal centers, numerous mitotic figures, lymphoid cells with prominent nucleoli, plasma cells, histiocytes. Immunoperoxidase reaction for Ig showed polyclonality. Infiltrations in liver, spleen, bone marrow, meninges, heart.
8y/M	pos.	—	marked increase in IgM, slight increase in IgG, normal IgA	Heavy lymphoid infiltrations (numerous plasma cells) in thymus, spleen, liver, oesophagus, brain, kidney, pancreas, adrenals and myocardium.
Purtilo et al [19] 2 well-documented cases of IM in boys				Depletion of T cells in lymph nodes and spleen, plasmocytosis in haemopoietic organs and infiltration of brain and viscera by lymphocytes and plasma cells.
Purtilo et al [20]				
16y/M	pos.	pos.	T-cells were decreased in number and failed to respond to PHA	Proliferating plasma cells in all lymph nodes. An immunoblastic sarcoma of B-cells replaced 3 cervical lymph nodes. Infiltration in spleen and bone marrow. The lymphoblastoid cells contained EBNA

TABLE II (continued)

Authors Cases, age/sex	Serologic tests: Heterophil antibodies	Serologic tests: Spec. EBV	Immunological examinations	Histologic and necropsy findings
Britton et al [5] 3y/F	—	pos.	No finding suggestive of immunodeficiency	Distorted architecture of tonsils and lymph nodes. Atypical lymphocytes infiltrated the bone marrow, lungs, liver, kidney and thymus. EBNA positive cells in tissues of involved organs.
Robinson et al [23] 4y/F	—	pos.	Hyporeaction to PHA stimulation	Extensive polyclonal lymphoproliferation, in lymph nodes, spleen, liver, bone marrow, lungs and stomach. Poorly differentiated lymphoplasmocytic malignant process. Cells containing lambda light chains of Ig (70%) far outnumbered those with kappa light chains (17%). EBNA was detected in majority of mononuclear cells.
Thestrup-Pedersen et al [28] 6y/M	—	pos.	T-IgG-lymphocytes (suppressor) were decreased. Normal function of lymphocytes after stimulation with mitogen	Immunoblast-plasma cell infiltration and Reed-Sternberg-like cells in lymph nodes. Immunoperoxidase technique showed the polyclonality of cytoplasmic immunoglobulins. The generalized lymphoproliferation resembled histologically Burkitt lymphoma. Cells showed EBNA positivity in 50–95%.

Fatal IM is infrequent; in the USA, 30 to 35 cases occur in a year [4]. Until 1970 only 20 fatal cases with clinical, haematological and serological evidence of IM were reported [17] and during the ten-year period ending in 1976, 195 cases were recorded by the Center of Disease Control in Atlanta, Georgia [4]. The scarcity of reports of fatal IM may be due to erroneous diagnoses and a lack of unterstanding of the potential seriousness of the disease. Even the characteristic histological picture of immunoblastic proliferation with mononuclear cell differentiation to plasma cell and reactive macrophages may pass unrecognized and instead of IM certain types of malignant lymphoma may be diagnosed [23]. Experimental [1, 15] and clinical [18, 21, 22, 26] evidences support the notion that the EBV can become oncogenic in immunodeficient hosts. Renal transplant recipients and children with inherited immune deficiency are at high risk of lymphoma [19] and defective immune response to EBV may

initiate persistent polyclonal proliferation of B-cells [11, 18].

Conversion from polyclonal to monoclonal proliferation is probably the result of a cytogenetic error in the B-cell. In an individual with intact immune mechanisms, only a cytogenetically aberrant monoclonal cell line can probably escape the immunosurveillance.

IM and a recently described disease, immunoblastic lymphadenopathy, have many similarities; both simulate a graft-versus-host reaction [10]. Angioimmunoblastic lymphadenopathy can turn into malignant immunoblastic sarcoma [16]. IM may turn into immunoblastic B-cell sarcoma in patients with X-linked lymphoproliferative immunodeficiency syndrome and in renal transplant recipients. The unusual feature of immunoblastic sarcoma occurring in these conditions are a polyclonality of the B-cell [21].

In IM, the atypical lymphocyte population is potentially capable of self-replication and malignant growth [1]. Thus, IM could be regarded as a model of a neoplastic or preneoplastic process which is almost invariably regulated successfully. Study of this immunoregulatory mechanisms could well lead to a better understanding of the evolution of malignant lymphoid disease in man.

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Glycosaminoglycan and hydroxyproline excretion in diabetic children

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Glycosaminoglycan and hydroxyproline excretion was studied in 24 diabetic children. While diabetic children under satisfactory control had a normal excretion rate, hyperglycaemic, ketotic patients excreted more glycosaminoglycan and hydroxyproline than did the controls. The high urinary glycosaminoglycan excretion was found to be due to increased excretion of hyaluronic acid.

Metabolism of collagen, the major connective tissue protein, is affected in diabetes [2, 5]. According to recent data [11, 12] the excretion rate of the metabolic end product of the macromolecules is also altered. In addition, the excretion rate of hydroxyproline (HYP) and glycosaminoglycans (GAG) may also be influenced by growth retardation which is often associated with diabetes [7].

Material and Methods

Subjects. Twenty four children suffering from insulin-dependent diabetes mellitus (IDDM) and 62 healthy, age and sex matched children were studied. On the basis of the presence of ketone bodies in the urine sample at the day of examination, patients were divided into ketotic and non-ketotic group. The reason of hospitalization of the ketotic children was either the first admission for the diagnosis of diabetes mellitus (6 cases) or infections, diet-breaking resulting in hyperglycaemic ketosis. The non-ketotic children were under satisfactory control. Relevant data of the two groups are summarized in Table I.

Laboratory methods. Apart from routine sampling, 24 hours urine samples were collected and stored at —20 °C. Total and free HYP were determined according to Kivirikko et al. [8]. Macromolecular GAG was isolated by cetylpyridinium chloride (CPC). Fraction was done by cellulose-Celite column chromatography [13]. Excretion of total GAG was calculated as sum of GAG fractions and expressed as equivalent of uronic acid. Fragment GAG was isolated from the supernatant of CPC isolate on ion-exchange column [4]. Uronic acid was determined with borax-carbasole and glucose with the anthrone reaction.

Serum glucose was measured enzymatically (GOD-POD Galenofarm) and urinary sugar by the o-toluidine method. Ketone bodies were detected by Acetest (Ames). HbA_{1c} and serum glycosyl protein were measured by a modified colorimetric method [10].

For statistical analysis, Student's t test and linear regression analysis were used.

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday.

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TABLE I

Characteristics of the study groups

	No. of children	Age, years	Duration of the disease	Daily blood glucose, mean ± S. D. mmol/l	Urinary glucose, mmol/day	Urinary ketone	HbA_{1c}	Serum glycosylprotein, μmol/g protein
Diabetic, non-ketotic	17	10.2 3–16	3.1	10.11±2.91	604±428	—	4.6	1.43
Diabetic, ketotic	22	11.3 3–16	2.8	16.10±3.89	1908±1194	+ − ++++	5.7	1.70
Control	62	10.9 3–16	—	4.20±0.90	—	—	2.8	0.96

RESULTS

Hydroxyproline excretion

Individual hydroxyproline excretion rates are shown in relation to the normal range obtained in healthy children (Fig. 1). In the diabetic children high values were mainly found. There was no difference between sexes as well as between newly diagnosed cases and those under long-term treatment.

The diabetic and normal children were divided into three age groups in order to see the age-related changes in hydroxyproline excretion. From Table II it is evident that both in diabetic and normal children hydroxyproline excretion increases with age. Though the mean HYP excretion rate in the three age groups of diabetic children showed a somewhat higher level than in the controls, the difference was not found to be significant.

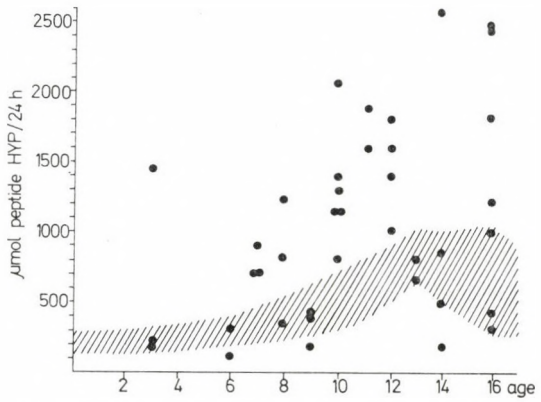

FIG. 1. Peptide hydroxyproline excretion. Normal range and individual values of diabetic children

TABLE II

Urinary HYP excretion in different age groups of diabetic and healthy children, (μ mol/24 h $\pm$ S. D.)

	Age groups		
	< 8 years	8–11 years	11–16 years
Diabetics	621.4$\pm$451.1 n = 12	665.0$\pm$497.3 n = 14	850.5$\pm$817.2 n = 21
Controls	270.1$\pm$98.0	560.1$\pm$110.3	720.2$\pm$396.3

TABLE III

Hydroxyproline excretion in normal, non-ketotic, and ketotic diabetic children

	Peptide HYP, μmol/24 h	Free HYP, μmol/24 h	Free HYP, per cent of total
Diabetic, non-ketotic	545.8$\pm$340.0	65.8$\pm$42.0	12.1
Diabetic, ketotic	1425.3$\pm$640.7	63.7$\pm$39.4	4.5
Normal	662.6$\pm$188.5	13.9$\pm$9.3	2.1

TABLE IV

Urinary GAG excretion in different age groups of diabetic and healthy children (μmol uronic acid/24 h $\pm$ S. D.)

	Age groups		
	< 8 years	8–11 years	11–16 years
Diabetics	33.4$\pm$27.5 n = 12	26.2$\pm$15.6 n = 14	25.4$\pm$18.4 n = 21
Controls	13.7$\pm$6.2	19.2$\pm$10.7	22.4$\pm$11.1

TABLE V

Glycosaminoglycan excretion in diabetic and normal children (mean, expressed as μmol uronic acid/day $\pm$ S. D.)

	Total GAG	Fragment GAG	Macromolecular/fragment GAGs	Hyaluronic acid	Chondroitin sulfate	Dermatan sulfate
Diabetic, non-ketotic	14.3$\pm$5.2	22.3$\pm$17.7	0.64	2.6$\pm$0.4	7.8$\pm$2.5	0.7$\pm$0.5
Diabetic, ketotic	44.9$\pm$20.4	43.3$\pm$24.7	1.03	25.2$\pm$15.9	13.3$\pm$4.6	1.4$\pm$1.3
Control	20.1$\pm$6.1	41.9$\pm$23.6	0.48	1.6$\pm$0.6	12.7$\pm$4.1	0.4$\pm$0.3

Evaluation according to the degree of metabolic control is shown in Table III. Peptide HYP excretion of ketotic, diabetic children was higher than either that of normal controls ($p < 0.01$) or that of diabetic but non-ketotic patients ($p < 0.01$). HYP excretion in non-ketotic children corresponded to the level of normal children.

As to free HYP in diabetic children, it was significantly higher than in the normal group. Free HYP excretion in non-ketotic diabetic children amounted to 12.1%.

Glycosaminoglycan excretion

The rate of total macromolecular GAG excretion showed a tendency similar to that of HYP (Fig. 2). GAG excretion rate seemed to be independent of sex and the duration of diabetes.

Mean excretion in different age groups is summarized in Table IV. Diabetic children excreted more GAG than controls but only in the youngest age group was the difference significant statistically.

Table V. summarizes the results according to the degree of metabolic control.

In the children with satisfactory metabolic control (non-ketotic group), GAG excretion was lower than in the controls but in the ketotic hyperglycaemic children it was significantly higher than in healthy ($p < < 0.001$) as well as in non-ketotic diabetic children ($p < 0.01$).

Excretion rates of CPC non-precipitable GAG (fragment GAGs) were the same in the three study groups. Mean values of the excreted quantities of fragment GAGs and their relation to the macromolecular GAG are given in Table V.

Individual GAG fractions showed characteristic patterns in the three groups (Fig. 3). The large amounts of GAG excreted by ketotic children differed substantially in their compo-

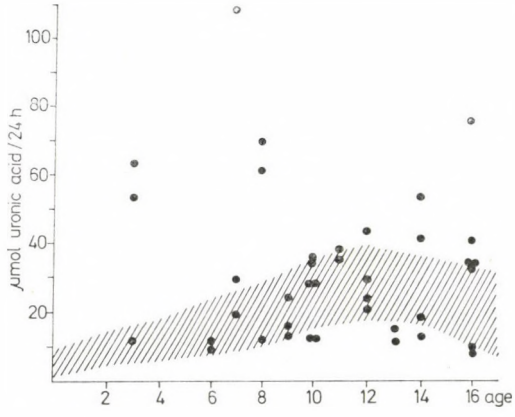

FIG. 2. Macromolecular glycosaminoglycan excretion. Normal range and individual values of diabetic children

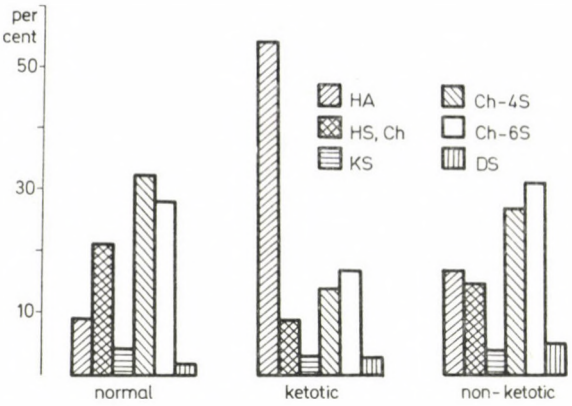

FIG. 3. Quantitative distribution of GAG components in the urine of normal and diabetic children. HA = hyaluronic acid, HS, Ch = heparan sulfate, chondroitin, KS = keratan sulfate, Ch-4S = chondroitin 4-sulfate, Ch-6S = chondroitin 6-sulfate, DS = dermatan sulfate

sition from that excreted by healthy or non-ketotic children. While in normal children, 30% of urinary GAG excretion was chondroitin sulfate, in the ketotic diabetics, hyaluronic acid constituted more than 50% of the urinary GAGs (range, 38.8% to 77.4%).

Due to the method applied, the "hyaluronic acid" fraction of the urinary GAGs is chemically heterogeneous. Besides hyaluronic acid it contains non-sulfated, low sulfated and low molecular weight GAG metabolites. There was no difference between the diabetic and control groups in the excretion rate of keratan sulfate, heparan sulfate and chondroitin. Though the ketotic group excreted three times more dermatan sulfate than did the other two, the difference was not significant statistically.

The quantity of urinary chondroitin sulfates was found to be the same in ketotic and control children. The somewhat lower excretion rate of chondroitin sulfates in the non-ketotic group turned out to be significant.

The hyaluronic acid fraction of GAGs was ten times higher in ketotic than in non-ketotic and control children ($p < 0.001$). The high GAG excretion in diabetes is mainly the consequence of the altered hyaluronic acid excretion.

Correlation between urinary connective tissue metabolites and other biochemical parameters in diabetes

A close correlation was observed between peptide HYP and total GAG excretion in diabetic children ($p < 0.01$). Furthermore, a significant correlation was found between

a) HYP excretion and the mean daily blood glucose values;

b) GAG excretion and the mean daily blood glucose values;

c) urinary HYP and daily sugar excretion (Fig. 4). On the other hand, there was no correlation between urinary metabolites (HYP, GAG) and HbA_{1c}, glycosylated serum protein levels, the daily insulin dose, and urinary glucose.

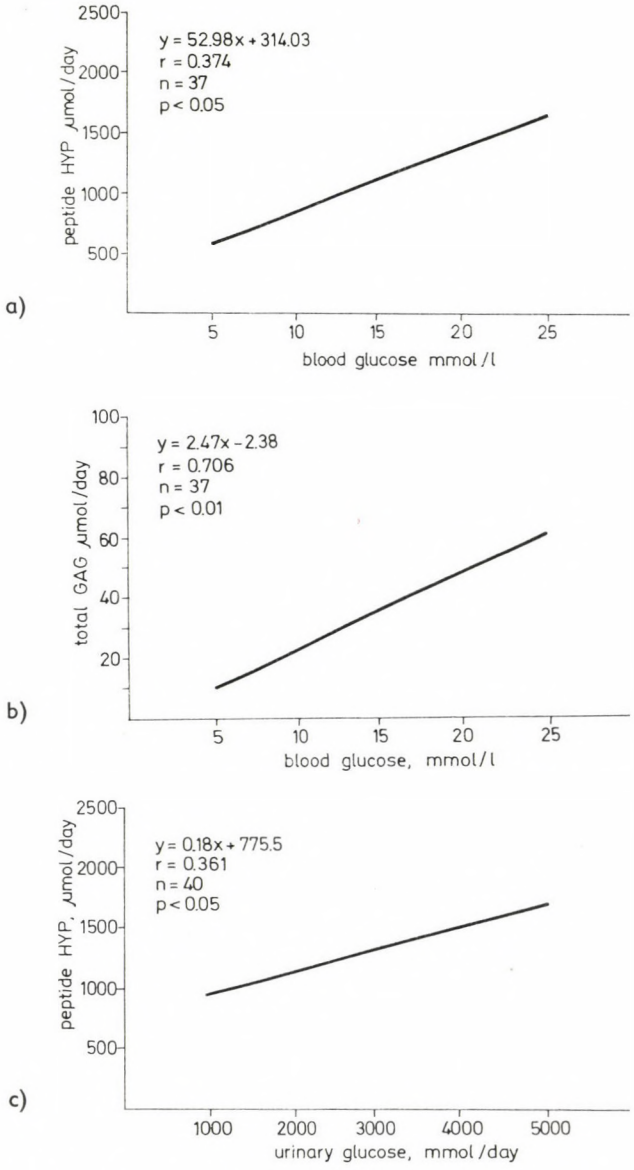

FIG. 4. Correlation between: *a)* Urinary peptide hydroxyproline excretion and mean blood glucose level. *b)* Urinary total glycosaminoglycan excretion and mean blood glucose level. *c)* Urinary peptide hydroxyproline and urinary sugar excretion

Discussion

Abnormalities in the metabolism of connective tissue macromolecules and the excretion rate of the metabolites in diabetes mellitus have been reported [1, 3, 6, 7, 11]. In animals with experimentally induced diabetes, the GAG content decreased and the soluble collagen increased [2]. Some authors, however, observed a higher hyaluronic acid content in "diabetic" than in normal cell line [5]. Total GAG excretion rate and the GAG/creatinine ratio were found to be higher in diabetic patients than in controls [6, 11]. Bodadonna et al reported decreased HYP excretion in diabetics [3], but other authors found it to be normal [1, 8] while o-hydroxylysyl glycosides, another characteristic collagenous metabolites present in urine, were excreted at lower rates than in controls [12].

On the basis of the present findings it has been concluded that in diabetic patients connective tissue metabolism and the excretion of different metabolites depended on the actual clinical and biochemical status. A diabetic child under good metabolic control grows normally, with undisturbed connective tissue metabolism and normal HYP and GAG excretion rates. During a one week period, patients may excrete HYP and GAG at high and normal rates, as we have frequently observed.

Connective tissue metabolism may rapidly change when HYP and GAG excretion correlates well with the rapidly changing parameters like blood glucose and urinary glucose excretion. In well-controlled out-patients, Lubec et al observed a correlation between GAG excretion and HbA_{1c} level [11].

Our results are in accordance with that obtained with cell lines of diabetic animals. These cultured cells not only contained but also excreted more hyaluronic acid than did normal cells [5]. It was interesting to note such a high hyaluronic acid level in the urine of intrauterine malnourished infants; in all probability it must have been due to the increased growth rate often seen in such neonates after birth [9].

The present results suggest that in diabetes the rate of connective tissue metabolism is altered and this is reflected in simultaneous changes in both HYP and GAG excretion. The high free HYP excretion in diabetic children is presumably caused not only by changes in collagen degradation, but by other metabolic alterations and changes in the renal threshold of this amino acid.

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A diagnostic survey of patients referred for chromosome analysis

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Out of 692 patients referred for chromosome analysis because of abnormal clinical features, 199 (28.7%) had chromosomal abnormalities. In addition, assessment of the abnormal phenotypic features and the data of other (laboratory, X-ray, etc.) examinations revealed 25 (3.6%) single gene disorders, 25 (3.6%) recognizable syndromes or associations of unknown aetiology, and in 4 cases (0.6%) environmental agents were established as possible aetiological factors. Altogether, of the 692 cases, 253 (36.5%) had a definitive diagnosis, while the remainder 439 were undiagnosed.

These results suggest that (*i*) chromosome analysis is worthwhile if the patient has significant clinical abnormalities, and (*ii*) a request for chromosome analysis should be viewed as one step in syndrome identification, so that a normal karyotype should stimulate the physician to further efforts to establish a diagnosis.

The present survey analyses the outcome of chromosome examinations of 692 referred cases (in and out-patients) over the eight-year period 1973–1980 in order to assess the contribution of chromosome analysis to the diagnosis of the malformed patients. The efficacy of our policy to establish diagnoses other than chromosomal aberration in patients referred for chromosome analysis is also evaluated.

Patients and Methods

During the 8-year period a total of 1048 chromosome examinations was carried out. Cases were classified into five groups: *A*) suspected of having chromosome anomalies, because of abnormal clinical features, 692 patients; *B*) children and adult family members of patients with chromosome aberrations or congenital malformations, 259 cases; *C*) adult volunteers, 38 cases; *D*) children screened by dermatoglyphic analysis, 39 cases; *E*) adult patients with chronic myelocytic leukaemia, 20 cases, with bone marrow examinations. In the following we shall deal only with group *A* (66% of the total of 1048 cases).

The majority of patients in group *A* were infants and older children referred by practising paediatricians for chromosome analysis. About a quarter of the cases were in-patients of our department.

All the karyotypes were analysed from lymphocyte cultures. At the beginning, G banding and other staining methods were used only where indicated, but in the last four years G and C banding was routinely carried out. A minimum of 12 metaphases were examined from each patient and

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday.

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the method of Bochkov et al. [1] was applied to exclude mosaicism. In addition, careful physical examinations were done repeatedly and further investigations (laboratory, X-ray, etc.) were carried out.

The results were assessed according to Winter et al. [7] with certain modifications. Cases were classified into groups according to the reason for referral. One of the following reasons for referral was usually given: 1. suspected chromosome abnormality specified by name; 2. multiple malformations; 3. children with odd-looking appearance and/or multiple minor anomalies; 4. single congenital (major or minor) anomaly; 5. mental retardation with no other malformation; 6. ambiguous genitalia or other sex anomalies; 7. miscellaneous (e.g. failure to thrive, dystrophy, etc.) including 9 cases with psychiatric disorders.

The final diagnoses were classified as 1. chromosome abnormalities; 2. single gene disorders; 3. recognized syndromes or associations of unknown aetiology; 4. environmental agents (e.g. serologically proved intrauterine infection, etc.). Cases with no chromosome aberration were classified into single gene disorders, recognised syndromes, or environmental agents only, when the physical features and/or the other findings strongly suggested the diagnosis in question. Patients undiagnosed at the time of chromosome analysis but subsequently given a diagnosis on reassessment at a later age are labelled by the final diagnosis.

TABLE I

Results of examinations of 692 patients referred for chromosome analysis because of abnormal clinical features

Reason for referral	No.	Abnormal karyotype	Single gene disorder	Syndrome or association of unknown aetiology	Environmental agent
Down syndrome	209	141 (67.5)			
Patau syndrome	11	5 (45.5)		1	
Edwards syndrome	17	8 (47.0)			
Mosaic 8-trisomy	2	1			
Cat eye syndrome	2				
Cri du chat syndrome	2	1			
Turner syndrome	38	17 (44.7)			
Klinefelter syndrome	19	6 (31.6)			
Multiple malformations	80	5 (6.2)	11 (13.7)	20 (25)	3
Odd-looking face and/or multiple minor anomaly	93	4 (4.3)	7 (7.5)	2	1
Single malformation or anomaly	40				
Mental retardation	54	1	1		
Ambiguous genitalia or other sex anomaly	64	7 (10.9)	6 (9.3)	1	
Miscellaneous	61	3			
Total	692	199 (28.7)	25 (3.6)	25 (3.6)	4 (0.6)

Results and Discussion

Of the 692 cases referred to us because of abnormal phenotypic features, 199 or *28.7%* had chromosomal abnormalities (see Table I). This frequency was similar to that observed in other studies [3, 5, 6]. Surprisingly, a higher rate of chromosomal abnormalities was found in females (32.5%) than in males (25.8%). A similar observation was reported by Singh [5] without any interpretation. These two data raise the question of a difference in the incidence of chromosome anomalies between males and females. This seems unlikely, especially as in the eight-year period we found chromosomal abnormalities in 102 males and 97 females, thus practically in equal number. More than twice as many males than females were, however, referred for chromosome analysis because of dysmorphic clinical features resulting in a difference in frequency between males and females observed. Therefore, the question is, are there more males than females with dysmorphic phenotype but normal chromosomes. Our observation and that of Singh [5] suggest that dysmorphic clinical features might be a slightly stronger indication for chromosome analysis in females than in males.

There were 209 patients who had the clinical features of *Down syndrome*. In 140 of these (66.9%) the initial suspicion was confirmed by the chromosome analysis. Of those referred because of suspected Down syndrome, only one out of the 209, a male infant with a small metacentric chromosome (47, XY, +mar) was given a definitive diagnosis other than 21-trisomy. All cases finally diagnosed as having 21-trisomy were referred with this diagnosis. Of the 140 cases with confirmed Down syndrome, ten (7.1%) were due to translocation (one D/G, one G/G, five 14/21, three 21/21), while three (2.1%) displayed a mosaic pattern.

Thirty-four cases were referred because of suspected *specific autosomal abnormalities* other than Down syndrome. About half of them had the specific chromosomal abnormality. Of the 11 cases referred because of suspected *Patau syndrome*, five were confirmed by chromosome analysis (one of them was due to translocation), while one infant had holoprosencephaly anomalad with normal karyotype. In one case finally diagnosed as having 13-trisomy, the specific diagnosis was not suggested by the referring clinician; the reason for referral was "multiple malformations".

Of those referred because of suspected *Edwards syndrome*, the clinical diagnosis could be confirmed in 7 out of 11 females, and in one out of 6 males, in accordance with the known preponderance of females to males in Edwards syndrome. Of these, two were mosaic normal/+18.

Out of 38 cases of suspected *Turner syndrome*, 17 (44.7%) had chromosomal abnormalities (of these, five had mosaic pattern). Apart from these 17 cases, two further cases, a newborn referred because of multiple malformations, and a 4-month-

old infant with ambiguous genitalia, were found to have a XO karyotype. Of those referred because of suspected Turner syndrome, only one case—an infant with Klippel-Feil anomalad—was given a definitive diagnosis other than Turner syndrome.

Out of 19 cases referred because of suspected *Klinefelter syndrome*, six (31.6%) had the specific chromosomal abnormality.

The 80 cases referred for chromosome analysis because of *multiple malformations* had a variety of "final diagnosis". Detailed diagnoses are given in the Appendix. Chromosomal abnormalities accounted for 6.2% of the group. In about two-fifths of the 80 cases a recognizable syndrome or association could be diagnosed, due to a single gene defect or of unknown aetiology. In three infants, environmental agents (two cytomegalovirus infections and one maternal diabetes) were the most probable aetiological factors responsible for the congenital abnormalities.

Of the 93 children referred because of *odd-looking face* and/or *multiple minor anomalies*, four (4.3%) had chromosome abnormalities, seven (7.5%) recognizable syndromes, while one case was diagnosed as fetal alcoholic syndrome (see Appendix).

No chromosomal abnormality could be observed in patients with single malformation or minor anomaly.

In the *mental retardation* referral group, one chromosomal aberration, a balanced 13/15 translocation, was found among the 54 cases. Although the aetiological role of this chromosomal abnormality is questionable, this was the only abnormal finding in this patient. In one referred case, a 2.5-year-old girl, Sanfilippo disease was finally diagnosed.

Of the 64 cases referred because of *ambiguous genitalia or some other sex anomaly*, seven had chromosomal abnormalities (2 XY/XO mosaicisms, 1 each XY/XX, XX/XXX, XY/XXY mosaicism, and 1—1 XO, XXXXY aneuploidy). Six further cases were diagnosed as single gene disorders, three adrenogenital syndromes, and three testicular feminisations, while in one boy referred because of cryptorchidism, the clinical features suggested the diagnosis of Noonan syndrome.

In the *miscellaneous* group, chromosomal abnormalities could be observed in three (two Yq deletions, one XYY) out of the 9 cases with psychiatric disorders.

Thus, of the 692 cases referred because of suspected chromosomal abnormalities, 28.7% had chromosomal aberrations, 3.6% single gene disorders, 3.6% recognised syndromes or associations of unknown aetiology, and in 0.6% of the cases environmental agents were the probable cause of congenital abnormalities. Altogether, of the 692 cases, 253 (36.5%) were given a definitive diagnosis, the remainder being classified under idiopathic congenital (major or minor) abnormalities, idiopathic mental retardation, or undiagnosed odd-looking babies.

Conclusions

Of the 692 cases with suspected chromosomal abnormalities, 28.7% had chromosomal aberrations. This high rate, comparing it to the 0.6% frequency of chromosomal aberrations in unselected newborn populations [2], demonstrates the diagnostic importance of cytogenetic examinations. Chromosome analysis is always indicated if the patient has significant clinical abnormalities. Our results, like those of others, also point to the low efficiency of karyotyping in patients with single malformations or mental retardation.

Generally, in 65—70% of congenital abnormalities the cause is unknown, and in the rest, genetic or preconceptual causes account for about 20%, chromosome or conceptual (cell division) causes for 3—5%, and known environmental (postconceptual) causes for 7—10% [3]. Although our cases cannot be regarded as a representative sample of congenital abnormalities in the population, it seems that in our material the percentage contribution of preconceptual (genetic) and environmental causes was low as compared to the total number of diagnosed cases. This would suggest that chromosome analysis should be regarded as one step in a general process of syndrome identification, so that a normal karyotype should stimulate the physician to further efforts to arrive at a reliable diagnosis.

Appendix

Classification of patients according to final diagnosis

I. Cases referred for multiple malformations

No.	Final diagnosis
	Chromosomal abnormalities:
1	46, XY, 6r
1	47, XYY
1	45, XO
1	46, XX, —13, +t(13q13q)
1	49, XXXXY
	Single gene disorders:
2	Holt–Oram syndrome
2	oral-facial-digital syndrome
1	achondroplasia syndrome
1	Smith–Lemli–Opitz syndrome
1	Laurence–Moon–Biedl syndrome
1	diastrophic dwarfism syndrome
1	Carpenter syndrome
1	Ellis–van Creveld syndrome
1	Jeune thoracic dystrophy syndrome
	Syndromes or associations of unknown aetiology:
6	VACTERL association
4	neural tube defect
2	Klippel–Feil anomalad
2	Goldenhar syndrome
1	Klippel–Trenaunay syndrome
1	Larsen syndrome
1	Beckwith–Wiedemann syndrome
1	ADAM syndrome
1	Robin anomalad
1	arthrogryposis
	Environmental agents:
2	congenital cytomegalovirus infection
1	maternal diabetes

II. Cases referred because of odd-looking face and/or multiple minor anomalies

No.	Final diagnosis
	Chromosomal abnormalities:
1	46, XX, 18p—
1	46, XX, 4r
1	45, XO

1 46, XX, 21r

Single gene disorders:

3 Saethre–Chotzen syndrome
1 ectodermal dysplasia
1 Albright syndrome
1 Seckel syndrome
1 Cockayne syndrome

Syndromes of unknown aetiology:

1 Silver–Russel syndrome
1 Cornelia de Lange syndrome

Environmental agents:

1 fetal alcoholic syndrome

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Normal chromosomes in liveborn neonates weighing less than 1000 g

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Cytogenetic investigations were performed in 37 phenotypically normal, liveborn immature neonates. Their mean birth weight was 758 g (range, 490–980 g), the mean gestational age was 24.2 weeks (range, 20–28 weeks). All the infants had normal karyotypes, no conspicuous structural aberrations and variants were found. It is presumed that while pregnancies with chromosomally abnormal zygotes are often terminated as fetal wastage or stillbirth, the phenotypically normal liveborn babies born even at a very early gestational age are likely to have normal chromosomes.

Several studies have shown that about 50% of first trimester abortions are associated with chromosome aberrations. The frequency of cytogenetic abnormalities sharply declines by advancing gestational age [3], however, as compared with normal unselected neonates, some surveys suggest a 3 to 4-fold incidence of chromosome abnormalities in premature infants [1, 4, 5]. This could not be confirmed by Bregman et al. [2], morover the materials of these studies had three essential limitations:

1. They were rather heterogeneous, involving both malformed and apparently healthy newborns.

2. Prematurity was diagnosed partly on the basis of clinical signs, partly considering the birth weight being under 2500 g.

3. The chromosome examinations were done mainly with traditional, non-banding procedures.

Thus, it can still not be judged whether prematurity or rather immaturity in itself is associated with an increased incidence of major or minor cytogenetic defects. The purpose of this study was to investigate by banding methods the karyotypes of apparently normal very low birth weight liveborn neonates.

Material and Methods

In our referral neonatal unit a total of 43 neonates whose birth weight was less than 1000 g, were karyotyped. Their gestational age was calculated from the mother's last normal menstruation. Two of them had major birth defects: anencephaly

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday.

and congenital heart malformation. Although their karyotype proved to be normal, they were excluded from the study. In four other cases no satisfactory preparations were obtained, thus we report here the results of 37 morphologically normal live-born immature neonates. The majority of these babies died in the perinatal period, at autopsy no inner malformations were found in any of them.

For cytogenetic investigations peripheral blood was taken on the 1st to 4th day of life. The whole blood lymphocyte cultures were harvested after 70 hours, 0.075 mol/l KCl solution was used for hypotonic shock. The air-dried preparations were routinely stained with traditional Giemsa and with a trypsin-Giemsa G-banding method. Twenty mitoses were microscopically examined and at least 3 conventionally stained and 3 G-banded cells were photographed. If there was a suspicion of structural aberration, additional slides were analysed with C and Q-banding techniques. The length of the Y-chromosome was measured in all boys in at least 5 conventionally stained mitoses, and expressed as $Y/F_{(mean)}$ ratio. A "long Y" variation was diagnosed when the Y/F ratio was greater than 1.00.

Results and Discussion

As shown in Table I, the karyotype was normal in every examined infant. The modal chromosome number was 46 in each case, the rate of hyper- and hypodiploid artifacts was less than 4% which corresponds to the standards in our laboratory.

Out of structural aberrations, translocations, ring chromosomes, exchange figures, inversions and insertions were not observed. Lacking an adequate control group, the number of chromatid and chromosome breaks and gaps, and of satellite associations was not evaluated, but their occurrence did not seem to exceed the usual level. Having had only a small number of late metaphase cells the exact sequence of centromere divisions could not be determined, yet, chromosomes No. 18 and 2 appeared to be the first, and the acrocentrics the last to separate. This pattern corresponded to that obtained previously in healthy control children and adults in different laboratories [6, 7, 11].

As regards to so-called normal variants, no conspicuous phenomena were seen among the autosomes. In one boy a "long Y" with a Y/F ratio of 1.10 was found. Because of the small number of observations, the 1/19, i.e. 5.2% frequency permits no statistical analysis and conclusion, but a significant difference from the Y-variant frequency of consecutive newborns seems rather unlikely. The mean $\pm$S.D. values of the Y/F ratios of all boys proved to be 0.89 ± 0.09 including the single long Y, and 0.88 ± 0.08 excluding it. These did not differ significantly from our normal value of 0.90 ± 0.09, previously determined in 20 healthy medical students.

Patil and Lubs [9] and Nielsen [8] described an association of long Y chromosomes with fetal loss. The present results did not confirm a correlation between long Y's and premature termination of pregnancy, however, further studies are needed to clarify the effect of the length of the Y chromosome on pregnancy in humans.

TABLE I

The applied cytogenetic methods and the karyotypes of the prematures examined

No.	Birth weight, g	Gestational age, week	Fate of neonate	Cytogenetic method	Karyotype
Girls					
1	500	?	died	T, G	46, XX
2	550	21	died	G, C	46, XX
3	600	23	died	T, G	46, XX
4	640	22	died	T, G	46, XX
5	650	22	died	T, G	46, XX
6	690	24	survived	T, G, C	46, XX
7	710	23	died	G	46, XX
8	750	24	died	T, G	46, XX
9	750	26	died	T, G	46, XX
10	800	24	survived	G	46, XX
11	800	?	died	T, G	46, XX
12	850	25	died	T, G	46, XX
13	850	24	died	T, G	46, XX
14	900	?	died	T, G	46, XX
15	900	26	survived	T, G	46, XX
16	900	25	died	T, G	46, XX
17	900	26	died	T, G	46, XX
18	910	28	died	T, G	46, XX
Boys					
19	490	?	died	T, G	46, XY
20	550	20	died	T, G	46, XY
21	590	21	died	T, G	46, XY
22	620	22	died	T, G, C	46, XY
23	650	?	died	T, G, Q	46, XY
24	700	24	died	T, G	46, XY
25	700	25	died	T, G	46, XY
26	700	?	died	T, G	46, XY
27	750	23	died	T, G, C	46, XY
28	800	25	died	T, G, Q	46, XYq+
29	800	25	survived	T, G, C	46, XY
30	800	24	died	T, G	46, XY
31	800	26	died	T, G	46, XY
32	830	25	survived	T, G, Q	46, XY
33	900	?	died	T, G	46, XY
34	900	25	died	T, G	46, XY
35	900	26	survived	T, G, C	46, XY
36	950	26	died	T, G	46, XY
37	980	28	survived	T, G, Q	46, XY

T = traditional (non-banding) Giemsa staining, G = trypsin-Giemsa G-banding, C = C-banding, Q = fluorescent Q-banding.

In conclusion, no chromosome abnormalities or conspicuous variability were found in a group of very low birth weight infants. The absence of major chromosome aberrations in our 37 patients excludes frequencies of more than 8% at the 95% confidence limit. According to gestational age these liveborn neonates were quite close to late midtrimester abortuses in whom significantly more chromosome abnormalities and structural varia-

tions have often been described. One may conclude that pregnancies bearing chromosomally abnormal zygotes develop abnormally and are terminated as fetal wastage or stillbirth, while the premature births of immature liveborns with normal phenotype are due to causes other than cytogenetic. This is in accordance with the late but still valid thesis of Ruzicska and Czeizel [10] that liveborn neonates represent a highly selected population.

From the practical point of view our findings suggest that in apparently normal liveborn neonates the very low birth weight in itself is no indication for routine cytogenetic investigations.

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Metabolic and hormonal effects of fasting in obese children

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A 30 hour fast induced a significant and comparable fall in blood glucose and branched-chain amino acid levels in both obese and control children, while the plasma levels of glycerol, lactate, pyruvate, cholesterol, HDL-cholesterol and the rest of the amino acids remained unchanged.

Sustained hyperinsulinism, sustained higher triglyceride levels and a lower degree of FFA mobilization characterized the plasma of obese children during fasting.

Obesity in childhood has become a significant problem in the developed countries. This is apparent when one realizes that 80% of overweight children become overweight adults [1]. In spite of the increasing problem of exogenous obesity, therapy is by no means solved. Radical therapies such as protein sparing fast, and prolonged total starvation should be approached with caution in children because of the known and unknown side-effects. Simple caloric restriction, e.g. 1200 or 1000 Kcal per day, hardly produces any weight loss in sedentary, grossly obese individuals. Heyden et al. [16] proposed low-calorie diet with intermittent fasting days each week. The inclusion of intermittent fasting showed impressive results and was acceptable for children after due instruction. Before applying the intermittent fast in the therapy of obesity, we wanted to investigate the metabolic and hormonal changes induced by a 30 h fast, since there is only one report on the metabolite and hormone levels during the first day of total starvation in obese children [5].

Material and Methods

Twenty obese (relative body weight beyond 120%) and 5 non-obese children were investigated. Their age and anthropometric parameters are given in Table I. The children were kept on a 80 Kcal/kg diet of constant composition (50% carbohydrate, 30% fat and 20% protein) for two days prior to the test. The 30 hour fast started at 6 a.m. after consumption of the breakfast and continued until noon of the second day. Blood samples were collected at 6, 12, 24 and 30 h for the

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday.

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TABLE I

Anthropometric data of obese and control children

	Control		Obese	
	Mean	± S. E.	Mean	± S. E.
Age, yr	10.6	0.63	10.7	0.45
Height, cm	142.7	3.01	148.4	2.63
Weight, kg	32.9	2.23	60.8	3.3
Triceps skinfold, mm	10.0	0.7	28.9	1.03
Biceps skinfold, mm	5.6	0.37	15.6	0.87
Subscapular skinfold, mm	8.3	0.66	31.5	1.01
Suprailiacal skinfold, mm	9.4	0.83	27.7	1.58
Body fat, per cent	20.01	1.29	40.5	0.77
Lean body mass, kg	26.2	1.58	37.1	2.03
Ideal body weight, per cent	89.9	2.5	148.9	3.77

measurement of glucose, immunoreactive insulin (IRI), FFA, lactate, pyruvate, cholesterol, high-density lipoproteincholesterol (HDL-cholesterol), triglyceride and glycerol. Anthropometric measurements and determination of plasma metabolites (glucose, FFA, lactate, pyruvate, IRI, cholesterol, triglyceride, glycerol) were made as described previously [23]. After heparin-manganese chloride precipitation [30] HDL-cholesterol was determined in the supernatant by the cholesterol oxidase method using the Galenofarm HDL-cholesterol test [22]. Ames Acetest tablets were used to assess urinary ketone excretion. Individual free amino acids were measured by automatic ion-exchange column chromatography.

The mean and standard error (S.E.) were calculated with standard methods. The statistical significance of the difference between the means of the groups were evaluated according the Student's t test.

RESULTS

The 6 hour fasting values for glucose, FFA, glycerol, lactate, pyruvate, cholesterol and triglyceride were not different in obese and control children. Plasma IRI was significantly higher, HDL-cholesterol significantly lower in the overweight children as compared to the controls at the 6th hour of fast (Figs 1 and 2).

Blood glucose decreased in both groups, but more significantly in the control children (Figure 1).

Decrease of the IRI concentration was marked already in the first 12 h of the fast in the controls, while in the adipose children it proved to be significant only after 30 h (Fig. 1). The IRI level of obese children remained significantly higher than that of controls throughout the whole fasting period.

The starvation-induced increase of FFA concentration was significant in the control children while the increase in glycerol was insignificant in both adipose children and the controls (Fig. 1).

Plasma lactate, pyruvate, cholesterol and HDL-cholesterol (Fig. 2) did

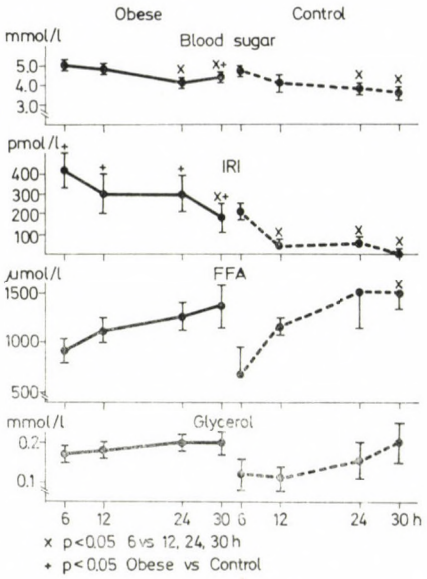

FIG. 1. Effect of fasting on blood glucose, IRI, FFA and glycerol levels (M ± S. E.)

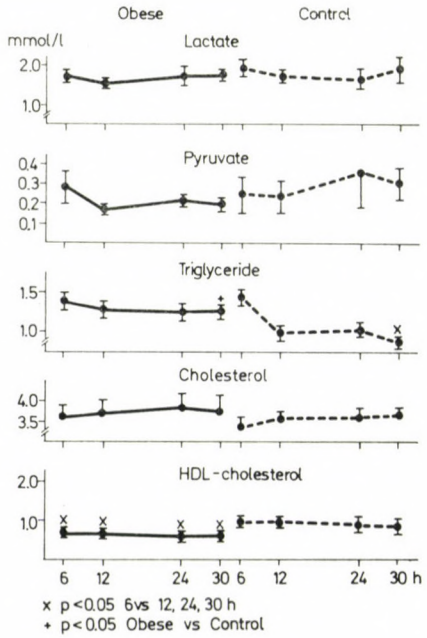

FIG. 2. Plasma levels of lactate, pyruvate, triglyceride, cholesterol and HDL-cholesterol during fasting (M ± S. E.)

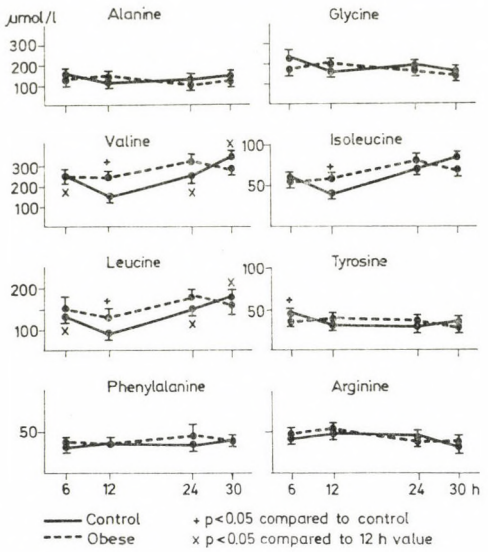

FIG. 3. Effect of fasting on the plasma free amino acids levels (M ± S. E.)

not change significantly during the 30 h fast in either group.

Triglyceride concentration fell significantly in the controls and remained unchanged in the obese children (Fig. 2). The triglyceride levels of overweight children were still significantly higher than that of the controls at the end of the 30 h fast.

The plasma levels of most amino acids were not modified by the fast in any of the groups (Fig. 3). Only the branched-chain amino acids showed an initial tendency to fall in the first 12 hours and a continuous rise throughout the next 18 hours. These changes were similar in the two groups, but proved to be significant only for leucine and valine in the control children.

Clinically, no side-effect was observed during the fast except for a mild ketonuria in 3 obese and 2 control children.

DISCUSSION

Blood glucose decreased during the fast in both obese and control children, but the fall in blood glucose was significantly less in obese children, in agreement with earlier reports [5, 13].

Hyperinsulinaemia [3, 6–8, 26 27,] one of the well known abnormalities in obesity, was apparent in the adipose children. It was shown previously [6, 10, 15, 19] that restoration of biochemical and hormonal changes including hyperinsulinaemia associated with obesity could be achieved by starvation, caloric restriction and/or weight reduction. Hyperinsulinaemia decreased significantly during a 30 h starvation in the present study, but plasma IRI levels were still above normal at the end of the brief starvation period. So it was shown that a 30 h fast was capable of reducing

the insulin level in obese patients as it has been reported in obese and diabetic obese adults [13, 14].

The blunted increase of FFA and glycerol during starvation in the obese group, together with our previous observation of a negative correlation between fasting FFA and adiposity [23] and an insignificant rise of FFA at the end of the glucose tolerance test [24], are suggestive of an impaired lipolysis in overweight children. Apart from a slight FFA mobilization during short periods of starvation [12, 13, 18], a decreased fatty acid release from adipose tissue was found in genetically obese animals [21], but others could not confirm these observations [25, 17, 4].

It is generally accepted that starvation for 3—6 days causes a rise in serum cholesterol and no change or a rise in serum triglyceride concentration [20, 9, 29]. We did not find any change in the cholesterol level of either group, probably because of the shortness of the fast. The significantly lower HDL-cholesterol level found in the present investigation is in agreement with other reports [12, 28]. The short-term starvation was without effect on the HDL-cholesterol values in both the obese and the control children.

Similarly, few changes could be observed in the level of most of the amino acids studied. Only the branched-chain amino acid levels were modified characteristically but, in contrast with a previous observation [5], these changes were modest and not significant in the obese children. There was also a tendency to decline in the level of alanine, glycine and tyrosine, but these changes were not significant. The apparent difference between our findings and those of Chaussain et al [5] is difficult to explain. The different body fat content of the children in the two studies is also difficult to compare on the basis of the data available. Besides, the changes of most of the other metabolites and hormones studied were also slighter in our study than in that of Chaussain et al [5].

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Major anomalies of the genitourinary tract in the neonate

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Thirty-two neonates were investigated or operated upon for major anomalies of the genitourinary tract during the ten-year period up to June 1981. The infants ranged in age from 1 to 31 days. Obstructive uropathies and conditions which appear mainly in later infancy or childhood were excluded.

Diagnostic and therapeutic problems of renal dysplasia (multicystic kidney), infantile polycystic kidney disease, renal venous thrombosis, tumours, exstrophy of the bladder, combination of paediatric surgical and urological anomalies, and sex assignment in infants are discussed with some case reports.

Data are scarce concerning the surgical management of malformations of the genitourinary tract in the neonate [5]. Analysis of the records of all neonates in whom a malformation of the genitourinary tract required investigation and/or surgery has allowed to draw conclusions as to the diagnostic manoeuvres and management of neonatal genitourinary surgical diseases.

MATERIAL

During the ten-year period up to June 1981, 32 neonates were investigated or operated upon for major anomalies of the genitourinary tract. The age of the infants ranged from 1 to 31 days.

Obstructive uropathies, which comprise nearly half of the urologic diseases in the newborn and present the most challenging problem to those responsible for the care of these patients, were excluded from the study. Furthermore, conditions which appear mainly in later infancy or childhood and usually do not require emergency surgery were also neglected, thus undescended testicles, testicular torsion, inguinal hernia, hydrocele, phimosis, hydrocolpos, vulvar synechia, hymenal polyp, vaginal and paraurethral cysts.

Renal dysplasia (multicystic kidney)

Although surgically managable cystic diseases of the kidney are uncommon in early infancy, the possibility should be taken into account, especially in the case of a palpable abdominal mass.

Of the group of renal dysplasias multicystic kidney is the most frequent abdominal mass palpable in newborn infants and the most common cystic lesion of the neonatal kidney. In this condition the kidney

s composed of cysts of variable size and is usually larger than normal (Fig. 1). During excretory urography contrast medium could not be visualized on the affected side, while the unaffected side had a normal pyelographic appearance. In view of the risk of injury, hypertension or infection and the possibility of a neoplasm, the mass is usually removed surgically.

All of our 6 neonates with multicystic kidneys were treated by nephrectomy and all of them are thriving.

Polycystic kidney

The term "polycystic kidney disease" includes more than one syndrome. Any classification of these diseases is only an arbitrary frame to work with and evaluate data. Most workers distinguish between an infantile and an adult type of polycystic kidney. The two entities differ greatly in pathology, genetics, clinical picture, X-ray appearance and prognosis.

Infantile polycystic kidney disease

This condition is considered to have a single, autosomal, recessive mode of transmission. Blyth and Ockenden divided it into four groups: — perinatal, neonatal, infantile and juvenile — according to the patient's age at the clinical presentation [3]. The kidneys in infantile polycystic disease are huge or enlarged and may almost fill the abdomen. Both cortex and medulla are filled with radially oriented small cysts. Death, often shortly after birth, is usually the result of respiratory distress, pulmonary hypoplasia, lung rupture, etc. Increasing renal insufficiency, pyuria, haematuria and hypertension develop in the infants who survive the neonatal period. The X-ray findings are striking. A plain film of the abdomen shows the massively enlarged kidneys as soft tissue masses. In the excretory urogram there is often a delay of several minutes or hours before the appearance of the contrast medium in the kidneys. The renal pelvis and calyces are distorted. In several cases



FIG. 1. Multicystic kidney

the dye never concentrates in the calyces or pelvis. Only a purely symptomatic treatment is a reality in the infantile and juvenile forms (increasing chronic renal insufficiency, hypertension, anaemia, respiratory problems, etc.). Diagnosis, treatment, genetic counselling and prognosis are mainly determined by the type of the disease.

In one of our neonates with polycystic kidney disease, genetic counselling and prognosis met with difficulties. The disease, infantile polycystic kidney, is known to be hereditary, and analysis of the family pedigree showed a dominant trait (adult type polycystic kidney). The boy was born of the 3rd pregnancy of the mother. The abdomen was protuberant, both kidneys were enlarged to 8×4 cm, with lobated contours (Fig. 2a). The urogram showed poor dye excretion. Angiographic studies via the umbilical artery confirmed a neonatal type polycystic kidney (Fig. 2b).

The maternal grandmother died of renal failure at 43 years of age. Post mortem revealed huge kidneys with numerous cysts of variable size, containing blood-tinged or clear fluid. The mother has elevated blood pressure, her left kidney is enlarged, the calyces are distended but clinically she is healthy. The patient's sister died at the age of 2 hours. Each of her kidneys was as large as a female fist, and they displayed countless small cysts in both cortex and medulla. The infant's hypertensive sibling, a 4-year old boy, is also affected; his left kidney is enlarged.

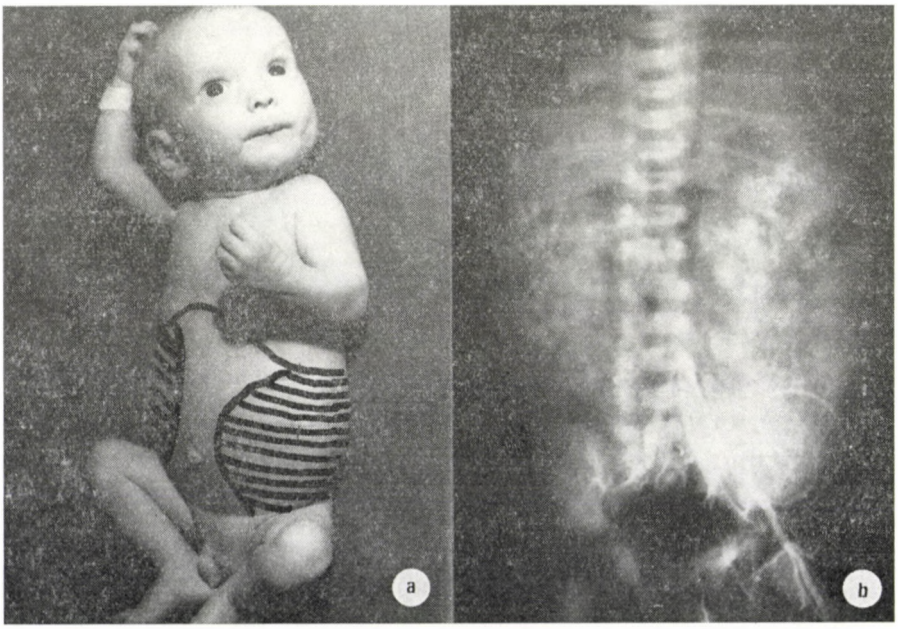

FIG. 2. Infantile polycystic kidney: *a)* hatched areas show the enlarged kidney *b)* angiography via umbilical artery

Renal venous thrombosis

Renal venous thrombosis is suspected in a neonate when haematuria and an enlarged kidney are observed shortly after a period of asphyxia, shock, dehydration or sepsis. The suggestion has been made that renal venous thrombosis is a bilateral disease and only the less involved kidney is overlooked [1].

Nephrectomy used to be advocated to prevent infection and the infrequent hypertension which usually means a serious problem after healing, and renal atrophy. Now it would seem that immediate nephrectomy, unless infection or some other untoward development occurs, is no longer the operation of choice for unilateral thrombosis since without thrombectomy some recovery of function may be achieved as a result of an incomplete or segmental involvement or subsequent recanalization [7]. Bilateral thrombosis may be treated by thrombectomy. During the ten-year period analysed, we observed 3 neonates with this condition. In one of them a non-surgical approach was chosen; this patient survived. In one of the nephrectomized infants the infection and thrombosis progressed, the thrombosis spread to both renal veins and inferior venae cavae. This patient died 8 days postoperatively.

TABLE I

Major genitourinary anomalies requiring surgical consideration in the neonate*

Anomalies	No. of cases	Total
Renal dysplasia (multicystic kidney)	6	
total		5
segmental		1
Polycystic renal disease	5	
infantile type		4
adult type		1
Renal vein thrombosis	3	
Tumour	5	
Wilms'		1
cong. mesoblast. nephroma		1
retroperit. neuroblast.		1
pelvic neuroblastoma		2
Exstrophy of bladder	1	
Combination of paediatric surgical and urological anomalies	7	
compatible with life		2
incompatible with life		5
Severe cases of ambiguous external genitalia	5	
Total	32	

* Obstructive uropathy, undescended testes, testicular torsion, inguinal hernia, hydrocele, phimosis, hydrocolpos, synechia vulvae, hymenal polyp, vaginal and paraurethral cysts, etc., have been excluded.

Tumours

A review of our records revealed an interesting fact: from 1960 to 1975 we had no patient with a malignant tumour at birth or within the first months of life, whereas in the past 5 years we have recorded eight such patients, five of them requiring urological management.

Two of the tumours arose from the kidneys. One of them was a Wilms tumour, the other a congenital mesoblastic nephroma. Physical examination and X-ray studies at birth detected in a 1650 g preterm infant a left renal tumour (Fig. 3a). The removed kidney with the tumour weighed 170 g (Fig. 3b). On the cut surface we saw a nearly complete replacement of the renal parenchyma by a bulging firm mass, presenting a yellowish silk pattern (Fig. 3c). Microscopic diagnosis was congenital mesoblastic nephroma. The infant is now 13 months old, weighs 11 kg and thrives well.

The third tumour also mimicked a kidney neoplasm on the intravenous urogram, but surgery proved it to be a retroperitoneal tumour located in front of the intact but displaced and compressed kidney. Histologically it was a neuroblastoma.

Two patients presented with urinary retention due to pelvic and presacral tumours, which had dislocated the rectum and obstructed the blad-

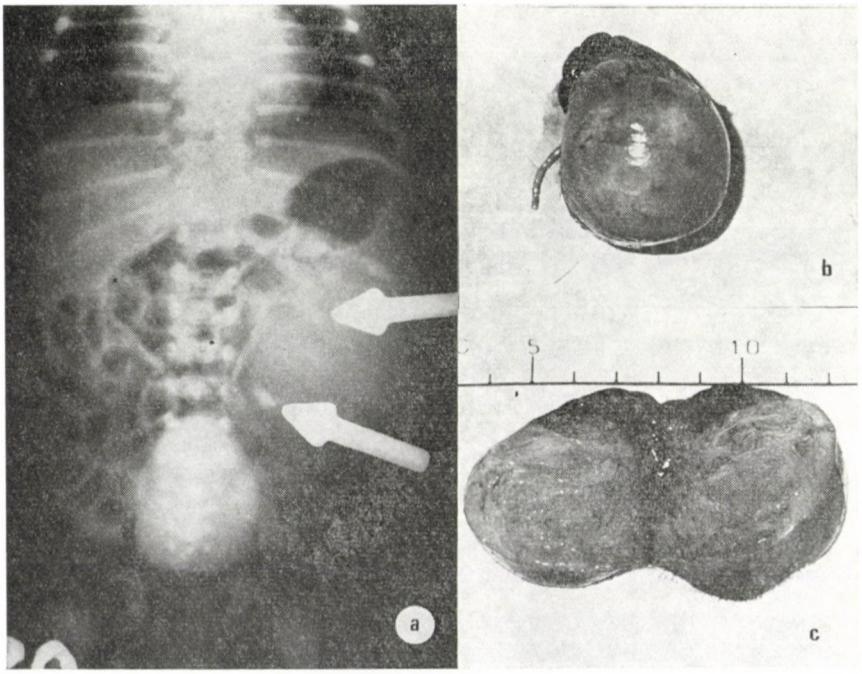

FIG. 3. Congenital mesoblastic nephroma: *a*) intravenous urogram: the distorted left renal pelvis is displaced medially and compressed (arrows), *b*) removed kidney with tumour below *c*) cut surface of tumour and kidney

der outlet. Histologically both tumours proved to be capsulated differentiated neuroblastomas.

In view of the age of these patients and the radical excision of the tumours, neither radiotherapy nor cytostatic drugs were given, but all patients were and still are followed regularly. Nowadays it is generally accepted that in the neonate and young infant aggressive multimodal therapy may be more dangerous than the disease itself [4].

Exstrophy of the bladder

Exstrophic conditions form a broad spectrum of developmental abnormalities: the lowest degree is represented by epispadias, while the highest is exstrophy of the cloaca or vesico-intestinal fissure.

The only treatment of bladder exstrophy applicable in newborns is vesical reconstruction. Several authors are of the opinion that such surgery should be carried out at the age of 2 to 3 years. However, according to a considerable number of other workers, the operation is more easily and effectively performed in the neonatal period when the wall of the bladder is still thin and pliable. Our attempts at such early reconstruction of the bladder have failed.

Combination of surgical and urological anomalies

Malformations compatible with life. We had only two neonates in whom both the paediatric and urological malformations required surgical correction within the first month of life. In one of them rectal agenesis and ureterovesical obstruction necessitated a pull-through operation and uretero-nephrectomy (Figs 4a and b).

Malformations incompatible with life. In four patients with multiple grave anomalies palliative surgical interventions were only feasible, after which

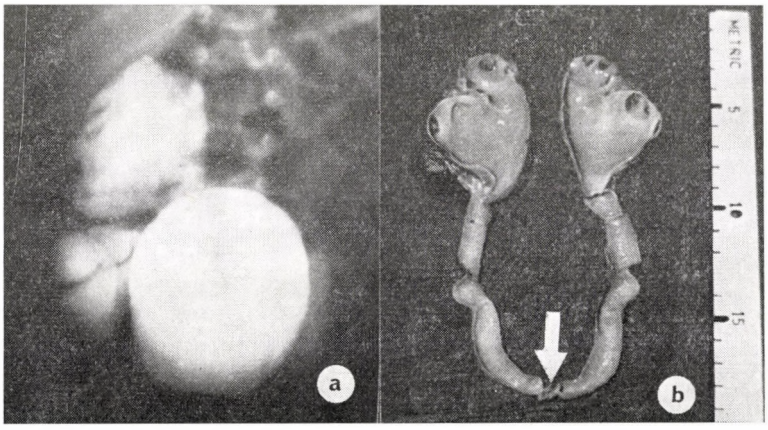

FIG. 4. Ureterovesical obstruction (↑) associated with rectal agenesis: *a*) cystogram: severe dilatation of the right upper urinary tract, *b*) removed kidney and ureter: hardly any renal parenchyma is visible

they died within a few days. In one of them the post mortem revealed partial agenesis of the colon, rectal agenesis, colo-vesical fistula, bipartite uterus, urogenital sinus, obstruction of the left pelviureteric junction and the uretero-vesical junction, with Meckel diverticulum and vertebral anomalies.

Another neonate had a megacystis-microcolon-hypoperistalsis syndrome, a condition described by Berdon et al in 1976, as a new cause of neonatal intestinal obstruction [2], a uniformly fatal combination of urological and intestinal abnormalities. The patient was a girl born at term; she was admitted with abdominal distension and bilious vomiting. The stomach was displaced upwards by the distended urinary bladder. Gastrographin enema demonstrated a microcolon. Meconium ileus or intestinal obstruction was suspected. At surgery the enlarged bladder was filled with urine and reached the umbilicus. The small intestine measured only 60 cm from the pylorus to the ileocoecal valve. Both kidneys were enlarged. A double-barrel ileostomy was prepared but satisfactory intestinal function never ensued and the infant died on the 28th postoperative day.

Sex assignment in the newborn with ambiguous external genitalia

Prompt and appropriate assignment of sex in the newborn infant with ambiguous genitalia is an important task. When confronted with such conditions the attending physicians including the paediatric surgeon should be aware of the anxiety imposed on the family.

The first task is to examine the external genitalia. One should appraise the anatomic abnormalities, noting the size of the phallus, the location of the urethral orifice, the degree of labioscrotal fold fusion, the presence or absence and the size and location of palpable gonads, and the presence of other abnormalities. The findings are usually confusing even for the experienced specialist. The continuum of changes between purely female and purely male external genitalia have been divided into five stages by Prader [6]. It should, however, be stressed that the findings obtained on examination of the external genitalia never allow a diagnosis to be made, since disorders with an identical external appearance may have different causes (Figs 5a and b).

Evaluation of the internal genitalia is more difficult. It should be known whether or not gonads are present and whether they are dysgenetic or histologically normal testes or ovaries. In addition, it is important to know whether or not an uterus and Fallopian tubes are present.

The urogenital sinus can be explored under general anaesthesia, and a catheter and an endoscope of 10 Ch may be helpful in finding the vaginal opening. Radiological examination of the urogenital sinus, using a viscid contrast medium, is often informative. In favourable cases the vagina and the narrow cavity of the uterus will be outlined.

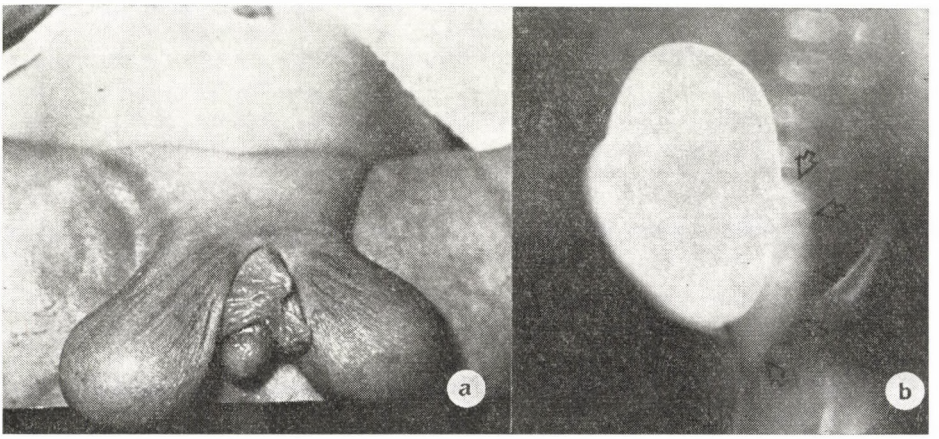

FIG. 5. Neonate with ambiguous genitalia: *a*) external appearance, *b*) genitography: uterus outlined by arrows (chromosomal analysis: 46 XY)

In order to ascertain the absence or presence of internal genitalia and to obtain biopsies from the gonads, a laparotomy or laparoscopy [8] has to be carried out even in small neonates. Nowadays, we give preference to the latter method.

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Energy metabolism, substrate utilization, metabolite and hormone levels in infants fed various parenteral solutions

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Previous observations on the effects of various types of parenteral nutrition on changes in oxygen consumption, respiratory quotient, total heat production, distribution of nutrient utilization, metabolic and hormonal levels in infants during the neonatal and postneonatal periods are reviewed.

The relevant findings obtained in fifty-two newborn infants infused with different types of metabolic solutions were as follows. Oxygen consumption increased during Aminosol-glucose and Intralipid-glucose infusion. The respiratory quotient varied according to the oxidation of the nutrients. A significant elevation was observed during Aminosol-glucose infusion, which tended to fall in response to Intralipid. Total heat production was increased during all types of parenteral nutrition, and substrate utilization depended on the quality and amount of nutrient intake. The magnitude of the changes in concentrations of metabolites (glucose, free fatty acids, alpha-amino-nitrogen) and insulin and growth hormone were smallest when the parenteral nutrition consisted of glucose, amino acids and lipid and the total caloric intake did not exceed the maintenance energy expenditure. On the basis of the results, during the neonatal period it seems advisable to administer as parenteral nutrition, about 70—75 kcal/kg/day in the form of 7.0—8.0 g/kg/day glucose, 1.7—2.0 g/kg/day amino acids and 3.0—4.0 g/kg/day lipid.

Nutrition by intravenous infusion of various nutritive mixtures appears to be of great importance for the adequate management of sick infants during the postneonatal period. Long-term parenteral nutrition by giving only an amino acid-glucose mixture or a glucose-amino acid-lipid solution can produce a gain in weight with a positive nitrogen balance [2, 8, 11, 13, 29). Some metabolic and biochemical studies, however, show that these feeding methods may cause adverse metabolic effects such as hyperammonaemia, hyperaminoacidaemia, increased osmolality, metabolic acidosis, etc. [4, 6, 7, 9, 14, 16, 20, 28, 30].

It is well-known that maintenance caloric requirement varies greatly according to gestational, postnatal age, body weight and numerous other factors [18, 19, 24, 25]. Knowledge of the caloric expenditure of newborns and the calorigenic effect of parenteral nutrition is also essential when alimentation takes place by intravenous route. In spite of many reports on the

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday.

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metabolic effects of intravenous nutrition and on the aspects of maintenance energy expenditure in the neonatal or postneonatal period, there is a dearth of data on the effect of various types of parenteral nutrition on heat production and substrate utilization. This report deals with the responses of energy metabolism and substrate utilization to various types of parenteral nutrition during the neonatal and postnatal periods. In view of the metabolic adaptation of the low-birth-weight infant it appeared important to determine the optimum amount of parenteral nutrient intakes which do not cause metabolic alterations but are still high enough to cover the maintenance energy expenditure of the newborn infant.

Methods

Clinical material. Fifty-two newborn infants divided into 5 series of 7–15 each (see Table I) were infused with five different types of nutritive solution.

The infants in the first four series (I–IV) were selected on the basis of their inability to tolerate oral or tube feeding without the danger of apnoeic spells and/or aspiration. The patients in the fifth series (V) had undergone surgery in the neonatal or postneonatal period and needed parenteral nutrition during intensive postoperative care.

The number of infants, mean ± SE body weight, gestational and postnatal age at examination are summarized in Table I.

Infusions. In the first three series (I, II, III) we investigated the acute effects of parenteral solutions using short infusion periods.

In the first series (I) Aminosol-glucose (Vitrum, Stockholm) was infused for 240 min after a 4-hour starving period. The infusion was discontinued for 2 h in order to study the metabolic response to interruption of the parenteral nutrition.

In series II the effect of a 28 hour Aminosol-glucose infusion was studied after a 12 h control period.

In series III, the infants received glucose as a basic solution infused at a steady rate throughout 42 hours. The first 12 hours, when the infants received only intravenous glucose, served as control period. Over the next 6 h, Intralipid (10%, Vitrum, Stockholm) was infused in addition to glucose. After termination of Intralipid supplementation, intravenous feeding with glucose was continued for two consecutive 12 h periods.

In series IV, the caloric intake was increased. Low-birth-weight infants were fed intravenously for altogether 36 hours divided into three 12 h periods. In the first 12 h 10% glucose was given; during the second 12 h period 10% glucose with 2.7% crystalline amino acids (Aminosol 2400, Vitrum, Stockholm) was infused thereafter, in addition to glucose and an amino acid mixture, 10% lipid solution (Intralipid, Vitrum, Stockholm) was infused by means of a second infusion pump. In brief, solutions containing glucose (regimen A), glucose and amino acids (regimen B), and glucose with amino acids and lipids (regimen C) were given.

In series V we compared three consecutive 24 h periods, in which the infusion contained the same caloric and volume intake, but the composition of the nutrients was changed. Regimen A contained 10% glucose, regimen B glucose-amino acids, and regimen C Intralipid and amino acids. The volume and energy content of the infusates were the same, and in the case of two mixtures care was taken to infuse identical amounts of amino acids (1.70 to 1.77 g/kg/day).

The daily caloric input and the nutrients infused are shown in Table I.

Determination of energy expenditure and substrate utilization. Oxygen consumption

TABLE I

Data of infants, amount of calories and nutrients infused

Parenteral nutrition programme	No. of infants	Gestational age, weeks	Postnatal age, h	Body weight, g	Caloric input, kcal/kg/day	Nutrients infused, g/kg/day		
						glucose	amino acids	lipid
I. Short period of Aminosol-glucose infusion	7	30.8±0.7	31.4±2.8	1570±87.8	52.6±2.3	8.6±0.3	5.6±0.2	—
II. Longer period of Aminosol-glucose infusion	15	30.1±0.9	20.9±2.1	1282±86.2	36.4±2.7	5.9±0.4	3.9±0.3	—
III. Intralipid-glucose infusion	12	33.4±0.6	11.7±1.2	1734±75.7	43.6±2.4	5.5±0.5	—	7.5±0.2
IV. Increasing caloric intake, variable composition of nutrients	8	32.0±1.0	18.2±5.9	1362±148.4				
Regimen A = glucose alone					28.7±1.2	7.1±0.3	—	—
Regimen B = glucose + amino acid					35.1±1.4	7.1±0.3	1.5±0.07	—
Regimen C = glucose + amino acid + lipid					70.3±1.4	7.1±0.3	1.5±0.07	3.1±0.2
V. Same caloric intake, variable composition of nutrients	10	38.4±0.5	19.0±7.1	3067±285.9				
Regimen A = glucose alone					56.2±2.2	14.0±0.5	—	—
Regimen B = glucose + amino acid					55.4±2.5	12.2±0.5	1.7±0.07	—
Regimen C = lipid + amino acid					56.3±1.7	—	1.7±0.06	4.1±0.1

and carbon dioxide production were measured using a Kipp diaferometer, which allowed continuous measurement of the respiratory gas exchange and the respiratory quotient. The Kcal value of oxygen used for calculation of heat production corresponded to the average RQ of the observation period. The relative values of substrate utilization were calculated from the oxygen consumption and CO_2 production after subtraction of the oxygen and carbon dioxide related to the protein metabolism, as determined by the urea nitrogen excretion [3]. For this purpose urine collections were made during the observation periods according to the infusion periods. The relative utilization of fat and carbohydrates was estimated from the non-protein RQ [22, 26].

Chemical monitoring. Before and at the end of the infusion periods, blood samples were drawn through an umbilical venous catheter in series I to IV, and from a peripheral vein in series V. The following metabolites were measured: glucose [21], free fatty acids [5, 17], alpha-amino-nitrogen [27]. Plasma insulin and growth hormone were estimated by the method of Hales and Randle [12], and Hunter and Greenwood [15].

For statistical analysis the means and standard errors were calculated; when it seemed necessary, significance was estimated by Student's *t*-test.

Results

Oxygen consumption and respiratory quotient

Mean oxygen consumption and respiratory quotient calculated for 30-min periods before, during and after Aminosol-glucose infusion as well as Intralipid-glucose infusion are shown in Fig. 1. The upward tendency of oxygen consumption was obvious in both cases; but owing to the great individual variation of the initial data and responses to the fat and amino-glucose mixtures, the increase turned out to be insignificant whichever period was used for comparison. It is seen from Fig. 1 that the low RQ of fasting prematures varied greatly according to the oxidation of the nutrients during the infusion periods. A significant elevation was observed during Aminosol-glucose infusion and the mean respiratory quotient tended to fall in response to Intralipid.

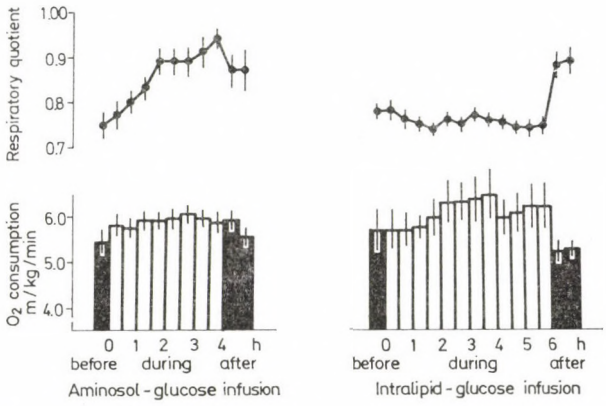

Fig. 1. Oxygen consumption and respiratory quotient during short periods of Aminosol-glucose and Intralipid-glucose infusion

Total heat production in response to various types of parenteral nutrition

Mean total heat production per kg body weight per day is shown in Fig. 2. During all types of parenteral nutrition there was a rise in total heat production, which reached the level of significance only in the case of the short Aminosol-glucose infusion period (I) when the amino acid input (see Table I) was the highest in the investigated series. Total metabolism during the longer Aminosol-glucose infusion (II), the Intralipid-glucose infusion (III), and in series V where caloric input and volume input were the same, did not appreciably exceed that obtained in the control periods.

Distribution of the chief nutrients in total heat production

To quantitate the nutrients utilized' the amount of protein was calculated from urinary nitrogen, and the amounts of carbohydrates and lipids were obtained from the non-protein respiratory quotient. The mean relative distribution of calories produced in series I, III and V is presented in Table II. Three individual examples of nutrient utilization during the longer Aminosol-glucose infusion are shown in Fig. 3.

It can be seen from Table II that the catabolism of amino acids had doubled, the amount of metabolized fat was still not negligible, and the amount of carbohydrates utilized was higher during the second half of the Aminosol-glucose infusion period (series I). When the Aminosol-glucose infusion was contained for a longer period of time (Fig. 3) the participation of carbohydrates increased, while fat metabolism became the smallest energy component, and the proportion of amino acid oxidation reached a high value, 60, 32 and 28% of total heat production during the 16—28 h of infusion. The effect of Intralipid on the distribution of the calories produced is also shown in Table II. It is seen (series III) that before Intralipid supplementation the metabolic state was dominated by fat utilization. The metabolic pattern during Intralipid-glucose infusion was characterized by an increase in fat

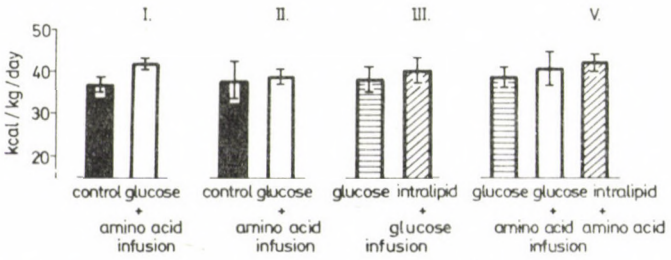

FIG. 2. Total heat production during various types of parenteral nutrition. I = short period of Aminosol-glucose; II = longer period of Aminosol-glucose; III = short period of Intralipid-glucose infusion; V = same caloric intake of different nutrients

TABLE II

Relative distribution of calories produced by fat, carbohydrate and protein during various types of parenteral nutrition

Parenteral nutrition programme	Periods of investigation	Relative distribution of calories produced by		
		Fat, per cent	Carbohydrate, per cent	Protein, per cent
Series I	0–120 min	48.96±6.43	44.96±6.57	6.07±0.68
	121–240 min	29.04±7.14	58.35±8.02	12.59±1.48
Series III	Glucose period	70.42±3.86	25.35±3.84	4.22±0.92
	Intralipid+glucose period	76.34±3.92	14.87±3.91	8.77±1.73
	Glucose period	38.49±7.62	53.06±8.80	8.44±1.98
	Glucose period	34.96±9.71	56.58±10.45	8.45±1.78
Series V	Glucose period	33.31±6.06	58.53±5.84	8.19±1.43
	Glucose+amino acid period	29.90±5.81	63.55±5.85	6.53±1.52
	Amino acid+lipid period	77.24±6.16	17.38±5.44	5.37±1.20

and protein oxidation. After termination of the infusion of fat, continuation of glucose infusion at the same rate markedly changed the distribution pattern of energy metabolism; the contribution of carbohydrate oxidation increased while that of fat decreased. Table II also shows the effect of parenteral nutrition on substrate utilization when caloric input and volume input were the same and the composition of the nutritive mixture was changed. It can be seen from the results of series V, that energy metabolism was dominated by carbohydrate utilization during the glucose infusion period and the addition of amino acids to glucose did not appreciably alter the distribution of substrate utilization. The distribution



FIG. 3. Relative distribution of calories produced by fat, carbohydrate and protein in three preterm infants receiving Aminosol-glucose infusion for longer period

pattern of energy metabolism changed markedly when lipid was administered together with an amino acid solution; then heat production was dominated by fat utilization.

Metabolic and hormonal responses to infused calories not exceeding maintenance energy expenditure

The changes in total heat production and substrate utilization showed that the former parameter slightly increased and the latter depended on the quality and amount of nutrient intake. In order to investigate what kind of metabolic and hormonal changes were caused by a type of parenteral nutrition which covered the maintenance energy expenditure but, at the same time did not exceed the intravenously infused calories, three consecutive 12 h periods (infusion of glucose, glucose + amino acid, glucose + amino acid + lipid) were investigated.

Metabolic responses. Figure 4 shows that the concentration of glucose at the end of the three infusion periods was higher than the control level ($p < 0.01$ and < 0.05). The greatest rise was seen during the glucose period (A), thereafter the addition of an amino acid mixture to glucose resulted in a fall (regimen B), and supplementation with lipid (regimen C) did not greatly influence the blood glucose level. The mean concentration of plasma free fatty acids fell in response to glucose, the infusion of

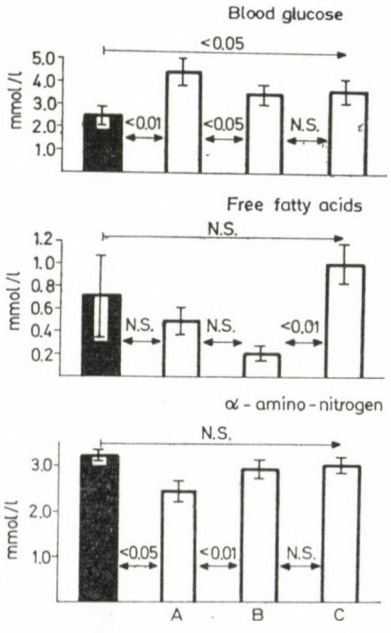

FIG. 4. Response of blood glucose, free fatty acids and alpha-amino-nitrogen to infusions of (A) 10% glucose, (B) 10% glucose + 2.7% amino acid, and (C) 10% glucose + 2.7% amino acid + 10% lipid

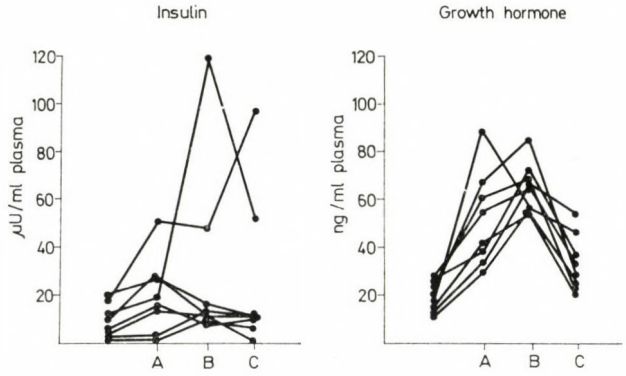

FIG. 5. Individual changes of plasma insulin and growth hormone during 10% glucose (A), 10% glucose + 2.7% amino acid (B), and 10% glucose + 2.7% amino acid + 10% lipid (C) infusion

amino acids in addition to glucose caused a further decrease in plasma FFA, and supplementation with lipid was, as expected, followed by a rise in FFA concentration. The changes in alpha-amino-nitrogen are shown in Fig. 4. The mean level of alpha-amino-nitrogen fell significantly in response to glucose ($p < 0.05$), but returned to the fasting level when an amino acid mixture was added to the glucose. The supplementation with lipid solution did not affect this level.

Insulin and growth hormone response. It is seen from Fig. 5 that the magnitude and time course of the individual changes in plasma insulin were varied. The changes in growth hormone were uniform: the definite increase elicited by glucose was followed by a further pronounced increase in response to the combined infusion of glucose and amino acids, and the addition of lipid was associated with a pronounced fall.

DISCUSSION AND CONCLUSIONS

Earlier investigations on the maintenance energy expenditure of low-birth-weight infants kept at neutral temperature had pointed to the need of an individual approach to the estimation of calorie requirement [18, 24], but it was obvious that total heat production was low during the neonatal period. By direct measurements of the components of total heat production (basal metabolic rate, specific dynamic action, activity, thermoregulatory heat production) it was found [18, 19] that the energy need of very low-birth-weight infants was about 50–65 kcal/kg/day.

These observations are of great importance for the parenteral alimentation of newborn infants since by using the conventional estimates of maintenance energy expenditure, an excessive caloric load may be imposed, leading to dangerous complica-

tions such as hyperammonaemia, hyperaminoacidaemia and metabolic acidosis [4, 9, 23]. Besides, the specific dynamic action of a protein-rich formula greatly contributes to the extra calories produced above the basal requirement [19], and non-protein energy sources such as glucose or fat [10] may improve the nitrogen balance. Still, even an energy input exceeding energy expenditure cannot maintain nitrogen equilibrium in the absence of nitrogen input [1, 26].

In the present study, heat production increased in every case of parenteral nutrition, but was significant only during series I, when amino acid input was highest (5.69 g/kg/day). Protein breakdown was also important when only the glucose-amino acid mixture was given over a longer period in series II with a higher amino acid input (3.95 g/kg/day) and a lower glucose input (5.94 g/kg/day). When fat or glucose were given as non-protein energy source with lower nitrogen input (1.70—1.77 g/kg/day as amino acid), the proportion of protein in the total heat production decreased as compared with the glucose period (series V).

The changes in the concentration of metabolites (glucose, free fatty acids and alpha-amino-nitrogen) and hormones (insulin and growth hormone) were smallest in the last infusion period of series IV, when parenteral nutrition consisted of glucose, amino acids and lipid, but the total caloric input did not exceed the maintenance energy expenditure.

On the basis of the results it seems advisable during the neonatal period to administer about 70–75 kcal/kg/day in the form of 7.0–8.0 g/kg/day glucose, 1.7–2.0 g/kg/day amino acids and 3.0–4.0 g/kg/day lipid as parenteral nutrition. In view of the usual absence of growth in low-birth-weight neonates during the first 10 days of life, it appears unnecessary to increase the caloric input above the maintenance requirement, otherwise most of the extra calories and substrates would probably be rapidly oxidized rather than incorporated in the body tissues.

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Cardiomegaly in hypoglycaemic small-for-gestational-age infants

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The chest X-rays of 15 hypoglycaemic small-for-gestational-age infants showed cardiomegaly in 10 infants, 4 had heart failure. None of the infants had respiratory distress syndrome, congenital heart disease, septicaemia, anaemia or polycythaemia; infants of diabetic mothers were excluded. Cardiomegaly disappeared with the normalization of blood glucose in most of the cases. The cause of the cardiomegaly and heart failure might be related to insufficient cardiac energy substrates in small-for-gestational-age infants. This condition should also be considered in the differential diagnosis of cardiomegaly and heart failure.

Hypoglycaemia is frequent in small-for-gestational-age (SGA) infants in the immediate postnatal period. The majority of its symptoms is related to the central nervous system [4, 11]. There have been few reports on the effects of hypoglycaemia manifesting with cardiomegaly and heart failure [1, 7, 8, 14]. These observations were made mainly in SGA infants.

A retrospective study of the chest X-rays of hypoglycaemic SGA infants admitted to our neonatal ward has therefore been performed.

Material and Method

During the five year period from January 1975, to December 1979, 32 hypoglycaemic SGA infants were admitted to our intensive care unit. Hypoglycaemia was diagnosed when the blood sugar was less than 20 mg/dl in preterm and less then 30 mg/dl in term infants. In 15 infants chest radiography was done at the onset of hypoglycaemia. Birthweight of these infants was below the tenth percentile according to our local intrauterine growth chart and they showed the typical signs of intrauterine malnutrition. Mean birthweight and gestational age of the infants are shown in Table I.

Table I

Birth weight and gestational age of the infants studied (Means and ranges)

Gestational age, week	38.8	(36–42)
Birth weight, g	2077	(1750–2650)
No. of infants	15	

Chest Y-rays were made in supine position antero-posteriorly. The transverse diameter of the chest and heart was measured as described earlier [12]. The cardiothoracic ratio was also calculated. Heart

This work is dedicated to Professor Julius Mestyán on the occasion of his 60th birthday.

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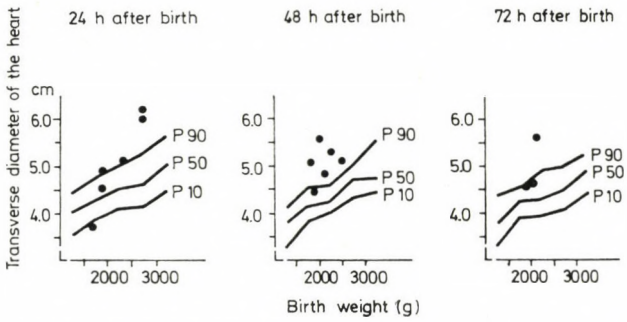

FIG. 1. Percentile position of heart size (transverse diameter of the heart) in 15 SGA infants with hypoglycaemia, according to our local standard

failure was diagnosed on the basis of the following symptoms: dyspnoea and tachypnoea (respiratory rate > 50/min); radiographic evidence of heart enlargement and pulmonary congestion; hepatomegaly. Heart enlargement was diagnosed when the transverse diameter was above the 90th percentile of our local standard and the cardio-thoracic ratio was greater than 0.52 (normal mean 0.50 ± 0.02). None of the infants had respiratory distress syndrome, congenital heart disease, septicaemia, anaemia or polycythaemia, conditions which may be associated with cardiac enlargement.

RESULTS

Ten of the 15 hypoglycaemic infants had cardiomegaly based on chest radiographs taken within three days postnatally, when hypoglycaemia had developed. The transverse diameter of the heart was above the 90th percentile [12]. We use different standards for each day of early postnatal life in view of the physiological changes in heart size after birth (Fig. 1). The cardiothoracic index ranged between 0.52–0.60.

TABLE II

Clinical data and radiographic findings in 10 small-for-gestational-age infants with hypoglycaemia and cardiac enlargement

No.	Birth weight, g	Gestational age, week	Labour and delivery	Apgar score 1 min	Apgar score 5 min	Blood glucose, mg/dl	Cardiothoracic index	Heart failure	Age at onset of hypoglycaemia, day
1	2650	42	normal	10	10	6	0.55	+	1
2	2200	39	vacuum extractiom	4	8	4	0.60	+	1
3	1750	—	toxaemia	10	10	12	0.52	—	1
4	2300	—	cord around the neck	4	8	29	0.53	—	1
5	2020	36	normal	9	10	24	0.60	+	2
6	2330	37	toxaemia	9	10	17	0.54	—	2
7	2140	38	normal	10	10	18	0.56	—	2
8	2450	40	cesarean section	6	10	30	0.52	—	2
9	1840	—	normal	7	8	20	0.56	—	2
10	2170	40	normal	8	10	25	0.58	+	3

The clinical data and the radiographic findings of the 10 SGA infants with cardiomegaly are summarized in Table II. Four infants with cardiomegaly had symptoms suggestive of congestive heart failure (Cases 1, 2, 5 and 10) manifesting with tachypnoea, tachycardia and hepatomegaly. These infants required a short period of digitalis therapy. In all infants the hypoglycaemia was treated with 5–10% dextrose infusion. The infants, who had cardiomegaly without heart failure improved rapidly, and showed a normal heart size after correction of the hypoglycaemia.

The course of one of the infants is shown in Figure 2, at 2 days of age showing enlarged heart, and at 5 days of age showing a normal heart. Of those with cardiac failure 1 infant improved rapidly, in the other 3 infants the cardiomegaly persisted for

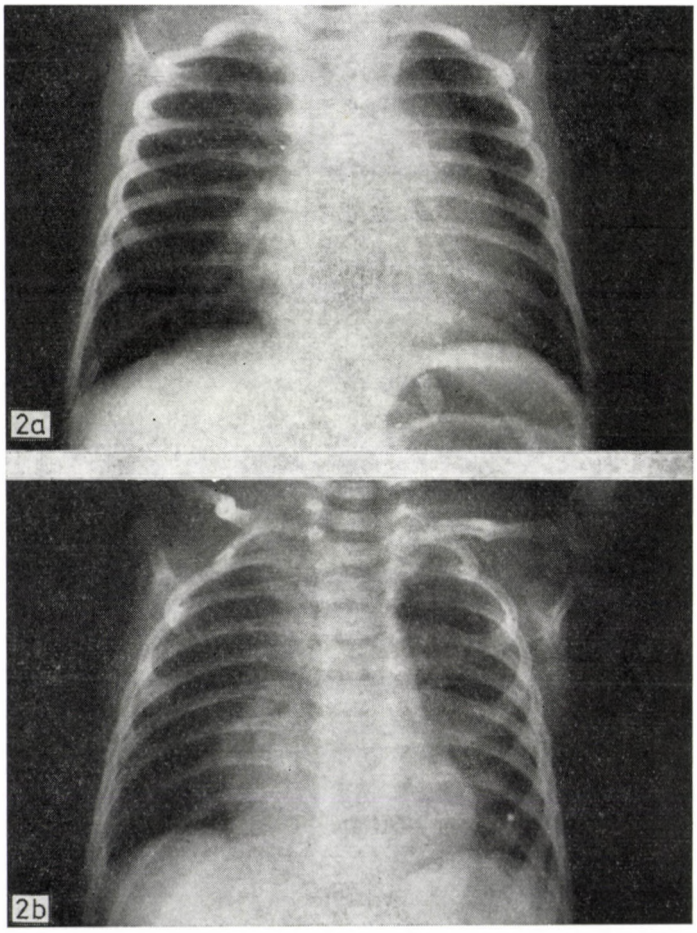

FIG. 2. Chest X-rays of SGA infant with hypoglycaemia and cardiomegaly. *a*) At 2 days of age showing enlarged heart; *b*) at 5 days of age showing normal heart

7–21 days. All four patients with cardiac failure had repeated ECG-s, which were abnormal showing peaked P waves in lead I, wide QRS complex, long QT interval. None of the hypoglycaemic SGA infants died.

Discussion

As to the association of hypoglycaemia and heart enlargement in the newborn infant, Benzing et al [2] described 27 infants with heart failure and hypoglycaemia; 26 had severe heart malformations. Reilly et al [8] were the first to report on the association of heart failure and neonatal hypoglycaemia in infants without cardiac malformations. Wyler et al [14] comparing the chest X-rays of 24 hypoglycaemic newborns with those of a matched control group of infants with normal blood glucose levels, found that heart size was significantly greater in the hypoglycaemic than in the control group. A retrospective review [1] of chest X-rays of 16 infants showed cardiomegaly in 12 infants; 6 of them were SGA. McReid et al [8] found 6 small-for-date babies with hypoglycaemia; they all had radiographic heart enlargement. Infants of diabetic mothers, infants with cardiac malformations and with polycythaemia were excluded from the studies.

Cardiac enlargement not due to congenital heart disease or maternal diabetes may occur in the newborn infant after perinatal asphyxia [3, 13], haemolytic disease [6], or polycythaemia [5]. Yao et al [15] demonstrated that small-for-date infants may have increased blood, red cell and plasma volumes.

Asphyxia may have been responsible for the cardiomegaly in three of our patients whose one minute Apgar score was less than 7, but not in the rest. The haematocrit value was within normal limits, therefore polycythaemia or anaemia was not a factor in the development of heart enlargement.

Since cardiomegaly mostly disappeared with the normalization of the blood glucose level, the cause of heart failure may be related to insufficient cardiac energy substrates. The small-for-date infant has low carbohydrate stores in the liver and heart [9, 10], which are rapidly depleted after birth, especially during hypoxic episodes [10]. Deprivation of cardiac glycogen might result therefore in functional insufficiency.

On the basis of our findings, hypoglycaemia should be considered in the differential diagnosis of cardiomegaly and heart failure in newborn infants.

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Relationship between metabolic control and plasma lipoprotein level in diabetic children

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All lipid and lipoprotein fractions except for total cholesterol were found to be high in diabetic children under long-term treatment. There was a positive correlation between HDL-cholesterol, HbA_{1a-c} and the insulin dose.

In untreated diabetic children, plasma triglyceride and its subfractions were also high, but phospholipids were normal. Total plasma cholesterol was normal, but its VLDL + LDL subfraction was increased and HDL-cholesterol low. After two months of insulin-treatment, parallel with the decrease of HbA_{1a-c}, the HDL-cholesterol increased and there was a drop in the level of triglycerides.

It is concluded that HDL-cholesterol is a good parameter of metabolic control during the initial therapy of diabetes. In patients under long-term treatment there is no such a simple relationship between control and HDL-cholesterol and the daily insulin-dose seems to play an important role in the regulation of plasma HDL-cholesterol.

Atherosclerotic cardiovascular disease is a leading cause of death in insulin-dependent, juvenile onset diabetics. In view of the importance of HDL-cholesterol as a negative risk factor in the development of ischaemic heart disease [7, 21], the relationship between metabolic control and serum lipids and lipoproteins has become an important aspect of diabetes.

In most surveys the incidence of fasting hyperlipoproteinaemia in diabetics has been 30 to 40% [15, 16, 19]. Beside the most frequently elevated triglyceride and cholesterol [1, 15, 16, 20, 29, 35], recently high HDL-cholesterol levels have also been described in insulin-treated maturity onset and juvenile diabetes [3, 7, 10, 17, 18, 22]. A low HDL-cholesterol level has been observed in newly diagnosed insulin deficient diabetes [31] and in maturity onset diabetes [3, 7, 17, 21]. Since HDL-cholesterol showed a significant positive correlation with the insulin sensitive lipoprotein lipase activity [22], and since HDL-cholesterol increased after one year insulin treatment in adults [25], insulin therapy was assumed to play a role in the regulation of HDL-

This work is dedicated to Professor Julius Mestyán on the occasion of this 60th birthday.

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cholesterol level in diabetics. Taskinen and Nikkilä [31] could not, however, demonstrate any increase of HDL-cholesterol level after two weeks insulin therapy in insulin deficient adult diabetics.

Insufficient control of diabetes is associated with increased lipid levels [5, 22, 28, 33]. The triglyceride and cholesterol levels showed a significant positive correlation with HbA_{1a-c} [9, 27], the fasting blood glucose level [8] and daily urinary glucose output [8] in diabetics. In a previous study [18] a similar significant positive correlation was demonstrated between HbA_{1a-c} and plasma HDL-cholesterol level in diabetic children.

The aim of the present study was to analyse the relationship between plasma lipoproteins and diabetes control, and to investigate the mechanism responsible for the elevation of HDL-cholesterol in juvenile diabetes.

Materials and Methods

Thirty two diabetic children (19 boys and 13 girls) and 48 non-diabetic children (29 boys and 19 girls) were studied. The means and ranges of ages were similar in the two groups of children, 10.1 (2–15) *vs.* 9.9 (2–15) years. Most diabetic children were followed as outpatients and some of them were admitted for checking their metabolic control. In 5 children diabetes was newly diagnosed, and in the remainder the average duration was 3.8 years (range, 1–12 years). The newly diagnosed diabetic children were treated as a separate group from those having already been treated for more than 1 year. The daily insulin dose at the time of the study varied from 0 to 1.96 U/kg. In the diabetic children the metabolic control was checked 4–5 times every year. The non-diabetic children were admitted for minor operations or with mild upper respiratory disease and blood samples taken for routine laboratory measurements were used for the purposes of this study. In diabetic children blood samples were collected after overnight fast and before the injection of the morning dose of insulin. Blood samples were taken in EDTA tubes, and plasma was separated immediately in a refrigerated centrifuge, and stored at −20 °C until estimation.

Blood glucose was estimated with the glucose oxidase method and urinary glucose was measured by the o-toluidine method. Ames Acetest tablets were used to assess urinary ketone excretion. Glycosylated haemoglobin was separated on Biorex 70 ion exchange columns. Cholesterol and triglyceride were measured enzymatically with Galenofarm and Boehringer tests. Phospholipids were measured colorimetrically [3]. HDL and VLDL + LDL were separated by heparin manganese chloride precipitation [2, 6, 7, 34]. HDL was determined in the supernatant and the combined concentration of VLDL + LDL was measured from the precipitate [2].

Results

Lipid and lipoprotein levels in diabetic children

All lipid and lipoprotein fractions except for cholesterol were significantly higher in chronic insulin treated diabetic children than in the controls (Table I).

In newly diagnosed, untreated diabetic-children plasma triglyceride and its HDL and VLDL + LDL-subfractions were significantly increased. Total cholesterol was not significantly different from controls, but VLDL +

TABLE I

	N. D. Diabetics		C. T. Diabetics		Controls	
	No.	Mean ± S. E.	No.	Mean ± S. E.	No.	Mean ± S. E.
Trigliceride, mmol/l	5	1.7±0.151[c,d]	108	1.36±0.1[b]	48	1.03±0.05
Cholesterol, mmol/l	5	4.3±0.23	122	4.1±0.14	48	3.8±0.114
Phospholipid, mmol/l	5	2.12±0.2	75	2.52±0.13[c]	48	1.72±0.09
HDL-triglyceride, mmol/l	5	0.63±0.09[c]	74	0.59±0.026[c]	48	0.38±0.02
HDL-cholesterol, mmol/l	5	0.74±0.12[c,d]	118	1.5±0.04[b]	48	1.26±0.07
HDL-phospholipid, mmol/l	5	1.12±0.15	73	1.46±0.08[c]	48	1.09±0.09
VLDL + LDL-triglyceride, mmol/l	5	0.96±0.1[b]	72	0.97±0.06[c]	48	0.72±0.045
VLDL + LDL-cholesterol, mmol'l	5	2.6±0.21[b]	72	2.37±0.12[a]	48	1.91±0.1
VLDL + LDL-phospholipid, mmol/l	5	0.9±0,1	72	0.97±0.066[a]	48	0.75±0.06

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ for the difference between cases and controls, [d] $p < 0.001$ for the difference between N. D. and C. T. diabetic groups. N. D.: newly diagnosed, C. T.: chronically treated

LDL-cholesterol was higher and HDL-cholesterol was much lower than in the controls. There was no change in plasma phospholipid and its HDL and VLDL + LDL subfractions (Table I).

In the untreated diabetics, high total triglyceride and low HDL-cholesterol were the two characteristic differences in the lipoprotein profiles of the two diabetic groups (Table I).

Plasma lipoprotein level and the degree of metabolic control

"Chronic" insulin treated children. Dividing the "chronic" insulin treated diabetic children into groups of satisfactory versus poor control on the basis of urinary glucose excretion, fasting blood glucose and HbA_{1a-c}, no differences in the plasma lipoproteins could be demonstrated except for the higher triglyceride level in the group with poor control (Table II).

However, when all values of the two groups were analysed, a significant positive correlation was observed between plasma cholesterol, HDL-cholesterol and glycosylated haemoglobin (Figs 1, 2). No such relationship existed between HbA_{1a-c} and triglyceride, phospholipid and their HDL- and VLDL + LDL subfractions.

Newly diagnosed diabetics. The metabolic control as judged by HbA_{1a-c}, fasting blood glucose and urinary glucose excretion, improved dramatically as a result of insulin treatment

TABLE II

Influence of satisfactory versus poor control of diabetes on plasma lipid and lipoprotein concentrations

	Satisfactory control		Poor control		
	No.	Mean ± S. E.	No.	Mean ± S. E.	p
HbA_{1a-c} per cent	51	10.2±0.5	71	18.5±0.6	<0.01
Fasting blood, glucose mmol/l	51	8.7±0.32	71	15.69±1.5	<0.01
Urinary glucose, g/day	51	12.8±2.1	71	54.0±5.4	<0.01
Urinary ketone bodies	51	neg.	71	+ − +	+++
Triglyceride, mmol/l	45	1.076±0.08	63	1.56±0.15	<0.02
Cholesterol, mml/ol	51	3.9±0.19	71	4.34±0.17	ns
Phospholipid, mmol/l	34	2.61±0.14	41	2.45±0.17	ns
HDL-triglyceride, mmol/l	33	0.57±0.04	41	0.6±0.04	ns
HDL-cholesterol, mmol/l	53	1.48±0.06	65	1.52±0.05	ns
HDL-phospholipid, mmol/l	35	1.35±0.1	38	1.55±0.12	ns
VLDL + LDL-trigliceride, mmol/l	33	0.89±0.08	39	1.02±0.08	ns
VLDL + LDL-cholesterol, mmol/l	33	2.43±0.18	39	2.31±0.15	ns
VLDL + LDL-phospholipid, mmol/l	33	0.94±0.07	39	0.99±0.097	ns

TABLE III

Effect of insulin on metabolic control and lipoprotein levels in newly diagnosed diabetics

	Before insulin		After insulin*		
	No.	Mean ± S. E.	No.	Mean ± S. E.	p
HbA_{1a-c}, per cent	5	14.56±0.6	5	10.02±1.02	<0.01
Fasting blood glucose, mmol/l	5	12.34±1.01	5	8.42±1.02	<0.01
Urinary glucose, g/day	5	37.1 ±5.4	5	19.4±4.1	<0.05
Triglyceride, mmol/l	5	1.7±0.15	5	1.13±0.1	<0.05
Cholesterol, mmol/l	5	4.3±0.23	5	3.89±0.2	ns
Phospholipid, mmol/l	5	2.12±0.2	5	2.2±0.23	ns
HDL-triglyceride, mmol/l	5	0.63±0.09	5	0.43±0.08	ns
HDL-cholesterol, mmol/l	5	0.74±0.12	5	1.36±0.13	<0.01
HDL-phospholipid, mmol/l	5	1.12±0.15	5	0.05±0.21	ns
VLDL + LDL-triglyceride, mmol/l	5	0.96±0.1	5	0.8±0.1	ns
VLDL + LDL-cholesterol, mmol/l	5	2.60±0.21	5	2.4±0.22	ns

* After two months of insulin therapy.

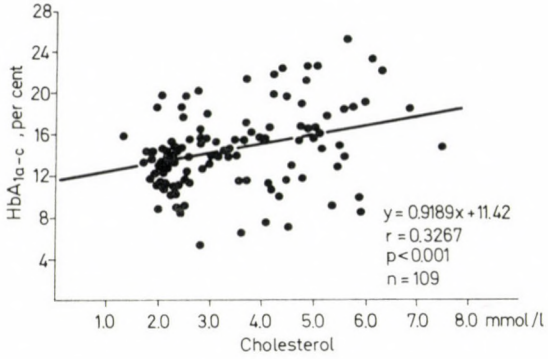

FIG. 1. Relatonship between glycosylated Hb and plasma cholesterol

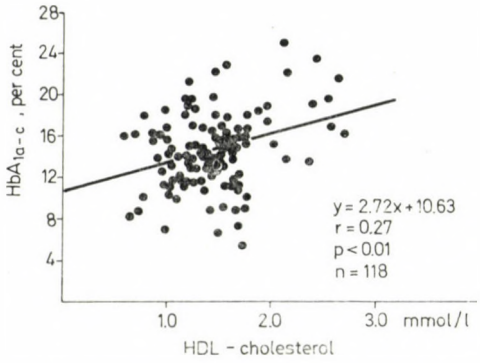

FIG. 2. Relationship between glycosylated Hb and HDL-cholesterol

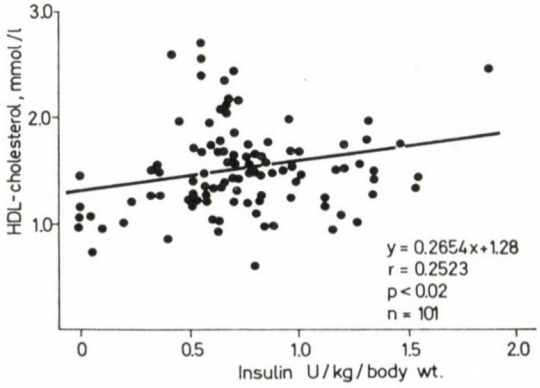

FIG. 3. Correlation between HDL-cholesterol and daily insulin dose

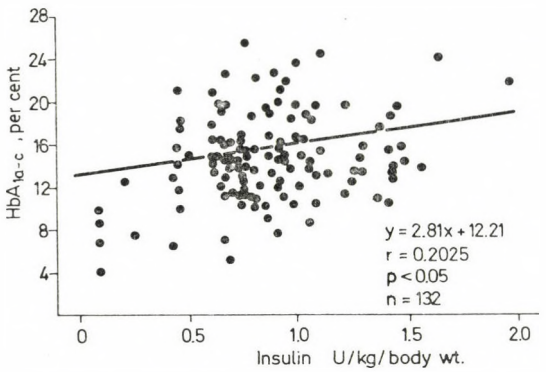

FIG. 4. Correlation between glycosylated Hb and daily insulin dose

and diet (Table III). Parallel with the improvement, HDL-cholesterol increased significantly and triglyceride decreased. The other lipids and lipoprotein subfractions did not change significantly (Table III).

In addition to the relationship between metabolic control and lipoprotein levels, the possible correlation between insulin therapy and lipoprotein levels has also been investigated.

Daily insulin dose, glycosylated Hb and plasma lipoprotein levels

The daily insulin dose showed a significant positive correlation with HDL-cholesterol (Fig. 3) in the "chronic" insulin treated diabetics suggesting that the more the insulin, the higher the HDL-cholesterol level. Higher insulin doses did not necessarily mean a better control. On the contrary, there was a positive correlation between Hba_{1a-c} and the dose of insulin (Fig. 4).

DISCUSSION

The significantly elevated lipid and lipoprotein levels observed in this study were in accordance with previous findings [8, 15, 16, 19]. There are, however, controversial data in the literature on the plasma level of HDL-cholesterol in diabetics and on its relationship with metabolic control. Significantly lower plasma HDL-cholesterol levels have been reported in maturity-onset [3, 7, 17, 21], and in untreated insulin deficient [31] diabetes. High or normal values have been observed in insulin treated maturity-onset and juvenile diabetes [3, 7, 10, 17, 22]. Elkeles et al [12] and Yudkin et al [36] found a positive, while Calvert et al [7] a negative correlation between HDL-cholesterol and glycosylated haemoglobin in diabetics. Barta et al [4] found an increase in HDL-cholesterol concentration parallel with the improvement of metabolic control, while others [31] failed to do so.

In the present study plasma HDL-cholesterol was significantly higher in "chronic" insulin treated and significantly lower in untreated diabetic children than in age-matched controls.

In addition to the fundamental difference in the plasma HDL-cholesterol levels of the two diabetic groups, further differences were revealed when the relationship between HbA_{1a-c} and HDL-cholesterol was studied. There was a positive correlation between HDL-cholesterol and HbA_{1a-c} in "chronic" insulin treated diabetic children confirming our previous observation [18]. In newly diagnosed diabetic children, however, the commencement of insulin therapy was associated with a decrease of HbA_{1a-c} and with an increase of the HDL-cholesterol level. In other words, an increased HDL-cholesterol would indicate poor control in the first and improving metabolic control in the second group. Or else, the relationship between diabetic metabolic control and the plasma HDL-cholesterol levels is a complex one and HDL-cholesterol could not be regarded as an independent parameter of the quality of control.

The significant positive correlation between HDL-cholesterol and daily insulin dose in "chronic" insulin treated children suggests that insulin therapy itself could play an important role in the regulation of plasma HDL-cholesterol level in diabetic children.

A remarkable part of HDL-cholesterol is a product of the catabolism of triglyceride-rich lipoproteins [11, 14]. Lipoprotein lipase, the main catabolic enzyme of the triglyceride rich lipoproteins is known to be highly sensitive to insulin [23, 26, 32]. Low lipoprotein lipase (LPL) activity with low HDL-cholesterol level in untreated insulin deficient diabetics [31], and high LPL activity with high HDL-cholesterol level in chronic insulin-treated diabetics [22] have been reported. LPL activity and HDL-cholesterol concentration showed a strong positive correlation [22, 31]. Thus, exogenous insulin probably elevates the HDL-cholesterol level by increasing LPL activity.

On the basis of our observations it seems likely that plasma HDL-cholesterol levels in children are influenced both by exogenous insulin as well as by other factors known to alter the plasma HDL-cholesterol concentration. On the one hand, insulin treatment was followed by a rapid improvement of metabolic control and an increase of HDL-cholesterol as shown in the newly-diagnosed group of diabetic children (Table III). High HDL-cholesterol levels, on the other hand, could also be associated with poor control despite the high dose of insulin as found in the "chronic" insulin treated group (Figs 3, 4). The opposite process, increasing HDL-cholesterol levels parallel with decreased insulin dosage has also been observed in diabetic children: Barta et al [4] observed a significant increase in plasma HDL-cholesterol during a summer camp for diabetic children when the insulin dose had been decreased.

Finally, our observations proved further data to the complexity of the independent and interlinked factors influencing plasma lipoprotein levels in diabetes. We are well aware that these observations require further confirmation in a more homogeneous group of young insulin dependent diabetics, especially in view of the conflicting findings of others.

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Energy cost of growth of premature infants

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The objective of this paper is to review approaches to the determination of the energy cost of growth in premature infants. Two approaches are compared: one based on the composition of weight gain, and one based on the determination of energy balance. Data are lacking on the composition of weight gained by the premature infant after birth, while the composition of fetal weight gain and its energy cost can be calculated from data on fetal body composition. These calculations show that energy storage amounts to less than 8.4 kJ/g weight gain below a body weight of 2 kg; the total energy cost of growth is less than 10.5 kJ/g. Estimates twice as high have been obtained from energy balance studies of growing premature infants and older infants. We conclude that the energy cost of growth in premature infants is still uncertain and requires further study.

The survival of the prematurely born infant is related to its ability to grow. However, premature birth poses a threat to the maintenance of energy intake, and as a consequence, to growth. It is therefore of obvious clinical interest to know how much energy is required by the premature infant in order to grow in the extrauterine environment.

This question can best be put within the context of overall energy balance. In the absence of external work, the energy balance of the whole body may be stated as follows: Gross Energy Intake = Energy Excretion + Energy Expenditure + Energy Storage

The energy is measured in units of kilojoules (kJ), which may be converted to kilocalories (kcal) by dividing by 4.18.

Gross, Digestible and Metabolizable Energy. The gross energy of a food is measured by determining the heat produced by its combustion in a bomb calorimeter. The gross energy of a food, determined by complete combustion in an atmosphere of compressed oxygen, is greater than the energy value of the food to the body for two reasons: (1) part of the food escapes absorption and is excreted in the stool, and (2) *in vivo* oxidation does not convert the nitrogen component of the food to oxide of nitrogen but leads to the formation of urea and other compounds which are excreted in the urine.

Digestible energy is defined as the gross energy of the diet minus the heat of combustion of the stool, and metabolizable energy is defined as digested energy minus the heat of combustion

of the urine. Digestible energy represents the fuel value of the food after taking into account losses due to incomplete digestion, and metabolizable energy represents the fuel value of the absorbed components of the food after taking into account the energy losses due to incomplete oxidation.

The Fate of Metabolizable Energy: Expenditure and Storage. In the non-working subject, metabolizable energy is expended as heat or stored. The expenditure of energy is conventionally divided into two major components: basal energy expenditure, and expenditure above the basal rate (for example, that due to activity or elicited by thermal stress) [5].

Basal energy expenditure cannot be measured in the infant. Growth itself requires the expenditure of energy, so the basal rate of energy expenditure cannot be measured in the growing infant. Moreover, in the non-growing adult, basal energy expenditure is measured under defined conditions which require the subject to be at rest in a post-absorptive state. There is no physiologically equivalent circumstance in the growing infant, who is either "digesting one meal or actively anticipating the next" [7].

In growing infants, the closest approximation to the basal energy expenditure is the "minimal" rate of energy expenditure measured when the infant is in quiet sleep in a thermoneutral environment. The minimal energy expenditure thus determined includes an increment above basal due to food intake and growth.

The presence of food in the gastrointestinal tract leads to an increase in the level of energy expenditure [6] due to the physiological work of digestion, absorption and assimilation. This increased rate of energy expenditure over the basal state is referred to as the heat increment of feeding.

In the growing baby, a fraction of the energy expenditure is used to support the biosynthetic processes involved in net tissue synthesis. This is part of the energy cost of growth. The other, and larger, part of the energy cost of growth consists of the energy stored in growing tissues and organs (chiefly as fat and protein); this component of the energy cost of growth is represented by the "energy storage" term of the energy balance equation.

In this paper, we review two approaches that have been used to estimate the energy cost of growth in premature infants:

1. The body composition approach, and
2. The clinical physiology approach.

Energy Cost of Growth as Estimated from Body Composition

If the net accretion rates of fat and protein in growing infants were known, energy storage could be calculated from the gross energy values for fat and protein respectively. Data on the body composition of full term newborns and infants provide a basis for calculating the composition of weight gain of such subjects during infancy.

The "male reference infant" of Fomon [2] is assumed to weigh 3.5 kg at birth and to double his weight by four months of age. The composition of weight gain during that four-month interval is 11.4% protein, 40.8% fat, and 45.3% water. Assuming gross energy values of 23.8 kJ/g for protein and 38.9 kJ/g for fat [4], the energy stored per gram of weight gain is therefore 18.6 kJ/g. The total energy cost of growth is the sum of the energy value of the tissues laid down, plus the energy expended in the anabolism of these tissue components from the materials absorbed from the digestive tract. The energy costs of protein and fat formation differ because of the difference in the complexities of the metabolic processes leading to their formation from their respective precursors. Kielanowski estimated the overall cost of depositing one gram of protein and of fat as 31.4 and 48.5 kJ/g respectively [3]. If we apply these factors, derived from experiments in baby pigs, to the male reference infant, we obtain a figure of 23.4 kJ/g for the total energy cost of growth.

Changes in body composition following premature birth are not accurately known; therefore, the composition of weight gain in growing premature infants cannot be calculated because of the lack of suitable data. However, the body composition of the growing fetus has been studied in considerable detail [10, 11]. Ziegler et al [11] summarized existing data and constructed from these data a "reference fetus" whose body composition changes during the latter part of gestation as shown in Fig. 1. As can be seen, the increase in size is accomplished by an increase in each of the major body components. However, as shown in Fig. 2, the composition of weight gain changes throughout the last part of gestation. It is of particular relevance to the topic of this paper to note that fat comprises

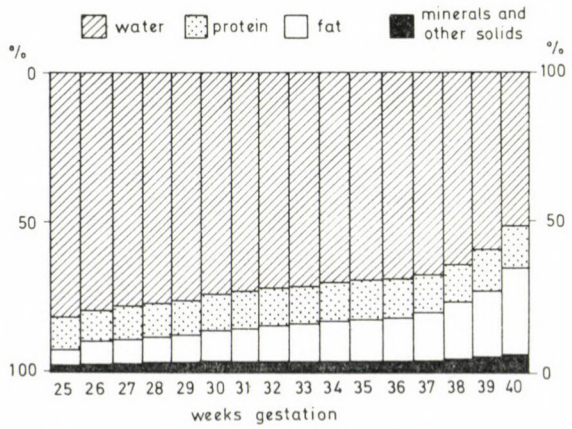

FIG. 1. Body composition of the human fetus at different gestational ages, from data of Ziegler et al [11]

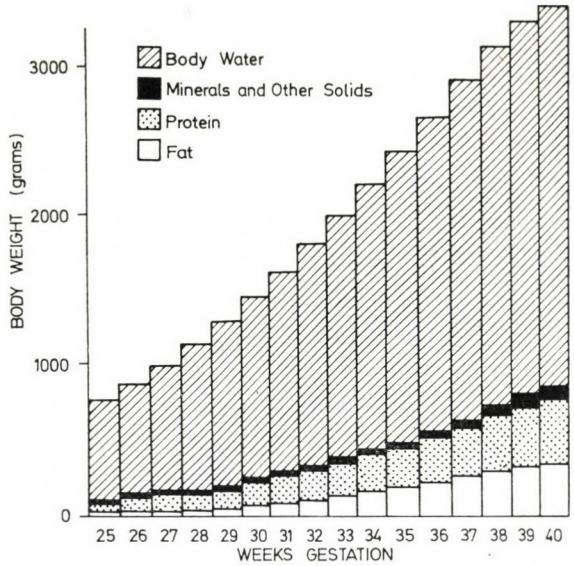

FIG. 2. Composition of weight gain in the reference fetus with gestational age, from data of Ziegler et al [11]

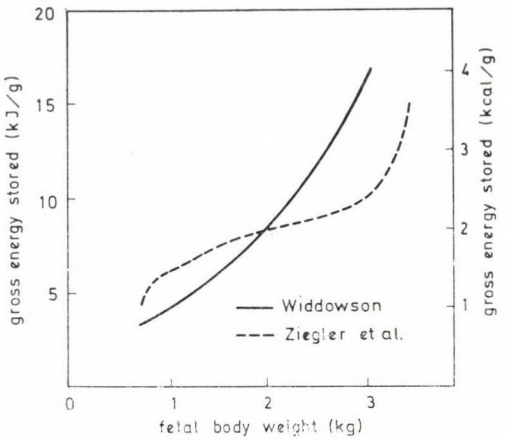

FIG. 3. Gross energy stored (kJ/g weight gain) as fetal weight increases. Calculations are based on the fetal body composition data of Ziegler et al [11] and Widdowson [10]. Assumed gross energy values (kJ/g) are 38.9 for fat, 23.8 for protein

a very small proportion of weight gain in the pre-term period, and that this proportion rises substantially as term approaches.

The energy equivalent of fetal weight gain can be calculated from such data on the rate of accretion of fat and protein. As shown in Fig. 3, the studies of both Ziegler et al [11] and Widdowson [10] predict that the energy stored per gram of fetal weight gain is not a constant figure, but

increases with fetal weight. Although the two studies do not agree closely (particularly in the upper range of fetal weight), there is reasonably close correspondence in the projection that below a fetal weight of 2 kg, energy storage varies between 4.2–8.4 kJ/g. Applying the factors of Kielanowski [3], it can be calculated that the total energy cost of growth varies between 5.0 and 10.5 kJ/g in the fetal weight range below 2 kg.

It is a major assumption that the composition of the premature infant's weight gain in the extrauterine environment is the same as that which would have occurred in utero over the same weight interval had the fetus not been delivered prematurely. Without making this assumption, it can be shown that during fetal growth the available data on body composition predict that the energy storage and the total energy cost of growth are considerably lower than the similarly calculated values for growth in infancy after full term birth. Moreover, during fetal growth, compositional data predict a varying energy storage due to the changing composition of weight gain. Below a body weight of 2 kg, the total energy cost of growth is calculated as less than 10.5 kJ/g weight gain. This low value is determined primarily by the relatively small lipid content of weight gain in the very low weight range.

The question then arises: in growing premature infants, are values for energy storage and the total energy cost of growth similar to those calculated from the body composition of the growing fetus? To answer this question we required clinical studies of the energy balance of growing premature infants.

Energy Cost of Growth as Estimated by Clinical Physiological Studies

The energy cost of growth can be determined from measurement of the energy balance in growing subjects. For such measurements of the energy cost of growth to be precise the energy storage must be a relatively large fraction of the gross energy intake; therefore, the subjects should be growing rapidly. During the late fetal and early postnatal periods, a growth rate of 1.5% per day (15 g/kg day) is typical. Thus, energy storage is measurable with reasonable precision in growing premature infants by applying the energy balance principle. The study should extend over a period that is long enough to measure weight gain precisely (10–14 days). In order to determine the increment in energy expenditure associated with growth, the subjects should be growing at different rates (i.e. there should be both fast growing and slowly growing or non-growing subjects included for comparison).

There are two general methods whereby the energy cost of growth has been deduced from energy balance.

1. The total energy cost of weight gain has been determined from the relationship between metabolizable energy intake and weight gain. The

slope of the regression of metabolizable energy intake on weight gain describes the increment in metabolizable energy intake per gram of weight gain and has been taken to represent the total energy cost in weight gain. It should be noted that this slope is determined by the sum of the increments in both energy storage and in total daily energy expenditure expressed per gram weight gain. The increment in total daily energy expenditure per gram weight gain is approximately equal to the energy expended for growth, although there may be additional factors to consider (see below).

2. The total energy cost of growth has been calculated by determining separately the energy storage and the energy expended for growth, and adding the two.

Energy storage is calculated from the energy balance:

Energy storage = Gross energy intake — Energy excretion — Energy expenditure.

The major technical obstacle is the measurement of total daily energy expenditure. Energy storage can be related to weight gain in two ways: (*1*) by the ratio method, whereby energy storage is divided by weight gain to obtain the energy stored per gram of weight gain (the ratio method is theoretically valid if, at zero weight gain, energy storage is zero [9]); and (*2*) by the regression method, whereby energy storage is plotted as a function of rate of weight gain, so that the slope of the linear regression gives energy stored per gram of weight gain.

The increase in energy expenditure in association with weight gain has been determined by the regression of total daily energy expenditure on weight gain. The slope of this line has been taken to give the increment in energy expenditure per gram of weight gain. This can be held to represent the energy expended in the net synthesis of new tissue if one assumes that other factors affecting total daily energy expenditure are constant over the range of growth rates studied. However, this assumption would not be valid if, for example, activity were to vary systematically with growth rate. Thus, the slope of the regression of total daily energy expenditure on weight gain gives a measure of the empirical relation between the two: determinants of this relationship include energy expended for growth and possibly other types of energy expenditure which vary systematically with growth rate.

The energy cost of growth has been calculated by the above methods in studies reported by Brooke et al for premature infants [1], and by Spady et al for malnourished older infants during recovery [8]. Both reports will be cited in order to establish certain similarities and differences.

* The infants were fed Cow and Gate® V Formula. The gross energy of V Formula as determined by Brooke et al [1] by bomb calorimetry, was 344 kJ/dl. This is 32% more than the label declaration for metabolizable energy (261 kJ/dl). This large discrepancy is unexplained; it should be borne in mind when considering the absolute values reported for gross energy intake, metabolizable energy and energy storage.

Brooke et al. [1] studied growing premature infants, including several under 1500 g in birth weight. Metabolizable energy intake of these infants averaged 601 kJ/kg.day.* Total energy expenditure amounted to 370 kJ/kg.day. This left 230 kJ/kg.day for energy storage, which represented 38% of metabolizable energy intake.

Brooke et al plotted weight gain (y-axis) as a function of metabolizable energy and stored energy (x-axis). We have re-analysed these data in order to display the energy variables as a function of weight gain (i.e. kJ energy/g weight gain).

Figures 4 and 5 show the relationships between metabolizable energy intake and weight gain and between stored energy and weight gain for the premature infants studied by Brooke et al. At zero weight gain, the metabolizable energy intake was 349 kJ/kg.day. Each gram of weight gain was associated with an increment of 18.3 kJ in metabolizable energy intake. This is one estimate of the total energy cost of weight gain. Each gram of weight gain was associated with an energy storage of 15.6 kJ.

For each gram of weight gain, the data of Brooke et al show a larger

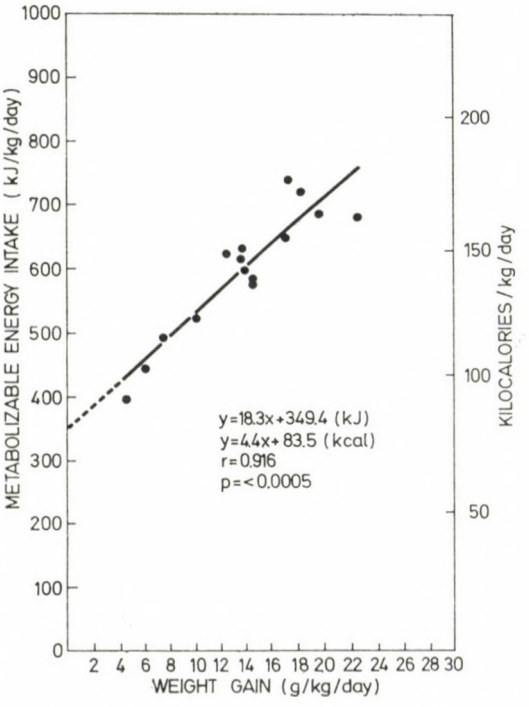

FIG. 4. Relation between metabolizable energy intake and weight gain in growing premature infants (data of Brooke et al, ref. 1). The intercept (349 kJ/kg day) defines the metabolizable energy intake at zero weight gain. The slope of the line (18.3 kJ/g) defines the total energy cost of weight gain

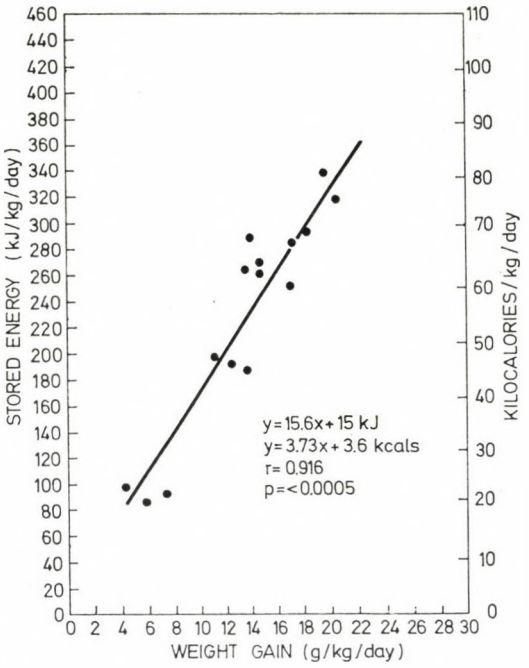

FIG. 5. Relation between energy storage and weight gain in growing premature infants (data of Brooke et al, ref. 1). The slope of line (15.6 kJ/g) defines the energy stored per gram weight gain. As predicted the energy stored at zero weight gain is not significantly different from zero !

increment in metabolizable energy intake (18.3 kJ) than could be accounted for as energy storage (15.6 kJ). Therefore, as rate of weight gain increased, energy expenditure must have increased as well.

We have performed further analyses of the data published by Brooke et al to explore the source of the increased energy expenditure of rapidly-growing infants. As rate of weight gain increased, postprandial energy expenditure increased significantly (0.7 kJ/g) (Fig. 6) and resting energy expenditure increased significantly (4.0 kJ/g) (Fig. 7). These values, when added to the energy storage equivalent of weight gain (15.6 kJ/g), give an estimate of the total energy cost of weight gain (20.3 kJ/g) which is greater than the increment of metabolizable energy intake (18.3 kJ/g). This difference may be due to the decline in activity energy expended by the babies with increasing weight gain; however, this relation was not significant statistically. In summary, as metabolizable energy intake increased, total energy expenditure increased, due to increases in both resting and postprandial energy expenditure. These increases were partially offset by a fall in energy expended in activity.

The results obtained by re-analysis of the data of Brooke et al for

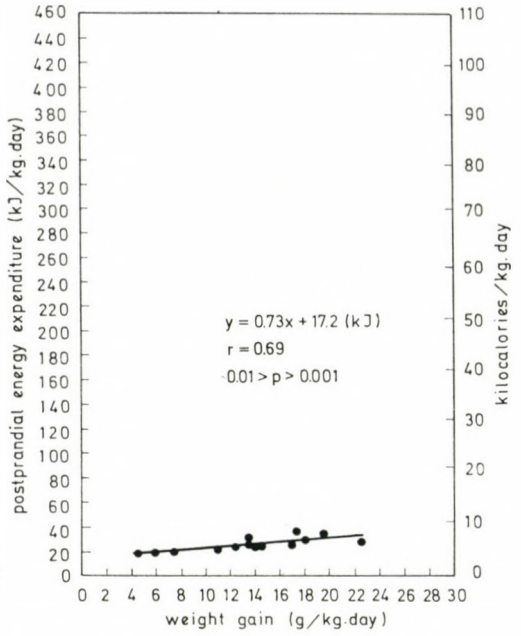

FIG. 6. Relation between postprandial energy expenditure and weight gain in growing premature infants (data of Brooke et al, ref. 1). Postprandial energy expenditure increases significantly (0.7 kJ/g) as rate of weight gain increases

growing premature babies may be contrasted with those obtained in an energy balance study of similar design in malnourished but recovering infants aged 8–18 months, reported earlier by Spady and co-workers [8] (Table I). The values for the two quite disparate groups are astonishingly similar.

By contrast, the values for the (total) energy cost of weight gain that we have calculated from the data of Brooke et al are considerably greater than those we have calculated on the basis of increments in fetal body composition in the low-weight range (Table II). In fact, the values obtained from the data of Brooke et al. closer to what would be predicted on the basis of body composition for the growing term infant during the first four months after birth. It is tempting to speculate that premature birth triggers a shift to a more "mature" composition of weight gain, comprising yet another example of precocious maturation. However, error could also account for the discrepancy. Three kinds of error must be mentioned in regard to the clinical study of energy balance.

First, the study of energy balance is beset by possibilities for error which all tend to over-estimate energy storage. This results because of systematic tendencies to over-estimate energy intake (e.g. because of milk remaining in the inside of tubes and

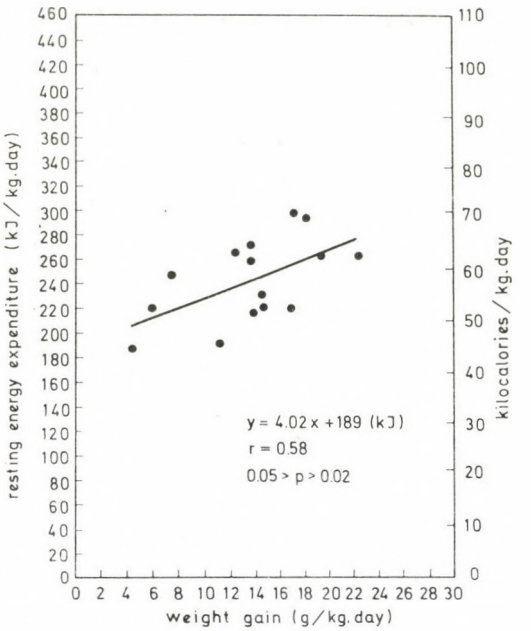

FIG. 7. Relation between resting energy expenditure and weight gain in growing premature infants (data of Brooke et al, ref. 1). Resting energy expenditure increases significantly (4.0 kJ/g) as rate of weight gain increases

syringes, undetected regurgitation) and to underestimate energy excretion (incomplete collection of stool or urine).

Second, there are numerous opportunities for experimental error in the study of energy balance which could affect the results in either direction. Examples include errors in the determination of gross energy intake (dilution error or non-homogeneity of milk feeding, error in bomb calorimetry)

TABLE I

Energy intake, energy storage, and weight gain in premature infants and 8–18 month infants

	Subjects	
	Premature infants (ref. 1)	8–18 month infants recovering from malnutrition (ref. 8)
Maintenance energy requirement, kJ/kg day	349	357
Metabolizable energy intake per gram weight gain, kJ/g	18.3	18.4
Energy storage per gram weight gain, kJ/g	15.6	16.7

TABLE II

Energy cost of weight gain (kJ/g)

	Basis of estimate		
	Body composition		Clinical experiment
	Term infant 0–4 month (ref. 2)	Premature infant (ref. 10, 11)	Premature infant (ref. 1)
Energy storage	18.6	4.2–8.4	15.6
Increment in energy expenditure	4.8	0.8–2.1	2.7–4.7
Total energy cost of weight gain	23.4	5.0–10.5	18.3–20.3

or errors in measurement of total daily energy expenditure. As regards the study of Brooke et al, we have noted previously the unexplained large discrepancy between the experimentally determined gross energy of the formula and the label declaration of (metabolizable) energy. A modest overestimation of gross energy intake would result in a relatively large overestimation of calculated energy storage.

Third, there are problems related to analysis. When an attempt is made to relate energy intake, expenditure or storage to weight gain, problems arise when other variables such as body weight, are related to both the independent and dependent variables. For example, there may be a positive correlation between metabolizable energy intake and both body weight gain, so that for babies of different weights regression analysis of metabolizable energy intake (kJ/day) against weight gain (g/day) could result in a positive relationship largely attributable to the relationship between metabolizable energy intake and body weight.

This problem is clearly recognized by both Brooke and Spady, who have sought to standardize for weight by expressing energy intake, energy expenditure, energy storage and weight gain in kilojoules/kilogram body weight or grams/kilogram body weight. The subsequent regression analysis of, say, metabolizable energy intake (kJ/kg.day) on weight gain (g/kg.d) produces a slope whose value is fallaciously expressed as kJ metabolizable energy intake/grams weight gain.

That the expression

$$\frac{\text{Mean kJ/kg}}{\text{Mean g/kg}} \quad \text{does not equal} \quad \frac{\text{Mean kJ}}{\text{Mean g}}$$

is demonstrated algebraically in the Appendix. The only conditions under which the two expressions are equivalent are obtained when either all babies are of the same weight or when the ratios of kilojoules metabolizable energy intake to grams weight gain are identical between each subject.

The appropriate way in which to examine such a relationship is to estimate a partial regression coefficient for each variable. Thus, for daily metabolizable energy intake

$$\text{Metabolizable Energy Intake (kJ)} = \\ = \text{Energy Expended (kJ)} + \\ + \text{Energy Stored (kJ)} \quad (1)$$

$$\text{if Energy Expenditure (kJ)} = \\ = a_1 + b_1 \text{ Body Weight (kg)} \quad (2)$$

$$\text{and Energy Storage (kJ)} = \\ = a_2 + b_2 \text{ Weight Gain (g)} \quad (3)$$

where a_1 and a_2 are constants and b_1 b_2 are the regression coefficients of the equation.

Then

$$\text{Metabolizable Energy Intake (kJ)} = \\ = a_1 + a_2 + b_1 \text{ Body Weight (kg)} + \\ + b_2 \text{ Weight Gain (g)} \quad (4)$$

Let

$$\text{constant } A = a_1 + a_2 \quad (5)$$

Then

$$\text{Metabolizable Energy Intake (kJ)} = \\ = A + b_1 \text{ Body Weight (kg)} + \\ + b_2 \text{ Weight Gain (g)} \quad (6)$$

The solution to this question is now available using multiple linear regression analysis. This will give a true estimate of the coefficient b_2, the slope of metabolizable energy intake on weight gain, in kilojoules per gram.

Unless the relationship between the dependent and independent variables is such that the line of best fit passes through the origin, quite different results may be anticipated from the two relationships:

$$y \text{ (kJ/kg)} = A + bx \text{ (g/kg)}$$

and

$$y' \text{ (kJ)} = A' + b_1 x' \text{ (g)} + b_2 \text{ Wt (kg)}$$

Certainly the regression equation between metabolizable energy intake and weight gain has a substantial intercept quite different from the origin (Fig. 4) and we may expect the regression coefficients (kJ/kg on g/kg, versus kJ on g) to be quite different.

On the other hand where the line of best fit passes close to the origin (i.e. where A approximates zero) we may expect the regression coefficients to be similar for both calculations.

The multiple linear regression model allows to make estimates (by extrapolation) of metabolizable energy intake when weight gain is zero for infants of specified weight — thus, from equation 6 at zero weight gain

$$\text{Metabolizable Energy Intake (kJ)} = \\ = A + b_1 \text{ Wt (kg)}.$$

The multiple linear regression model allows for adjustment of these regression coefficients as further variables are identified and added.

We are unable to re-examine the data of Brooke or Spady by multiple linear regression, as daily weights were not published. Further work on energy balance with close attention to the three major sources of error

described above will give closer estimates of the energy cost of growth. This information along with nitrogen balance will make it possible better to interpret the composition of weight gain in the low birthweight infant.

Appendix

Consider three subjects, of weight w_1, w_2 and w_3 kilograms respectively, whose daily weight gains are g_1, g_2 and g_3 grams per day and whose daily energy intakes are respectively e_1, e_2 and e_3 kJ/day.

Then energy intakes (kJ/kg.day) are

$$\frac{e_1}{w_1}, \frac{e_2}{w_2}, \frac{e_3}{w_3}$$

and weight gains (g/kg.day) are

$$\frac{g_1}{w_1}, \frac{g_2}{w_2}, \frac{g_3}{w_3}$$

so that mean energy intake in kJ/kg.day is

$$\left(\frac{e_1}{w_1} + \frac{e_2}{w_2} + \frac{e_3}{w_3}\right) \div 3$$

and mean weight gain in g/kg.day is

$$\left(\frac{g_1}{w_1} + \frac{g_2}{w_2} + \frac{g_3}{w_3}\right) \div 3$$

Therefore mean energy intake (kJ/kg.day) divided by mean weight gain (g/kg.day) is

$$\frac{\frac{e_1}{w_1} + \frac{e_2}{w_2} + \frac{e_3}{w_3}}{\frac{g_1}{w_1} + \frac{g_2}{w_2} + \frac{g_3}{w_3}} =$$

$$= \frac{e_1w_2w_3 + e_2w_1w_3 + e_3w_1w_2}{g_1w_2w_3 + g_2w_1w_3 + g_3w_1w_2}$$

Now mean daily energy intake (kJ) equals

$$\frac{e_1 + e_2 + e_3}{3}$$

and mean weight gain (grams) equals

$$\frac{g_1 + g_2 + g_3}{3}$$

so mean energy intake (kJ) divided by mean weight gain (g) is

$$\frac{e_1 + e_2 + e_3}{g_1 + g_2 + g_3}$$

Now

$$\frac{e_1w_2w_3 + e_2w_1w_3 + e_3w_1w_2}{g_1w_2w_3 + g_2w_1w_3 + g_ew_1w_w} \neq$$

$$\neq \frac{e_1 + e_2 + e_3}{g_1 + g_2 + g_3}$$

unless either $w_1 = w_2 = w_3$
and/or

$$\frac{e_1}{g_1} = \frac{e_2}{g_2} = \frac{e_3}{g_3}$$

It is a condition of the linear regression model that the line of best fit passes through the mean value for y and the mean value for x. As this point may differ according to the mode of expression of variables (kJ/kg or kJ, g/kg or g) then it follows that the linear regression model cannot be identical for both types of analysis, unless either of the two conditions above is present.

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Protein turnover rate in early life

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"Animal experiments are carried out to obtain vistas and to get ideas of the mechanisms of biological operation" (D. H. Barron, 1959).

It is a great pleasure and privilege to be invited to honour a good friend and distinguished medical scientist, Professor Julius Mestyán. We met more than ten years ago over our common interest in amino acid metabolism in the fetus and newborn. Subsequently, one of his colleagues, Dr. Gyula Soltész, joined us in the laboratory and introduced us to the measurement of protein synthetic rate *'in vivo'*. This had a profound influence on our work and a summary of the results in the fetal and newborn lamb, together with a short review of recent observations in the human newborn infant, is offered as a tribute to the stimulus from Pécs in Hungary.

THE NEWBORN

Two basic facts concerning protein metabolism in the young animal are well established. Firstly, the amino acid composition of their protein is similar to that of the adult. Secondly, and in contrast, the pattern of the free amino acids in both extracellular and intracellular fluids is different from the adult; moreover, the amino acid concentrations are highest in the fetus [50, 52]. These latter observations suggest that the balance of nitrogen metabolism between the organs is quite different in the growing animal. We thought that studies of the protein turnover rate in this period would provide a basis for some understanding of the amino acid requirements for growth and the effect of malnutrition at this time, and assist clinicians with their considerations for intravenous feeding of the newborn child and of nutritional supplementation for mothers at risk for delivering infants small for their gestational age.

A high turnover rate, as well as a faster deposition rate of protein might be anticipated in the growing animal because of the many structural rearrangements occurring at this time. Schoenheimer's concept of the dynamic state of the body protein may be exhibited to its full extent in the situation where the endocrine systems are relatively immature and the regulation of the biochemical processes not fully developed.

From general considerations of the evidence for the energy requirement of animals of different sizes, of the decline which takes place during growth and of the pattern of changes in the cellular and subcellular aspects of protein metabolism, tissue RNA content and enzyme activity, Munro

[29] and Young [58] anticipated a three to five fold level of protein turnover in the newborn human infant in comparison with the young adult. At the time of our studies Oja [32] had observed that labelled amino acid uptake by protein was very fast in liver homogenates of newborn rats in comparison with the adult, and Waterlow and Stephen [49] had found skeletal muscle protein synthesis "in vivo" to be inversely related to body weight in young rats.

Newborn Lambs

Our observations were made in the newborn lamb [44], which approximates to the size of the human infant but grows more quickly. The method of Waterlow and Stephen [49] was used to study the fractional synthesis rate of the mixed protein in some individual organs; the uptake of labelled ^{14}C leucine by protein was measured in the steady state judged from the plasma specific activity level; this plateau is reached before that of the intracellular pool, because it is smaller, and thereafter the amino acid uptake by protein is considered to be linear. The calculations were made from the ratio of the protein-bound (S_B) to free-intracellular (S_i) specific activity in the tissues at the end of five hours infusion using the equations for tissues with a fast turnover [11].

Newborn Clun lambs 2–5 days of age, weighing 2.5–7.0 kg, were studied about one hour after separation from their mothers. They were all well fed judging by the milk left in the rumen at the end of the observations, and although the blood glucose was not measured, subsequent measurement on suckling lambs of the same age and condition was 5.5 mM or greater. The carotid artery and jugular vein were catheterised under local anaesthesia for blood sampling and continuous infusion of the ^{14}C labelled leucine; the lambs slept throughout most of the experimental period. At the end of the infusion they were anaesthetised with pentobarbital, and samples of the organs taken as quickly as possible into ice-cold 10% T.C.A. for preparation for analysis of the free and bound cold and labelled isotope. The amino acids were measured by ion-exchange chromatography using a single column gradient elution technique (Technicon Analyser NCL 1). A fraction collector was used at the bottom of the column to collect the eluant fractions for the measurements of radioactivity.

The free amino acids in the tissues varied between the organs and were higher than in plasma (Table I). In contrast, the bound leucine was similar in the protein of each tissue studied. There was a gradient of specific activity from the plasma into the intracellular pools, and the specific activity in the liver was the lowest, only 40% of that in the plasma (Table II). The specific activity of leucine in liver protein was at least twice that in other tissues. This, combined with the low intracellular specific activity, provided the high $S_{B/Si}$

TABLE I

Leucine content of plasma and tissues in the newborn lamb (mean ± S. E.)

	Free μmol Kg^{-1} wet wt	Bound μmol g^{-1} protein
Plasma, μM	211 ± 55	—
Liver	522 ± 116	641 ± 12
Skeletal muscle	158 ± 30	614 ± 20
Cardiac muscle	286 ± 61	717 ± 30

TABLE II

Specific activity of leucine pools (mean ± S. E.)

	Free	Bound	$S_{B/SI}$
	cpm/nmol		
Plasma	8.8	—	—
Liver	3.4	0.62	0.184
Skeletal muscle	5.6	0.26	0.046
Cardiac muscle	5.2	0.33	0.064

TABLE III

Changes with age in mixed protein synthesis rate in sheep

	Fractional synthesis rate, k% day^{-1}	Half life, $t_{1/2}$ days	
	Lamb 2–3 days		Adult 1 year
Liver	105 ±7.0	0.7 ±0.05	6.9
Cardiac	34 ±6.5	2.3 ±0.19	21.6
Skeletal	23 ±1.5	3.1 ±0.42	40.9
'n'	6	6	4

ratio and "ks" values giving an average turnover rate of 105% day^{-1} for the mixed liver proteins, whilst that for skeletal and cardiac muscle was 23% and 34%, respectively. The half lives lay between 0.7 and 3.1 days (Table III).

At the time of our investigations there were no values for protein turnover rates in the adult sheep with which to compare our values in the lamb, but Buttery and his colleagues published data in 1975 [4] and these are also shown for comparison in Table III. The same method, a continuous infusion of labelled leucine was used, and it can be seen that the turnover rate in adult tissues is considerably less than in the newborn lamb. The relative lack of differentiation in turnover between the tissues which we observed in the young animal, may have been anticipated during rapid growth and the marked differences in half life between tissues in the ewe has also been observed in the adult mouse [22] and the rat [27].

Human Newborn

Young et al. [59] defined the relationship between protein and energy metabolism from an early period of growth and development through to old age in man; they used a modification of the continuous infusion method, by giving equal tracer doses of ^{15}N-glycine orally at intervals of three hours during a 60 hour period. Their results are shown graphically in Fig. 1, expressed as protein synthesis in g kg^{-1} day^{-1}. The premature infant has a very high synthetic rate, which falls quickly in the first 10–20 months of life, and declines more slowly during the subsequent years. Protein synthesis rate in the infant was three to five times that in the young adult, as these authors had predicted earlier; our results in the sheep are in accord with their findings.

Subsequently, Pencharz et al. [34] published a study on six premature human infants fed by mouth, assessing nitrogen metabolism by balance

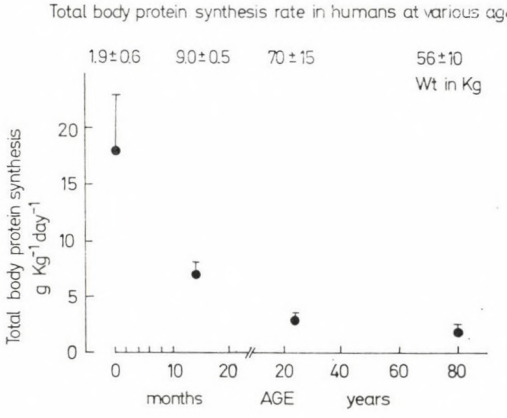

FIG. 1. To show the swift decline in protein synthetic rate in humans during the first year of life. After Young et al [59]

studies, obligatory nitrogen losses and determination of whole body nitrogen turnover, using ^{15}N glycine again. The infants were studied between 44 hours and 45 days after birth and weighed 1.78 ± 0.10 kg at the time of study. Whole body protein synthesis was 26.3 ± 7.0 g day^{-1} kg^{-1} and breakdown 23.8 ± 7.4 g day^{-1} kg^{-1}, a little higher than in the previous study. More recently, these authors have published data on the influence of birth weight and intra-uterine nutritional status on the total body protein turnover in a further group of premature infants of a similar weight range (35). In infants small for gestational age (S.F.D.) whole body protein synthesis and breakdown were 26 and 35% higher respectively ($p < < 0.01$) than in those whose weight was appropriate-for-gestational age. The higher values in the S.F.D. infants probably represent an adaptation to intra-uterine malnutrition similar to that observed in the undernourished 10–20 month old infants studied by Picou and Taylor-Roberts (37). Such an adaptation is also indicated in the experimental animal by the increased capacity of both the fetus and the placenta to take up a model, non-metabolisable, amino acid, α-amino isobutyric acid, in pregnant guinea-pigs fed deficient diets, particularly when the carbohydrate intake was low [57].

In further studies on the premature human infant fed intravenously, Pencharz and his colleagues [8] observed that protein synthesis was directly related with energy intake, and that protein intake regulated protein degradation. It is interesting to observe that all these infants had synthetic and catabolic rates which were half the values found in their two earlier studies. This may be related to the fact that these infants were fed intravenously and that the ^{15}N glycine was also given intravenously every three hours, urinary enrichment being measured as before. A reduction in whole body protein synthesis is also seen in the human adult during intravenous feeding and it has been suggested that it is due to a depression of intestinal protein synthesis. Alternatively, the effect may be methodological: during intestinal feeding all the amino acids pass through the portal vein to the liver and some may be metabolised and so dilute the ^{15}N enrichment giving a lower plateau which would raise the estimate of flux, protein synthesis and breakdown; however, the route of isotope administration has been shown to have no influence on protein synthesis calculations in the adult and no such data are available for the neonate.

The placenta

Protein Synthetic Rate

Joseph Barcroft [2b] introduced into fetal physiology Child's general hypothesis that the partition of nutrients between the different organs of the body was determined by their metabolic rates. In considering "the

relative claims of the fetus and mother to available nutritive material", the metabolic rate of the placenta may be expected to set the metabolic priority of the concept within the uterus particularly in the early stages of gestation. Late in gestation there is evidence from "in vivo" and "in vitro" studies which indicates that both oxygen consumption and glucose utilization of the trophoblast are high in comparison with the fetus; the mixed protein turnover rate of a tissue might give a more specific indication of its metabolic activity than these more familiar criteria.

Accordingly, calculations were made of protein synthetic rate in the placental cotyledon of the pregnant ewe during the investigation of turnover rate in the individual organs of the fetus described on page 13 [56]. The continuous infusion method of Waterlow and Stephen [49] was employed using ^{14}C lysine, in ewes 130—140 days' pregnant (term 147 days). The calculations, again, were made from the ratio protein of the bound to free-intracellular specific activity in the trophoblast following a six hour infusion. The fractional synthesis rate of 60% day^{-1} of the mixed placental protein was two-thirds of that observed for the fetal lamb's liver and heart in the same study, similar to that of brain and about five times faster than muscle (Table IV). The placental cotyledons in the sheep contain both maternal and fetal tissues, which are not separable, and the synthesis rate measured represents their combined metabolic activity; this is five times that for liver in the twelve months' old sheep (Table III) and provides the priority of the feto-placental unit for the capture of amino acids from the maternal circulation. The partition of amino acids between an equivalent weight of placenta and maternal liver will be of the order of 5 : 1 in the sheep; the absolute distribution is of the same order, due to the similar weights of the

TABLE IV

Mixed protein synthetic rate in the sheep placenta
(All values mean ± S. E.)

	Lysine specific activity, c.p.m./nmol		K % day^{-1}	$t_{1/2}$ days
	S_B	S_i		
Control	0.90	7.58	60	1.32
'n' = 6	±0.08	±0.69	±13	±0.23
Insulin infused	0.68	3.76	121	0.66
'n' = 4	±0.20	±0.37	±23	0.17
			$p < 0.05$	< 0.1

S_B = bound in protein, S_i = free, intracellular.

organs in this species, about 500 g. In the guinea-pig, a 3.5 : 1 ratio between the uptake of a non-metabolisable amino aid (AIB) per unit weight of placenta and maternal liver was found, and the ratio of the absolute amounts was 2 : 1 [57].

In four fetuses, insulin was infused at a rate of 0.2–1.6 U/kg/h, to give plasma levels ranging from 65–450 μU/ml, and these relatively high concentrations apparently doubled the placental protein fractional synthesis rate (Table IV). So that the bound and free specific activities measurement would be comparable between the control and experimental groups each value was corrected from the known isotope infusion value of 6 μCi kg^{-1} h^{-1}. It is seen in Table IV that the bound specific activity was lower during the insulin infusion, but not significantly so; the free specific activity was, however, very low giving a high "k" value and turnover rate. This observation that insulin may stimulate protein turnover in the placenta suggests that the hormone might be responsible for the heavy placentae found in diabetic patients whose blood sugar is not controlled, and account for their apparently large functional capacity.

A possible link between placental protein synthetic rate, fetal size at delivery and maternal nutrition has been investigated by two groups. An increase in energy metabolism and synthetic rate was found in placentas from low birth weight infants by Metcoff and his colleagues [26]. Such an adaptation to adverse conditions might be comparable to the increased amino acid uptake by the placentas of guinea-pigs fed on a diet low in protein but adequate in calories observed by Young and Widdowson [57] and the high turnover rate found by Pencharz in premature infants small for their gestational age [35]. In contrast, however, Munro's group found that trophoblast protein synthetic rate was directly related to the size of the organ in women from two different socio-economic groups in Boston and from a rural area in Guatemala [21].

Transfer of Amino Acids

The presence of the trophoblast with a high metabolic activity is known to influence the maternal artery to umbilical vein "downhill" gradients for oxygen and glucose across the placental membrane in experimental animals. The high fetal to maternal plasma gradients for amino acids make such a relationship less clear. Early observations on uterine and umbilical A–V differences in the human subject at vaginal delivery and Caesarean section indicated that there was some modification of proportions of the amino acids by the trophoblast during transfer, but the most definitive data have been obtained from the pregnant ewe, with indwelling catheters in the umbilical vessels in chronic preparations, during the third trimester of gestation [17, 23]. In Fig. 2 the naturally occurring uterine arterio-venous (A-V) differences across the ewe's placenta

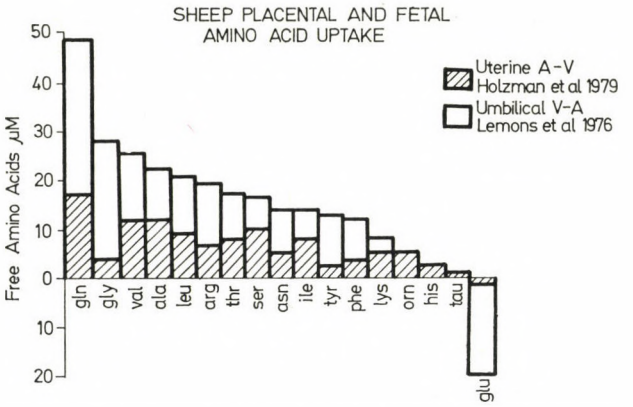

FIG. 2. Fetal and placental amino acid uptake in the sheep; a comparison of umbilical V-A differences with the uterine A-V differences. After Lemons et al [23] and Holzman et al [17]

are compared with the umbilical venous-arterial (V-A) differences. The extraction is at most 10% for any amino acid by the maternal side of the placenta, and the input into the umbilical vein blood is 18% of the fetal circulating levels. The maternal A-V differences are about one-half of the maternal V-A differences because the blood flow to the placenta on the maternal side of the placenta is double that on the fetal side at this time in gestation. Of great interest is the large output of glutamine and glycine into the fetal circulation in comparison with the maternal uptake, thus clearly indicating synthesis of these two molecules by the trophoblast, probably from essential amino acids taken up from the maternal circulation.

In Fig. 3 a comparison of the umbilical amino acid V-A differences in the fetal lamb in the third trimester and premature human infant at vaginal delivery is shown [14]. The data from the human subject must be complicated by anaesthesia and labour, but an interesting species difference is nevertheless apparent, namely, that less glutamine and more alanine is transferred in the human infant than in the lamb.

Fig. 2 shows that there is negligibe uptake of glutamate by the maternal side of the placenta and, apparently, considerable uptake of glutamate by the fetal surface. Such a negative A-V for glutamate is seen across some other tissues and the fetal side of the human placenta, and Hayashi and Sanada [13] have more recently observed that as gestation proceeds in the human subject glycine, serine and glutamine are also taken up by the fetal side of the placenta.

Free and Bound Amino Acid Relationships

The relationship of the relative proportions of the amino acids trans-

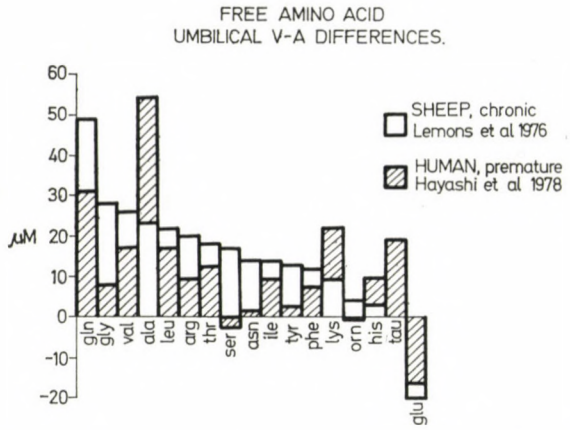

FIG. 3. A comparison of the umbilical V-A differences across the sheep and human placenta in premature deliveriese. After Lemons et al [23] and Hayashi et al [14]

ferred from the maternal to fetal circulation to those in the fetal protein is of particular interest. Table V shows quite clearly for a few amino acids, representative of the four main transport groups, that there is no relationship between the umbilical V-A difference and their proportions in protein; in particular, the non-essential acidic and neutral straight chain amino acids have small and very varied umbilical V-A differences yet are present in greater proportion in the protein than the branched chain

TABLE V

Amino acids in human tissues

	Fetal protein mmol kg^{-1}	Free in placenta μmol kg^{-1} wet wt	Umbilical V-A differences μmol
Glutamate	700	3450	—17
Aspartate	800	5679	0
Alanine	650	1987	55
Glycine	700	1655	8
Valine	500	229	17
Leucine	500	258	17
Lysine	450	339	22
Arginine	300	280	10

A comparison of the relative proportions of two examples from each of the four main groups of amino acids, acidic, neutral straight chain and branched chain, and basic in fetal protein, the free precursor placental pool and the umbilical venous-arterial differences (see Young [52]).

and basic, essential, amino acids. Further inspection of the free amino acid concentrations in a fetal tissue, the trophoblast, shows that the proportion of the non-essential to essential amino acids, are greater still than their proportion in protein; this indicates that the non-essential are synthesised "de novo" within the cell, since they are taken up relatively slowly by the trophoblast. Such considerations must form the basis of Munro's [30] observations, from nutritional studies, that there is a greater proportional, as well as absolute, need of essential amino acids in young growing animals in comparison with the adult. Further, the so-called non-essential amino acids, besides being present in greater amounts in protein than the essential amino acids, are important constituents of purine and pyrimidine molecules, and therefore necessary for DNA and RNA synthesis. These conversions of essential to non-essential amino acids will be effected by the wide range of transaminases and deaminases known to be present in fetal tissues.

The fetal lamb

Protein Synthetic Rates in Some Fetal Organs

Following our experiences with the measurement of protein synthetic rate in the newborn lamb, we made similar studies in the fetal lamb "in utero" [31]. Chronic preparations were prepared in ewes, with indwelling catheters in the fetal carotid artery and jugular vein inserted under Ketalar anaesthesia 24 to 48 hours before the synthesis rate measurements. This time interval was chosen for purely practical reasons and with the full knowledge that the plasma free amino acids in both mother and fetus were slightly reduced following operative procedures, indicating some disturbance of amino acid metabolism, and that full recovery did not occur for between five to seven days [55]. The acid base balance of the fetus was good at the time of the protein synthetic rate measurements. ^{14}C-labelled lysine was used instead of leucine.

The first experiments were carried out in Border Leicester ewes, 123–130 days' pregnant with fetuses weighing 2.41 ± 0.17 kg. The results are shown in Table VI and seen to be little different from those obtained in the newborn lamb (Table III). Again, the specific activities in liver protein were greater than in the other protein pools, and the "k" value depended upon this rather than that of the free pool. The free pools themselves were higher in the fetus than in the newborn lamb, though that of the plasma was lower. There was considerable metabolism of the lysine, the counts associated with it ranging from 13% of the total counts in liver to 38% in muscle. The calculated flux of lysine was, therefore, large as for leucine in the newborn lamb, 600 μmol/kg/h, equivalent to five times the daily increment of protein during the last trimester of gestation in the

TABLE VI

Fractional synthesis rate, K %, of mixed protein in fetal organs and the corresponding half life, $t_{1/2}$. Means ± S. E.

	K % day^{-1}	$t_{1/2}$ days	'n'
Brain	82 ±22	1.62 ±0.41	6
Liver	127 ±18	0.67 ±0.15	6
Cardiac muscle	99 ±24	1.0 ±0.24	6
Skeletal muscle	40* ±19	7.81* ±2.96	5

* $p < 0.02$

lamb, namely, 10 g [2a]. Loss into the mother across the placental membrane may have contributed to the large flux though isotope was never detected in the uterine venous blood. Meier et al. [25] have published smaller values for lysine flux in the fetal lamb, 360 μmol/kg/h at 110 days' gestation and half this value at 145 days. Any loss due to metabolism does not influence the value for fractional synthesis rate because this depends only on the ratio of specific activities in the free and bound pools.

The Influence of Insulin and Metabolite Levels on Protein Synthetic Rate

The suggestion that an increased production rate of insulin by the fetal islets may be responsible for the macrosomia of the large for gestational age infant, born of diabetic mothers whose blood sugar is not well controlled, is widely held [12, 16]. The experimental evidence for the influence of insulin on protein synthetic rate was, until recently, conflicting. Picon [36] had observed that, when fetal rats were given insulin intramuscularly "in utero", they were heavier than control littermates, contained more nitrogen, and had a greater lipid ratio in their carcasse. Many in vitro observations also showed that insulin would stimulate the uptake of labelled amino acids by isolated tissues, but in vivo measurement of protein turnover rate, using the continuous infusion method, did not show any such effect [33]. More recently, McCormick et al. [24] confirmed Picon's findings, using fetal rhesus monkeys made continuously hyperinsulinaemic "in utero" by the subcutaneous implantation of a mini pump delivering the hormone continuously for 21 days in the last third

of gestation; however, exceedingly high plasma insulin values were attained. In preliminary studies of our own, using the chronic preparation in pregnant ewes as described previously, we observed a big reduction in the free amino acid concentration in the fetal plasmas, as well as that of glucose [7, 34] which probably reduced the precursors available for synthesis. We therefore undertook a study in which an attempt was made to maintain the fetal plasma glucose concentration and the free amino acid concentration during the insulin infusions [18].

The observations were made in Welsh mountain crossbred ewes, 130–140 days' pregnant; the mean weight of the control fetuses not given insulin was 2.74 $\pm$ 0.2 kg, and in the experimental groups 3.24 $\pm$ 0.4 kg. Insulin monocomponent crystalline bovine was infused at rates of 0.2–1.6 U/kg/h and gave plasma levels ranging from 65–450 μU/ml in comparison with the controls of 8.0 μU to 56 μU/ml. Plasma glucose was maintained at about 2.4 mM, three times the resting level, by an infusion at the rate of 1.2 g/kg/h, and an increase in plasma free amino acids was achieved by an infusion of Aminosol Vitrum at the rate of 60 mg N/kg/h.

Insulin had no influence on the synthesis rate in any of the organs studied (Fig. 4); the exception was skeletal muscle, in which fractional synthesis rate was stimulated in the presence of high free amino acids, the $t_{1/2}$ of the mixed protein being reduced by a factor of three. When glucose was infused alone, it had no influence on protein synthetic rate, except in the brain. Amino acids alone inhibited synthesis rate in all the organs with the exception of skeletal muscle. These results emphasise, once more, the importance of glucose for brain metabolism. The

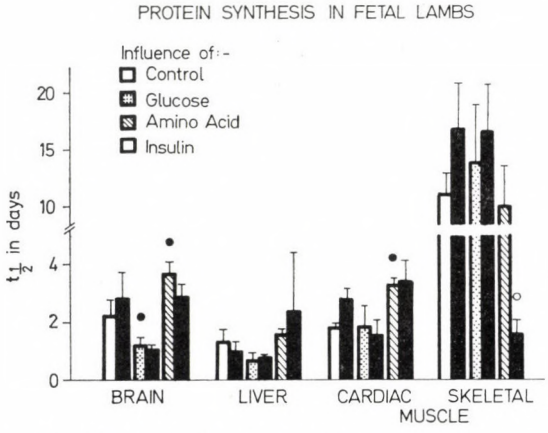

FIG. 4. The influence of insulin, a rise in plasma glucose and amino acid levels on the $t_{½}$ of mixed proteins in fetal lamb organs, compared with control values. Glucose stimulated synthesis in the brain ($p > 0.03$) and amino acids depressed synthesis in the brain and heart ($p > 0.05$). Insulin increased synthesis rate in the muscle in the presence of high free amino acid concentrations ($p > 0.02$). After Horn et al [18]

inhibitory influence of high free amino acid concentrations on synthesis rate in brain, heart, and liver may explain the low turnover rate in the premature infants studied by Duffy et al. [8] receiving intravenous feeding in comparison with those fed by mouth [34, 35], for it is known from previous observations that the plasma free amino acids are usually raised by such procedures [39]. The results may also explain why protein supplements in the maternal diet are not associated with any improvement in the weight of the human infant [42] and in the rhesus monkey [40]; plasma amino acid estimations were not, however, made in these last two studies.

The absence of any influence of insulin on protein synthetic rate, except in muscle when the plasma free amino acids were raised, agrees with the observations of Pain and Garlick [33] in adult rat skeletal muscle. Both these authors and ourselves used infusions of insulin which were nearer to those that might occur under physiological circumstances; the observations were also made over a very much shorter time period than those of Picon [36] or McCormick et al. [24]. In our experiments, no marked fall in plasma or tissue free amino acids were observed in the fetus, suggesting that the hormone had not reduced the catabolic portion of protein turnover known to occur in adult muscle [5] which suggests that the hormone may not be capable of influencing this portion of the fast anabolic/catabolic equilibrium which characterizes the quick turnover rate of fetal protein.

Metabolism of insulin in the pregnant ewe and her fetus

The metabolism of insulin in the fetus has received comparatively little attention in comparison with its effect on fetal glucose levels and utilization in the lamb [6, 9, 38]. A few isolated studies have indicated that insulin may have a longer half life in the fetal lamb than in the ewe [9, 38]. The probable immaturity of the fetal degrading processes for insulin and the presence of the placenta may both contribute to these differences. The purpose of this preliminary study was, therefore, to make a direct comparison between mother and fetus of the metabolic clearance rate of the hormone, the half life and the distribution space, using unlabelled ovine insulin.

Chronic preparations were, again, set up in Welsh mountain ewes 120–134 days' pregnant, with indwelling catheters in the fetal carotid artery and jugular vein, and in the two jugular veins of the mother, and the first observations were made one and two days after the operation [46]. Metabolic clearance rate (MCR) was measured from several steady state levels attained by incremental constant infusions, ranging from 0.05 to 0.3 U/kg/h according to the method used by Sonksen et al. [45]. In a few studies, a priming dose was used to reduce the time required to achieve a satisfactory steady state. Normoglycaemia was maintained by a glucose clamp technique to protect the fetus from the effects of prolonged

hypoglycaemia. The half life ($t_{1/2}$) of the hormone was measured from the decay curve after the infusion had been stopped at the highest steady state level. The MCR was estimated from the infusion rate divided by the steady state plasma hormone concentration, and defined as the volume of blood completely and irreversibly cleared of the hormone per unit time. It is also equal to the distribution space (DS) and the fractional clearance (k). K was measured from the initial gradient of the insulin decay on a semi log plot and the half life calculated from the equation $k = 0.693/t_{1/2}$. Knowing MCR and K, the distribution space is calculated from DS = = MCR/K. Insulin was measured by an adaptation of the double antibody radioimmunoassay technique.

The results for MCR, t_1 and apparent DS are summarized in Table VII for 5–7 fetuses and ewes. The basal MCR of 16.3 ± 2.4 ml/kg/min for the fetus was not significantly greater than for the mother, 14.5 ± 2.6 ml/kg/min. The shape of the curves, with MCR plotted against plasma concentrations up to 800 μU/ml showed that a saturable process was concerned in the clearance for both (Fig. 5). The half life for insulin was, however, significantly longer in the fetus, 16.8 min in comparison with the 9.3 min in the mother. Conversely, the DS was much greater in the fetus than in the mother, 251 ml/kg in comparison with 119 ml/kg. A fetal DS twice the size of the maternal might be accounted for by the placenta, and by the presence of a greater number of receptors in the fetal tissue such as has been observed for the monocytes in human cord blood [47] and for the red blood cells in the human infant [15]. However, in spite of this negative correlation of intensity of insulin binding with age, insulin would not appear to be broken down so readily in the fetus for the long half life indicates immaturity of the degrading systems in comparison with the mother.

Fig. 6 shows three decay curves for insulin in one fetus, two were

TABLE VII

Comparison of fetal and maternal sheep insulin metabolism

	Basal MCR, ml/min/kg	$T_{1/2}$, min	DS, ml/kg
Fetal	16.29 ±2.42	16.8 ±2.17	251 ±14
Maternal	14.46 ±2.63	9.29 ±1.12	119 ±17
n pairs	5	7	6
P	> 0.5	< 0.01	< 0.0001

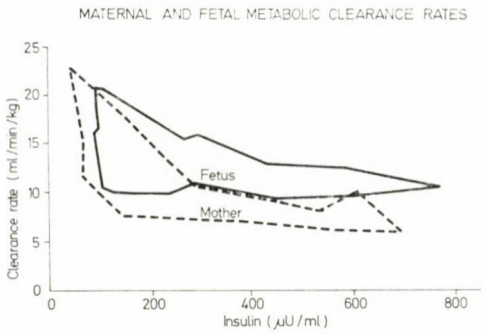

FIG. 5. Insulin clearance rate is plotted against plasma concentration and shows that a saturable process is concerned in both the fetus and mother

repeated at intervals of 2 and 4 days following the initial observation 24 hours after the insertion of catheters. The increasing decay rates suggest that the insulin degrading processes might be stimulated "in utero" by each preceding infusion: alternatively, the fetus might be recovering from the effects of the surgery, or simply growing up. Should this apparent stimulation of degrading processes be reproducible, it would suggest that premature sensitization of the degrading process might occur in the infant of a diabetic mother whose glucose was not well controlled during pregnancy; fetal insulin release is continually stimulated by the reflection of the high maternal blood sugar in the fetal plasma. Fowden (9) also found that fetal insulin release, in response to arginine, was enhanced prematurely in the lamb by previous injections.

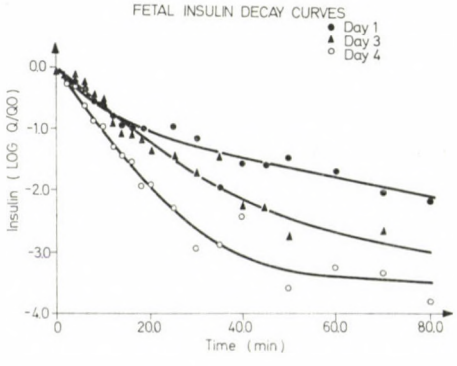

FIG. 6. Plasma decay curves in a single fetus 1, 3 and 4 days, following the operative procedure for introduction of catheters, showing a possible sensitization of the insulin degrading process by a preceding infusion

CONTINUATION

Precursor Pools for the Calculation of Protein Turnover Rate

The values for protein turnover rate presented in these studies in the whole animal have been calculated using the specific activity of the intracellular pool assuming this to be the precursor substrate for synthesis, as originally suggested by Waterlow and Stephen [49]; the values may, therefore, be greater by a factor of up to two than if the specific activity of the plasma free amino acid had been used in the calculations (see Table II and [7]). Until recently, there has been no uniformity of opinion concerning the origin of the precursor pool; the early observations, on isolated tissues, revolved around whether or not there was a delay before linear incorporation of a labelled isotope took place from the extracellular fluid into the protein [41]. But evidence has been provided for a mixture of both extracellular and intracellular amino acids contributing to the plasma membrane compartment which funnels precursors towards the protein synthesis site [1,28]. Khairallah and his colleagues [20] have used the immediate precursor specific activity of the amino acyl t RNA in their measurements of protein synthetic rates and found it to have a specific activity midway between that in the extra- and intracellular fluids.

In a critical discussion of the subject of protein turnover measurements, Waterlow et al. [48] and Garlick [10] have pointed out that estimations of synthesis rate should be carried out by at least two methods to assess the real value; further, that the uptake methods measure synthesis rate only, and that protein accumulation rate must also be measured to obtain by subtraction, the value for catabolic rate. Such assays can at the present time, only be carried out in animal models.

The Association of Fast Protein Turnover and Deposition Rates and the Action of Insulin during Growth

The tissue forming the functional mass of the body consists of a heterogeneous mixture of proteins with varying half-lives and Lajtha et al. [22] have shown that the relative proportions of those with long and short half-lives differ in each tissue; for instance, 94% of the mixed proteins in the brain have a long half-life of 10 days, whilst in the liver, no protein has a half-life longer than 26 hours. No such information is available for developing tissues. Jefferson [19] considers that all proteins have an inherently fast turnover rate and each may or may not be gradually restrained by hormones as the orchestra of endocrine function comes into full play during development. The lack of effect of insulin on synthesis rate in the protein of fetal organs over a short time period suggests that it may not act as a general anabolic hormone at this stage of development. However, Picon [36] and

McCormick [24] could increase nitrogen accretion in fetuses by insulin infusions given over prolonged periods, in which there would be time for the stimulation of receptor analogous to the sensitization of degrading processes for the hormone by previous insulin infusions suggested by our studies on insulin metabolism. Again, animal models only can help us understand the mechanism of biological action with such important clinical implications.

Acknowledgements

Many thanks are due to the co-authors with whom the work was carried out: to Dr. Peter Garlick for helpful discussions on protein synthesis rate measurement; to Drs. Norbert Freinkel, and Boyd Metzger and Professor Peter Sonksen for good discussion on the mechanism of action of insulin; and, finally, to the Wellcome Trust and A.R.C. for financial support.

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Professor J. Mestyán 60 Years



The papers in this issue have been dedicated to Julius Mestyán by his pupils and some of his scientific friends. This time it was really the pupils who had devised this kind of celebration; as they say they could find no other means to express their affection and liking for their chief and their attachment to him. For he does not support praise: he would just regard it as blarney.

Julius Mestyán was born at Pécs, from a family of musicians. He started to play the violin but was then attracted to biology and as a medical student he soon joined the Institute of Pathophysiology. He worked there for 12 years and had the reputation of a brilliant physiologist. His main fields of interest were the regulation of temperature and energy metabolism in the newborn, neonatal paren-

teral nutrition and the problems of intrauterine life, especially growth. These were the subjects he studied as a Nuffield scholar in Oxford and on these did he later lecture upon as a visiting professor in Sheffield and at many ESPR and other meetings. In 1957, Edmund Kerpel-Fronius, himself an old addict to physiology, who then held the chair of paediatrics, invited Mestyán to join the staff of the department. It is there that he still works, now as the head of the institution.

During the many years of his paediatric activity he has given up all his hobbies. The one that remains is the science and practice of paediatrics and his only ambition is the excellence and success of his department. For many years he has not taken a holiday and the nurses often see him walking around the wards at night or carrying an attaché-case packed tight with periodicals. But in the last years he acquired a small garden in the outskirts of Pécs. If he is too tired to work at home, he goes there to dig the soil and to dress the few vines he has. Some months ago he offered me a glass of grape juice: it was the best chasselas I have ever tasted. This was only natural. Those who know Julius know well that he pursuits excellence.

P. V. VÉGHELYI

Book reviews

Forschung im Dienst der jungen Generation. Herausgegeben von E. SCHMIDT-KOLMER 193 Seiten mit 34 Abbildungen und 33 Tabellen. Verlag Volk und Gesundheit, Berlin 1980. Preis: M 12,—

Im Zeichen des Internationalen Jahres des Kindes wurden die neuesten Forschungsergebnisse und wissenschaftlichen Abhandlungen von 20 Autoren, Mitarbeitern des Instituts für Hygiene des Kindes- und Jugendalters der DDR und Partnern aus dem Forschungsprojekt »Gesundheitsschutz der jungen Generation« zusammengestellt.

Die Beiträge geben eine Übersicht über wissenschaftliche Probleme, mit denen sich Sozialhygieniker, Kommunalhygieniker, Pädiater, Psychologen und Pädagogen mit dem Ziel einer optimalen Förderung der Gesundheit und allseitigen Entwicklung der Kinder und Jugendlichen der DDR auseinanderzusetzen haben.

Es handelt sich einerseits um Bemühungen zur Erhöhung der Effektivität der periodischen gesundheitlichen Überwachungen der 0- bis 18jährigen mit dem Ziel der Verhütung bzw. der Früherkennung, -erfassung und -behandlung jener Erkrankungen und Schädigungen, die sich aus der noch ungenügenden Gewährleistung optimaler hygienischer Lebensbedingungen und einer gesundheits- und entwicklungsgerechten Lebensweise der Kinder und Jugendlichen in den Familien wie in den Einrichtungen der gesellschaftlichen Erziehung ergeben. Weiterhin sind es hygienische, psychologische und pädagogische Probleme, die für die Förderung der Gesundheit und Entwicklung der Kinder in den Krippen von besonderer Bedeutung sind, wie Häufigkeit der Erkrankungen der Atemwege zu verringern und das Niveau der Erziehungsarbeit zu erhöhen. Dabei wird die enge Verflechtung von gesundhetlicher Betreuung und pflegeerzieherischer Arbeit gerade in der frühen Kindheit besonders offensichtlich.

J. FALK

L'avortement provoqué: guide pratique des soins et services requis. Organisation Mondiale de la Santé, Geneva 1979. WHO publication No. 49, pp. 79. Price: SwFr. 6.—

This little pamphlet offers practical information on the most important points of artificial abortion. The main chapters are, the hygienic relations of induced abortion and its legal status in the different countries; legalized abortion; the methods and possible complications of the termination of pregnancy; and, finally, the methods of contraception after artificial abortion.

It is interesting to understand that 9% of our Earth's population lives in countries where there are no means whatever for a legal abortion, and 11% in countries where an endangerment of the mother's life is the only possibility for an abortion

being permitted. Not more than 40% of the governments have taken a "liberal" attitude in this question. In the booklet, emphasis is laid on the effects of criminal abortion endangering the mother's life and causing many deaths. Fortunately, the frequency of such interventions is declining.

Finally, the early and late complication of induced abortion are discussed, such as haemorrhage, injuries, infections, cervical injury, etc., and it is emphasized that abortions in the earliest phase of pregnancy mean the least danger.

L. LAMPÉ

O. THALHAMMER: *Phenylalaninblutspiegel-unabhängige Wirkung der Phenylketonurie-Mutante.* 16 Seiten mit 4 Abbildungen und 8 Tabellen. J. A. Barth, Leipzig 1980. Preis: M 6,40

Der Inhalt der Monographie ist Professor Thalhammers Vortrag an der Deutschen Akademie der Naturforscher Leopoldina im Oktober 1979.

Unter den Problemen der Phenylketonurie ist heute wohl jene Erkennung von dringlichster Bedeutung, daß sich der IQ der entsprechend behandelten Kinder mit fortschreitendem Alter stets verschlechtert. Im Rahmen der gut organisierten und seit längerer Zeit funktionierenden Reihenuntersuchungen in Österreich wurde auch die IQ-Untersuchung der Eltern von PKU-Kindern durchgeführt. Es stellte sich heraus, daß der IQ der obligaten Heterozygoten subnormal war, obwohl sich ihr Phenylalanin- und Tyrosinspiegel im Serum normal gestaltete. Aus diesen Beobachtungen hat Professor Thalhammer den Schluß gezogen, daß die 50%-ige Enzymaktivität der Heterozygoten doch nicht zur Metabolisierung des Phenylalanins genügt und daß sich dieses intrazellulär anhäuft. Diese Hypothese wurde mit geistreichen Experimenten in Lymphozyten bekräftigt. Es wurde beobachtet, daß das intrazelluläre Phenylalanin und Tyrosin in Heterozygoten signifikant höher war als bei gesunden Personen, und die intrazelluläre Anhäufung des Phenylalanins könnte die Zellfunktionen schädlich beeinflussen.

Es ist lohnend, auf diese Feststellungen hinzuhören, da es sich um eine grundlegende Erklärung der komplexen Fragen der Phenylketonurie-Therapie handeln dürfte.

P. KISS

Fetal and neonatal physiological measurements. Edited by P. ROLFE. Pitman Medical, Bath 1980. pp. 449. Price: £ 30

This book is based on an international conference held in Oxford in September 1979. It contains almost all the papers presented at the nine sessions as well as the posters suitable for full publication. This was the first international conference to bring together clinicians, physiologists, bioengineers and physicists concerned with the development of techniques of physiological measurements and their application to the fetus and the newborn infant.

The topics include many of the most important aspects of physiological measurements applied in perinatal medicine: fetal cardiac assessment, measurement of neonatal cardiovascular function ,measurement of uterine activity, fetal breathing, the neonatal respiratory system, cerebral measurements, fetal and neonatal blood gas and pH measurement.

Major bioengineering interests including ultrasound, microprocessor signal analysis, and mass spectrometric and electro-chemical gas analysis, are given wide coverage in the 62 papers.

With the many authors the style and quality of the papers is necessarily varied. Some authors have written a long text with numerous references; others have restricted their report to less than three pages. Nearly half of the papers deal with fetal and neonatal cardiovascular function and blood gas and pH measure-

ments, which are clinically perhaps the two most important aspects of neonatal physiological measurements. There is e.g. an interesting report on the possibility to detect and follow up iatrogenic hypoxaemia and right-to-left ductal shunting by the use of double site transcutaneous PO_2 monitoring (J. F. Lucey) and the first account of an electrochemical sensor for simultaneous transcutaneous measurement of PO_2 and PCO_2 (D. Halsall et al.). In the section of General Topics, Yamanouchi et al. describe their first experience with a transcutaneous bilirubin meter produced in conjunction with the Minolta Camera Company. The recording and analysis of fetal and neonatal EEG and other non-invasive techniques of cerebral measurements have improved and now prove to be useful tools in assessing the degree of cerebral injury very early in newborn infants.

The references after most papers are adequate and the book is well produced, a success for the publishers as well as for the host institute of the conference, the Bio-Engineering Unit of the University Department of Paediatrics, John Radcliffe Hospital, Oxford. Unfortunately, the reader is prevented from following the comments and criticism which were raised in the discussions, although some of these were amalgamated in the individual papers.

All in all, this volume will be of particular interest to obstetric and neonatal paediatric staff, fetal and neonatal physiologists, bioengineers and medical physicists.

G. SOLTÉSZ

Fractures in Children. Edited by G. CHAPCHAL. Georg Thieme Verlag, Stuttgart—New York 1981. pp. xii + 260, 289 figures and 94 tables. Price: DM 98.—

This book contains the papers on fractures in children, given at the 9th International Symposium on Topical Problems in Orthopaedic Surgery held in Luzern, Switzerland, in 1979. The text of the 83 papers has been carefully edited and is well illustrated. They give useful information on many problems associated with fractures.

The book is divided into chapters according to the sessions of the symposium. In the first part we find general observations, the characteristics of fractures in general and the reactions of the bones to lesions and the process of healing. A paper from the Department of Orthopaedics in Würzburg, FRG, reports on 45 fractures during delivery; 19 were clavicular fractures and 15 fractures of the humerus. Several reports deal with epiphyseal fractures, the resulting disturbances of growth, and their prevention and treatment. Separate papers have been devoted to the possibilities of correction of the inequal length of the lower extremity, i.e. stimulation of growth, epiphyseodesis, and surgical lengthening.

The next section deals with the different body areas. First, the growth disturbances following injuries of the proximal end of the femur, the different types of condylar fracture of the femur and tibia, and the secondary deformities and functional disturbances are discussed. Some infrequent but interesting epiphyseolyses, the mechanism of injuries and the different therapeutic methods are dealt with.

Most papers in the 3rd section discuss the treatment of fractures. In spite of the advances in interior stabilization techniques, 80 to 90% childhood fractures are treated by conservative means. The cause of this is explained by the many complications due to incorrect methods of treatment.

In the 4th section the fractures of the elbow area are discussed. These fractures are most common in the paediatric age group and present the majority of problems. This type of fracture calls for precise anatomical reconstruction and internal or percutaneous stabilization. With correct treatment growth disturbances and axis deviations will be a rare occurrence.

The last section deals with some uncommon fracture types. The authors reported on 20 pelvic fractures of which 15 were due to traffic accidents, while, paradoxically, traffic accidents were the cause of not more than 7 among 58 cases of spinal fracture; among these, 18 were due to sport accidents and 21 to falls from more than 1 m height. In spite of its low frequency, fractures of the hip and the head of the femur were the subject of several papers, together with their complications and the results of treatment. The results achieved with tibial fractures were presented by authors from Vienna on the basis of 400 cases.

Presenting some new observations and details of interest, the book will be useful for the specialist of paediatric traumatology.

T. VIZKELETY

O. CRAIG. *Childhood Diabetes and its Management.* Butterworths, London 1981. pp. 315. Price: Ł 17.50

In this book it is attempted to give an overall picture of childhood diabetes from the point of view of the practising paediatrician. The text is excellent, well-readable, illustrated with many case reports with advice on how to cope with medical problems of the diabetics and hints at how to deal with problems raised by the patients' parents. After describing the nature and details of maturity diabetes in the young, it is shown how this form can be trated without insulin. As to the much-discussed glucose tolerance test, it is recommended to give a dose of 2 g/kg below 3 years of age, 1.75 g/kg between 3 and 9, and a total dose of 50 g after the age of 10 years. The difficulty of diagnosing diabetes on the basis of the tolerance test is emphasized. As to the diet, a limited carbohydrate one without any free sugar is recommended but it is stressed that it should be prescribed in accordance with the possibilities available in the child's home. A well-constructed table demonstrates the close connection between systematic control and the patient's condition. The use of HbA_1 and its place in control are discussed in some detail. Much space is devoted to insulin and its different forms. Their choice and administration are explained meticulously, and even the home care of the equipment is explained with advice when to report at the outpatient clinic and what to take there. As regards the use of oral antidiabetic agents, it is said that they have no part to play in the management of juvenile diabetes.

A chapter is devoted to the behaviour of the diabetic child and of his family, illustrated by a number of characteristic histories demonstrating cases of over-protection, adolescent denial, disordered behaviour, of patients from poor families, of the connections of emotions with blood sugar, etc. Then follows a chapter on communication and education and this includes pieces of wise advice how the nurse, the ward sister, the registrar, the dietitian, the consultant and the family doctor should communicate with the patient and his mother. This is followed by a description of a morning in the diabetic clinic and then a chapter on some talking points in the clinic; these points are holidays, dependence of the patient on his parents, smoking, the teeth, what occupations the diabetic should choose, and whether he is allowed to drive a car.

The second half of the book contains a long chapter on insulin and metabolism. Then ketoacidosis, precoma and coma are explained and their treatment is surveyed, and then the course and aetiology of the disease are discussed. Finally, the various diseases associated with diabetes, its usual complications and the foods for the diabetic are reviewed.

It is no wonder that this book, written in a clear colloquial and often witty style, should now live to its second edition. It will be welcome by all doctors who have diabetic children under their care. Should

they have some practical problem with the patient or his family, they can be certain to find the right answer in this book.

L. Barta

Zur Kompensation und Dekompensation in der kindlichen Entwicklung. Herausgegeben von G. Göllnitz, J. Külz, G. K. Uschakov. 220 Seiten mit 37 Abbildungen und 79 Tabellen. G. Fischer Verlag, Jena 1980. Preis: M 44,—

Das beachtenswerte Buch besteht aus 24 Abhandlungen, die einer mehrjährigen Zusammenarbeit auf dem Gebiet des entwicklungsgeschädigten Kindes und Jugendlichen zwischen den Lehrstühlen für Pädiatrie, Psychiatrie und Kinderneuropsychiatrie von Rostock und den Lehrstühlen für Psychiatrie und Jugendhygiene des 2. Moskauer Medizinischen Instituts sowie den Neurophysiologischen Laboratorium des Instituts für Hygiene des Kindes- und Jugendsalters des Gesundheitsministeriums der UdSSR entspringen.

Die vielfältigen interdisziplinär erarbeiteten Befunde und Erfahrungen sind unter dem Gesichtspunkt von Kompensation und Dekompensation, einschließlich der Problematik Adaptation des Neugeborenen und sich entwickelnden Kindes zusammengestellt worden, um darin zu unterstreiche, daß die funktionellen Systeme des Menschen und sener Leistungen in einer engen somatischen und psychisch-reaktiven Wechselwirkung sind. Schäden und Störungen aus beiden Bereichen sind nicht statisch sondern dynamisch sowohl in Diagnostik als auch in Therapie zu betrachten.

In pädiatrischen Anteil des Buches werden Probleme der Adaptation, Desadaptation und Readaptation besprochen, wobei der Schwerpunkt auf Fragen der Temperatur, Stoffwechselregulation und Entwicklungsbesonderheiten bei Kindern mit Herzfehlern, Nierenerkrankungen und Diabetes liegt. Der neuropsychiatrische, neurophysiologische und entwicklungspsychologische Anteil geht auf Kompensation und Dekompensation von Leistungen bei hirngeschädigten Kindern im Rahmen ihrer Entwicklung ein. Im Mittelpunkt stehen Folgeerscheinigungen nach prä- und perinatalen Hirnschädigungen, späten Schädel-Hirn-Traumen und Anfallsleiden. Psychosoziale Bedingungen können Folgezustände nach organischen Schäden im günstigen Fall mildern, im ungünstigen Fall verstärken und Fehlentwicklung der kindlichen Persönlichkeit provozieren. Das Buch bietet für Forschung und Praxis eine Fülle von Anregungen und Diskussionsmaterial.

J. Falk

J. Oehme und P. Gutjahr: *Krebs bei Kindern und Jugendlichen.* 428 Seiten mit 90 Abbildungen und 80 Tabellen. Deutscher Ärzte-Verlag GmbH, Köln 1981. Preis: DM 68,–

In diesem kleinen, handlichen Fach-Taschenbuch, Nr. 39. der Series »für Lehre und Praxis« werden die mit den malignen Tumoren und leukämischen Erkrankungen des Kindesalters zusammenhängenden grundlegenden Kenntnisse zusammengefaßt. Der Allgemeine Teil bespricht die Epidemiologie, Ätiologie, Immunologie, Histopathologie, die Fragen der Klassifizierung, ferner die chirurgischen, radio- und chemotherapeutischen Probleme. Abschließend werden die bekannteren Zytostatika und deren Indikationen, Dosierung und Nebenwirkungen erläutert. Im Speziellen Teil werden die Leukämie und dreizehn maligne Tumorformen eingehend behandelt und auch auf die Metastasenbildung und die Schweregrad-Einteilung hingewiesen. Im letzten Teil folgen wiederum allgemeinere, doch neben den heutigen therapeutischen Erfolgen äußerst wichtige Kapitel: Zusammenarbeit zwischen Allgemeinarzt und Tumorzentrum, die ambulante Behandlung. Wertvoll sind auch die Beiträge über die Spätfolgen und die psychosozialen Probleme; die Wichtigkeit

der psychischen Betreuung wird hervorgehoben, — die Bedeutung der kreativen Aktivität der Patienten jedoch nicht erwähnt.

Durch die straffe und didaktische Struktur und zahlreiche demonstrative Abbildungen und Tabellen eignet sich das Buch vorzüglich, jene Ärzte (Pädiater, Radiologe, Chirurg, Psychologe usw.), die mit diesen Erkrankungen konfrontiert werden, mit der zeitgemäßen Problematik bekannt zu machen.

Mit Recht betonen die Autoren, daß nur durch eine frühe Diagnose, Therapie in den großen Zentren und entsprechend Heimbetreuung die heute erreichbaren optimalsten Heilergebnisse gesichert werden können, und zu diesem Zweck ist die entsprechende Information und Orientierung der erwähnten Mediziner unerläßlich.

Die Verfasser heben hervor, daß es nicht ihr Ziel war, alle theoretischen Aspekte, Kenntnisse und Literaturhinweise in diesem kurzen »Leitfaden« anzugeben, das ist die Aufgabe der großen Handbücher der pädiatrischen Onkologie. Als Leitfaden für den in der Praxis und Klinik tätigen Arzt wird sich das Buch zweifellos ausgezeichnet bewähren.

D. SCHULER

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CONTENTS

Cerebrospinal fluid concentration in preterm and full term newborn infants. *M Fekete, E Harangi* 1

Intrauterine weight retardation and the concomitant length retardation: A statistical analysis of anthropometric data. *L Járai* 9

Fatal infectious mononucleosis. Report of a case with a review of the literature. *P Kajtár, S Molnár, M Várbiró* 17

Glycosaminoglycan and hydroxyproline excretion in diabetic children. *L Klujber, R Szücs, Zs Baranyai, J Gabulya, Gy Soltész* 27

A diagnostic survey of patients referred for chromosome analysis. *G Kosztolányi* ... 35

Normal chromosomes in liveborn neonates weighing less than 1000 g. *K Méhes, K Bajnóczky* 41

Metabolic and hormonal effects of fasting in obese children. *D Molnár, G Soltész* 45

Major anomalies of the genitourinary tract in the neonate. *A Pintér* 51

Energy metabolism, substrate utilization, metabolite and hormone levels in infants fed various parenteral solutions. *I Rubecz* 59

Cardiomegaly in hypoglycaemic small-for-gestational-age infants. *K Schultz, J Weisenbach* 69

Relationship between metabolic control and plasma lipoprotein level in diabetic children. *G Soltész, D Molnár, L Klujber, M Kardos* 75

Energy cost of growth of premature infants. *P K Whyte, J C Sinclair, H S Bayley, D Campbell, J Singer* 85

Protein turnover rate in early life. *M Young* 99

Professor J Mestyán 60 years 119

Book reviews 121

Index: 26.021 **HU ISSN 0001–6527**

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Intravenous glucose tolerance test in childhood obesity: Metabolite levels and their relation to glucose utilization rate (K_G)*

D. Molnár, Maria Kardos, G. Soltész, Susan Baranyai

Department of Paediatrics, University Medical School, Pécs

Intravenous glucose tolerance tests were performed in 33 obese and 12 nonobese children. In addition to the glucose disappearance rate the changes in response to the glucose load in plasma insulin, FFA, glycerol, cholesterol, triglyceride, lactate and pyruvate were examined. The relationship between biochemical parameters and the glucose disappearance rate was also studied.

1. Reduced glucose tolerance, basal and glucose-induced hyperinsulinaemia were frequent in the obese children.

2. Normal or reduced glucose tolerance in spite of the apparent hyperinsulinaemia and the negative correlation between fasting insulin level and K_G in the nonhypertriglyceridaemic obese group was the marker of insulin resistance in overweight children.

3. The reduced elevation of FFA in the 2nd hour of the intravenous glucose tolerance test might be the sign of an impaired lipolysis in obesity.

4. The significant negative correlation found between K_G, fasting FFA and triglyceride levels in certain obese subgroups suggested the importance of FFA and triglyceride in the regulation of peripheral glucose utilization.

Obesity is characterized by basal and reactive hyperinsulinaemia (3, 8–10, 26, 27). In spite of the increased insulin secretion, abnormalities in glucose metabolism and increased frequency of impaired glucose tolerance have been observed in obese children (3, 13, 20, 24, 36) and adults (29, 34), but normal results have also been found (6, 15, 35).

In the present study the changes of plasma glucose, insulin, FFA, glycerol, cholesterol, triglyceride, lactate and pyruvate were measured during a standard intravenous glucose tolerance test (IVGTT). The rate of disappearance of glucose (K_G) was calculated and the relationship between biochemical parameters and K_G was also studied. The correlation between anthropometric parameters and K_G has been described previously (23).

Materials and Methods

Investigations were carried out in 12 non-obese and 33 obese children weighing more than 20% in excess of their ideal body weight as given in the tables of Maaser (21). In cases with height exceeding 160 cm the ideal body weight was calculated according to the formula: (height — 100)×0.9. The anthropometric

* Supported by the Scientific Research Council, Hungarian Ministry of Health.

TABLE I

Anthropometric characteristics of the obese and control children

	Obese			Control		
	No.	Mean	SE	No.	Mean	SE
Age, yr	33	10.7	0.45	12	11.5	0.74
Height, cm	33	148.4	2.63	12	146.8	3.25
Weight, kg	33	60.8	3.3	12	36.5	3.06
Ideal body weight, per cent	33	148.9	3.77	12	92.2	3.95
Sum of four skinfolds, mm	33	104.7	3.61	12	35.9	3.66
Body fat, per cent	33	40.5	0.77	12	20.8	2.15

measurements, the calculation of body fat and the determination of plasma insulin and metabolites were done as described previously (23). The characteristic anthropometric data of obese and control children are shown in Table I.

IVGTT was carried out at 08 a.m. after an overnight fast. 0.5 g/kg glucose in 40% solution was injected into the cubital vein in approximately 2 minutes. Blood samples were drawn from the other arm just before and 10, 20, 40, 60, 90, 120 min after the glucose load. K_G was calculated according to the formula $\ln 2/T\frac{1}{2} \times 100$, where $T\frac{1}{2}$ was obtained from the semilogarithmic plot of total blood glucose by the graphical method. The formula was applied to the segment from 20 to 60 min of the disappearance curve.

Fasting triglyceride values above 1.7 mmol/l were considered hypertriglyceridaemic and glucose-induced insulin response above 956 pmol/l (mean insulin response to i.v. glucose load in obese children) was taken as hyperinsulinaemic. K_G values of 1.3 or less were taken as impaired. According to these criteria, the following obese subgroups were formed: hypertriglyceridaemic (HT), non-hypertriglyceridaemic (NHT), hyperinsulinaemic (HI), non-hyperinsulinaemic (NHI), a group with impaired glucose tolerance (IGT) and with normal glucose tolerance (NGT).

The mean, standard error (SE) and correlation coefficient were calculated with standard methods. Statistical significance of the difference between the means of various groups was evaluated according to Student's *t*-test.

RESULTS

Glucose utilization rate (K_G) *in obese children*

The blood glucose concentration of obese children after overnight fast and the peak of glycaemia after the intravenous glucose load were not significantly different from those of the controls (Fig. 1). As a result of the more rapid decline of the blood glucose concentration in the non-obese patients, the blood glucose level was significantly higher in the obese children between 20 and 80 min, but it was similar to the controls after 100 min.

The K_G value was lower (1.55 ± 0.1) in obese children than in the controls (1.77 ± 0.18), but the difference was not significant. Impaired glucose tol-

erance was found in 23% of obese children.

Insulin response to i.v. glucose load

Basal and reactive hyperinsulinaemia could be demonstrated in the obese patients (Fig. 2). All insulin levels determined during IVGTT were significantly higher in overweight children. The degree of hyperinsulinaemia and the course of the insulin curve were similar in the different obese subgroups (HT, NHT, HI, NHI) except in the obese children with IGT. Strikingly higher insulin levels were observed in the late phase (60 and 100 min.) of the IVGTT in children with IGT compared to those with NGT (Fig. 3).

Lactate, pyruvate, FFA, glycerol, cholesterol and triglyceride levels during glucose tolerance test

Basal levels of these metabolites were similar in obese children and the controls (Fig. 4).

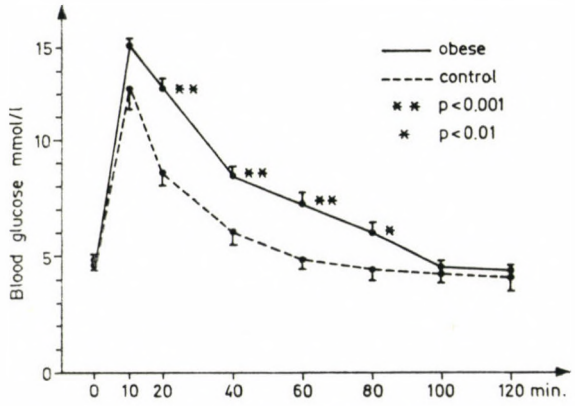

FIG. 1. Mean ± SE blood glucose of 12 control and 33 obese children during i.v. glucose tolerance test

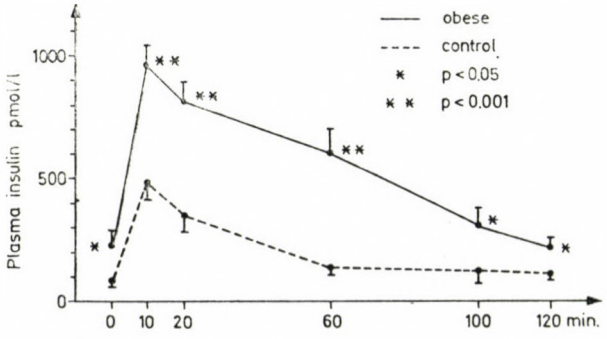

FIG. 2. Mean ± SE plasma insulin of 12 control and 33 obese children during i.v. glucose tolerance test

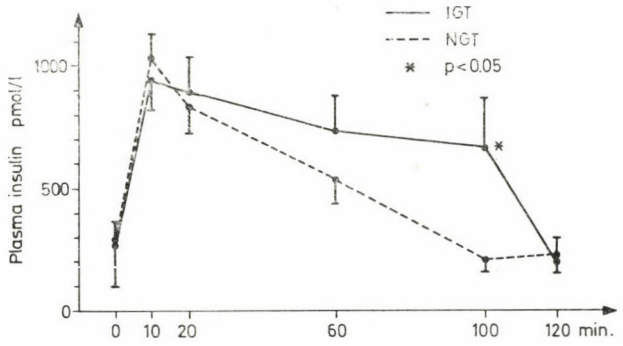

FIG. 3. Mean ± SE plasma insulin of 25 obese children with NGT and 8 obese children with IGT during i.v. glucose tolerance test

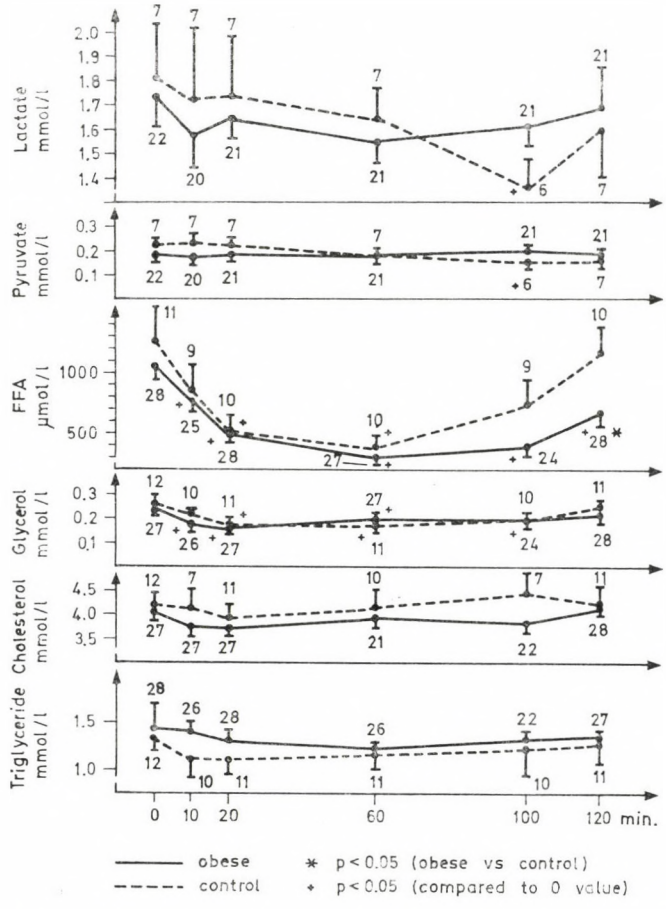

FIG. 4. Mean ± SE blood lactate, pyruvate, glycerol, plasma FFA, cholesterol and triglyceride of control and obese children during i.v. glucose tolerance test

The plasma lactate and pyruvate levels during IVGTT decreased significantly in the controls, but not in the obese group. The glucose-induced drop of FFA and glycerol was similar in the obese and control children, but a delayed increase of glycerol and especially of FFA in the 2nd hour of IVGTT was seen in the obese patients.

Cholesterol and triglyceride decreased in the 1st hour of IVGTT in both the obese and control children, but the decrease was not significant statistically (Fig. 4). In the levels of these metabolites, no differences were found in the obese subgroups.

Interrelationships between glucose tolerance and biochemical parameters

The results of correlative studies concerning K_G, fasting blood glucose, insulin, insulin 0—10 (insulin level at 0 min — insulin level at 10 min), FFA, FFA 0—60 (FFA level at 0 min—FFA level at 60 min), triglyceride, cholesterol and glycerol can be seen in Table II. No relationship was found between insulin 0–10, FFA 0–60, cholesterol, glycerol and K_G.

Fasting blood glucose correlated negatively with K_G, the correlation was, however, significant only in the NHI, HI obese subgroups and in the controls. Basal insulin concentration showed a significant negative correlation with K_G only in the NHT obese children. Between the fasting FFA level and K_G a significant negative correlation could be demonstrated in the HI obese subgroup and when obese and control children were considered as one group. The relationship of K_G and triglyceride concentration revealed a significant correlation between these two parameters in the controls and the NHT children.

Discussion

In obese children as a whole the glycaemia curves were higher, while the K_G values were not significantly lower, than in the controls. Glucose tolerance was impaired in 23% of the cases. Thus obesity is usually not associated with impaired glucose tolerance, but the frequency of impaired glucose tolerance is high in children with weight excess.

Most of the papers in the literature reported higher K_G values for healthy children (13, 19, 20) than those found in this study. Higher T1/2 and lower K_G were, however, found when the glucose load was 0.5 g/kg instead of 0.33 g/kg (12), since there is some correlation between the applied dose of glucose and the glucose disappearance rate (16). The controversy in the literature concerning glucose tolerance in obesity can be due to differences in methods (i.v. or oral administration, and dose of glucose, etc.), differences in calculations ("absolute" or "excess" blood glucose values, T1/2 or other methods), and possible differences in the degree of fatness, age, etc.

The basal and reactive hyperinsulinaemia found in this study is in accordance with earlier reports

TABLE II

Correlation coefficients between biochemical parameters and K_G in obese (O), control (C) and NHT, HT, NHI, HI, NGT, IGT obese children

	O	C	O+C	NHT	HT	NHI	HI	NGT	IGT
Fasting blood glucose	−0.156	−0.787*	−0.23	−0.41	−0.65	−0.569*	−0.733**	−0.202	−0.128
Fasting insulin	−0.056	0.443	0.16	−0.447*	0.228	0.15	−0.096	−0.243	−0.205
Insulin 0–10	−0.06	−0.24	0.11	0.09	−0.102	−0.45	0.41	−0.03	−0.56
Fasting FFA	−0.173	−0.311	−0.33*	0.034	−0.539	−0.04	−0.643*	0.127	0.011
FFA 0–60	0.11	0.11	0.16	0.22	−0.34	−0.38	−0.08	−0.13	−0.16
Fasting triglyceride	−0.368	−0.695*	−0.20	−0.635**	0.364	−0.275	−0.085	−0.212	0.488
Fasting cholesterol	−0.094	−0.40	−0.18	−0.054	−0.074	0.007	0.333	−0.002	0.394
Fasting glycerol	−0.124	0.012	−0.08	0.017	0.357	−0.455	−0.216	0.188	−0.392

* $p < 0,05$
** $p < 0,01$
NHT non-hypertriglyceridaemic
HT hypertriglyceridaemic
NHI non-hyperinsulinaemic
HI hyperinsulinaemic
NGT normal glucose tolerance
IGT impaired glucose tolerance

(3, 8, 9, 10, 26, 27). The normal or impaired glucose tolerance in spite of the apparent hyperinsulinaemia and the more pronounced late phase hyperinsulinaemia in children with IGT suggests that childhood obesity is accompanied by insulin resistance, especially in cases with IGT.

By all odds, a further sign of insulin resistance is the smaller decrease after glucose load of plasma lactate and pyruvate in obese children than in the controls.

Earlier data concerning basal and glucose-induced drop of FFA in obese patients are contradictory (7, 8, 14, 26, 29). We found normal fasting levels and the glucose-induced decrease was comparable to that of the controls. The reduced rise of glycerol and especially of FFA in the 2nd hour of IVGTT coinciding with the fall of insulin level, is suggestive of an impaired lipolysis in overweight children. This is supported by some earlier reports (2, 11, 17, 22, 32) and by our recent finding (23) that the fasting FFA level is in negative correlation with the degree of obesity.

Fasting blood glucose levels, as it was expected, showed a negative correlation with the K_G value in the controls and the NHI and HI obese subgroups.

An obvious connection between early insulin response and K_G has been reported (3, 18, 29), but could not be demonstrated in our material. Our results showed a significant negative correlation in the NHT obese subgroup and a nearly significant positive correlation in the control group between K_G and fasting insulin concentration, in agreement with the finding of Ratzmann et al. (33). The negative correlation of K_G and fasting insulin in NHT obese patients may reflect the insulin resistance of obese subjects. Olefsky and Reaven (25) showed that the basal insulin concentration, but not the glucose-induced insulin response, was closely associated with the number of insulin receptors and with the degree of insulin resistance.

The influence of FFA (29, 31) and triglyceride (1) levels on peripheral glucose utilization has repeatedly been shown. In the present study, K_G correlated significantly with FFA in the obese and control children when these were regarded as one group and in HI children. The correlation of triglyceride and K_G was significant in the controls and NHT children. This suggests that fasting FFA and triglyceride are factors apt to influence K_G but their effect does not hold across the whole range of adiposity, only in certain obese subgroups.

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Effect of glucagon infusion on some plasma metabolites and hormones in obese children*

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The metabolic and hormonal effects of glucagon infusion (6 μg/kg/h) for three hours were studied in obese children. Glucagon caused a sustained hyperglycaemia and hyperinsulinaemia and a lower than normal (non-obese) growth hormone response. Plasma triglycerides, cholesterol, glycerol and the majority of the free amino acids showed a significant decrease in comparison with the controls, while free fatty acids showed a moderate decrease.

Glucagon administration revealed some hormonal and metabolic abnormalities of obesity. The effect of glucagon-induced insulin secretion and the action of pharmacologic doses of glucagon have, however, to be considered in the interpretation of the metabolic effect of glucagon.

Glucagon is considered a glucogenic hormone, and recent investigations (12) have shown that glucagon also modulates lipid metabolism and has important metabolic effects on the three principal lipid substrates in the blood, i.e. free fatty acids, ketone bodies and triglycerides. Glucagon has also been shown to stimulate the secretion of a number of hormones including growth hormone and insulin (9, 11).

The present investigation was undertaken to study the metabolic and hormonal effects of glucagon infusion in obese children.

Material and Methods

The investigations were carried out in 24 non-obese and 35 obese children weighing more than 20% in excess of their ideal body weight.

Blood glucose concentration was estimated by the glucose oxidase method. Plasma triglyceride, glycerol and cholesterol levels were determined enzymatically using Boehringer kits. Immunoreactive insulin and growth hormone levels were measured by double radioimmunoassay using commercially available kits of Radiochemical Centre Amersham for insulin and of Biodata for growth hormone determination. Plasma free amino acids were measured by automatic ion exchange chromatography (Beckman Multichrom). Lactate, pyruvate and free fatty acid determinations were made by standard methods described in a previous paper (10).

The mean, standard error and the correlation coefficient were calculated with standard methods. Statistical significance of the difference between the means of various groups were evaluated according to Student's *t*-test.

In this series of investigations postabsorptive venous blood sample after an overnight fast was taken prior to glucagon infusion for baseline metabolite and hormone estimation. Glucagon (Cristalline Glucagon, Eli Lilly, Indianapolis) was

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infused into an antecubital vein at a rate of 6 μg/kg/h for 180 min, by means of a constant infusion pump. Venous blood samples were drawn at 60, 120 and 180 min during the infusion.

Results

Blood glucose (Fig. 1). Fasting blood glucose concentration was similar in the control and obese group (76.9±3.6 *vs.* 83.4±2.9 mg/dl). After 60 min of glucagon infusion, blood glucose increased by nearly 50 mg/dl in both groups ($p < 0.001$), then it tended to fall during the 2nd and 3rd h in both control and obese children and in the control group it returned to the pre-infusion level. The fall of blood glucose was less marked in the obese children and they remained significantly hyperglycaemic throughout the whole infusion period.

Plasma immunoreactive insulin. The fasting plasma insulin level was significantly higher in the obese group (26±5.1 *vs.* 0.5±3.9 μU/ml). Glucagon infusion induced endogenous insulin secretion in both groups with peak values at 60 min, after which a continuous and significant decline was seen. But insulin secretion as judged by the plasma insulin levels was much higher in the obese group during the whole infusion period (Fig. 1).

Growth hormone (Fig. 1). Basal plasma growth hormone levels were similar in both groups (9.6±3.8 *vs.* 2.5±0.8 ng/ml). The hormone levels tended to increase during the infusion in both groups. The increment, however, reached the level of significance only at 180 min in the control group and at 120 and 180 min in the obese group.

Blood lactate, pyruvate and lactate/pyruvate ratio. Basal levels were

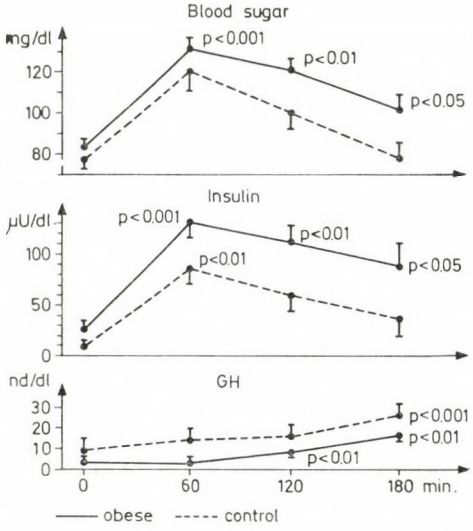

Fig. 1. Blood glucose, plasma insulin and growth hormone levels during glucagon infusion

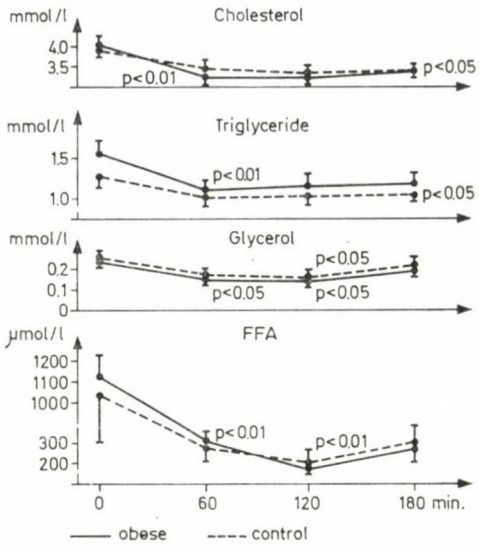

FIG. 2. Effect of glucagon infusion on plasma cholesterol, triglyceride, glycerol and FFA levels

similar in both groups and remained practically unchanged during the 3 h glucagon infusion.

Plasma free fatty acids (Fig. 2). There was no significant difference in fasting FFA levels between the control and obese children. Plasma FFA levels significantly decreased during the first and second hour of glucagon infusion in both groups with no apparent change in the third hour.

Cholesterol (Fig. 2). Baseline cholesterol levels were similar in the two groups. Cholesterol showed a tendency to fall during the first hour of glucagon infusion, but the decrease was significant only in the obese group. The decrease of plasma cholesterol reached significance at 120 min in the control group with no change thereafter. Plasma cholesterol remained unchanged between 60 and 180 min in the obese children.

Triglycerides (Fig. 2). Plasma fasting triglyceride levels were higher in the obese group (1.55 ± 0.1 *vs.* 1.25 ± 0.1 mmol/l). There was a significant fall during the first hour of infusion in the obese group with no change thereafter. There was also a tendency to decrease in the control group, but only the 180 min value was significantly below the starting level.

Glycerol (Fig. 2). The fasting levels were similar in both groups. Significantly lower levels could be observed at 120 min in the control group and at 60 and 120 min in the obese group.

Plasma amino acids (Fig. 3 and Table I). Fasting total free amino acid levels were somewhat but not significantly higher in the obese group. Among the individual free amino acids the concentration of alanine and lysine were significantly

elevated, but a number of other free amino acids also tended to increase (Fig. 4 and Table I).

More than half of the individual free amino acids measured showed a significant decline during the glucagon infusion, especially during the second and third hour. As far as the total free amino acid levels are concerned, the changes were similar in both groups but more pronounced in the obese children. Among the individual free amino acids, glycine, alanine, valine, isoleucine, leucine, tyrosine, lysine, ornithine and arginine decreased significantly in the obese group. The same amino acids showed a significant decline during glucagon infusion in the control group except for alanine, lysine and ornithine, the levels of which did not change significantly.

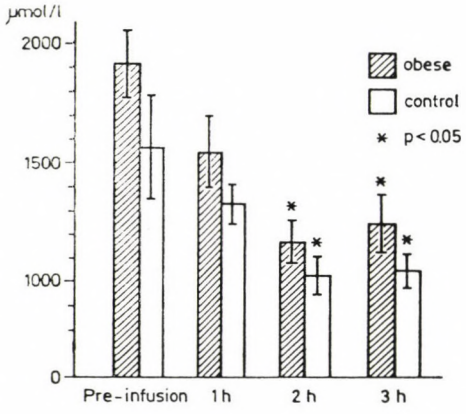

FIG. 3. Plasma total free amino acid levels during glucagon infusion

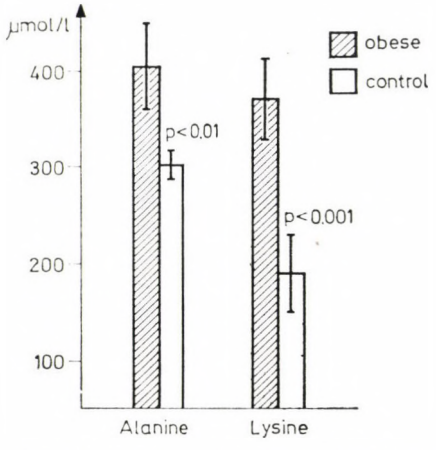

FIG. 4. Plasma alanine and lysine levels in obese and control children

TABLE I

Effect of exogeneous hyperglucagonaemia (continuous infusion of glucagon) on individual plasma amino acid levels in obese children (M ± SE)

		Pre-infusion	1 h	2 h	3 h
Taurine	Obese	66±11	45±10	46± 8	39± 9
	Control	51±18	56± 9	29± 9	87±14
Aspartate	O	34± 5	24± 2	28± 4	25± 4
	C	27± 9	35± 8	15± 8	19± 5
Glycine	O	214±16	147±10	112± 7***	101±10***
	C	178±37	151±34	114±35	92±15*
Alanine	O	402±42	310±24	264±18**	252±18**
	C	303±19	275±44	215±38	230±40
Cystine	O	85±19	106± 9	78±25	100±20
	C	178±101	137± 7	81±18	74±12
Valine	O	267±21	221±16	167±13***	187±17**
	C	252±44	233±40	169±24	142±19*
Methionine	O	10± 4	11± 3	4± 1	33± 8
	C	11± 1	11± 1	4± 1	33± 8
Isoleucine	O	68± 8	48± 3*	33± 3***	37±21
	C	50± 5	44± 8	28± 1*	28± 2*
Leucine	O	137± 9	108± 9	78±10***	87±11**
	C	119±19	105±26	57± 7	76± 4
Tyrosine	O	64±14	44± 5	28± 3*	23± 4*
	C	44± 9	40± 4	21± 1*	26± 1*
Phenylalanine	O	66±11	47± 4	56±11	53± 9
	C	66±10	34± 1	20± 1	36± 1
Lysine	O	376±39	300±45	203±26**	221±29**
	C	182±47	134± 4	203±23	128±37
Ornithine	O	64± 8	70±22	31± 5**	39±11
	C	36±13	28±17	29± 1	21± 4
Arginine	O	61± 6	48± 5	36± 5**	34± 4**
	C	63±22	30± 7	20± 5*	25± 2
Total	Obese	1914±122	1529±138	1164±102*	1234±112*
	Control	1560±182	1310± 88	1005± 83*	1017± 96*

*0.05; **$p < 0.01$; *** $p < 0.001$.

Discussion

One of the most striking effects of the continuous glucagon infusion was a marked insulin secretory response in both groups, especially in the obese children. The glucagon-induced insulin secretion greatly modified the effect of glucagon. The plasma FFA curve was the mirror image of that of the insulin curve (Fig. 2): the higher the insulin, the lower the FFA; in fact, the lipolytic effect of glucagon (11) seemed to be completely suppressed by the glucagon-induced insulin secretion.

Insulin is lipogenic, and there is a fall in FFA during insulin infusion, e.g. during the insulin treatment of diabetic ketoacidosis and during glucose tolerance tests. Although plasma glucagon levels were not measured during the infusion, they probably were high during the entire period, so it is possible that the ratio of these two antagonistic hormones have determined the overall metabolic effects. Some of the metabolites measured may also have been more "sensitive" to glucagon rather than to insulin levels. Thus, the fall in triglycerides may have been primarily a glucagon effect since glucagon may reduce the circulating plasma triglyceride concentration (11), and oral glucose administration results in a definite increase in plasma triglycerides (1).

Felig et al. (5, 6) were the first to report significantly higher levels of branched-chain amino acids, tyrosine and phenylalanine in obese adults. The hyperaminoacidaemia was interpreted as a manifestation of the insulin ineffectiveness characteristic of obesity. Furthermore, it was speculated that the hyperaminoacidaemia might have provided the feedback signal to the beta cells through which insulin resistance was accompanied by an appropriately augmented secretory rate of insulin. The increment of branched-chain amino acids and that of tyrosine and phenylalanine was not significant in our material. The difference may be explained by comparing the fasting plasma insulin levels of the two series of investigations. Fasting plasma insulin was only moderately increased in our obese children compared with the overweight adults of Felig et al. (5).

The recent date of Felig et al. (6) showed significantly higher alanine levels in a group of obese adults. Interestingly, the first paper of the same group (5) reported essentially similar alanine levels in obese and in control subjects.

Chaussain et al. (4) also found identical plasma alanine levels in obese and control children. In contrast, in the present study, alanine was one of the two amino acids which showed a significantly higher fasting level in obese children. The cause of hyperalaninaemia is far from clear. Since among the metabolic hormones regulating plasma amino acid levels glucagon seems to be the most important, the question arises whether or not the increased alanine level was due to hypoglucagonaemia. Fast-

ing plasma glucagon levels have not been measured in our patients, but alpha-cell function, as judged by basal levels and by responses to glucose and protein administration, was found to be normal in obese persons (7).

It is interesting that the hyperalaninaemia in the obese adult patients of Felig et al. (6) was accompanied by an increased splanchnic uptake of alanine. This association probably reflects an increased rate of gluconeogenesis.

The hypoaminoacidaemia induced by bolus injection or infusion of glucagon is well documented (2, 3, 8). In interpreting this metabolic effect, two important factors must be considered. The dose of glucagon infused was pharmacological (8) and the glucagon stimulated insulin secretion may greatly modify the effect of glucagon. Endogenous insulin secretion as seen after the administration of glucose (5), significantly reduced the levels of branched-chain amino acids, tyrosine and phenylalanine.

In conclusion, glucagon infusion had a profound effect on the plasma level of most investigated metabolites. Plasma triglycerides, cholesterol, glycerol and the majority of the amino acids decreased significantly, while free fatty acids showed a moderate decrease in comparison with the controls.

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Haemodialysis treatment of acute renal failure in hypertonic dehydration

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Acute renal failure caused by hypernatraemic, hypertonic dehydration was successfully treated in a five months old marasmic infant of 3500 g body weight. Haemothorax complicated the course but the patient recovered fully. If oliguria persists beyond 48 hours and kidney function deteriorates in spite of adequate fluid intake, dialysis remains the only therapeutic method in renal failure induced by hypertonic dehydration.

Acute renal failure in infancy often appears as a consequence of severe dehydration. Tubular necrosis or in the most malignant form of renal damage cortical necrosis may develop (14). Inadequate fluid replacement in dehydration may lead to these serious complications. In tropical countries, diarrhoea is the main factor causing dehydration. Besides, marasmus is also endemic in tropical countries and in marasmic infants dehydration caused by diarrhoea is always life-threatening (10). Hypernatraemic hypertonic type dehydration is frequently accompanied by extreme oliguria and in such cases only peritoneal dialysis may improve the patient's condition and kidney function (8). Ten to fifteen years ago haemodialysis did not seem to be a safe treatment for seriously ill children with acute renal failure. Nowadays, however, improved technical means, sophisticated instruments and accumulated experience have changed the situation. There are more and more optimistic opinions published also for the treatment of children (7).

Report of a Case

M.Md.M. a 5 months old boy was admitted with diarrhoea and severe dehydration. His body weight was 3470 g. The marasmic infant's condition was critical. The fontanel was deeply sunken and so were the eyes. The skin lost its elasticity, it could be pinched up easily in folds and regained the normal contour slowly. The oral mucosa and the tongue were dry. The patient was dyspnoeic with a respiratory rate of 80/min. Auscultation of the chest revealed fine moist rales on both sides. The pulse rate was 160/min. the first and second heart sounds were normal. The liver reached 3 cm below the costal margin. The baby was unconscious but reacted to painful stimuli. After blood sampling, intravenous fluid therapy was started with 0.18% NaCl–3.2%

TABLE I

	On admission	10 h	24 h after admission	48 h	1 h	22 h after dialysis	10 days
BUN, mg/dl	182	—	181	184	126	186	15
Serum creatinine mg/dl	2.3	—	2.6	4.6	3.0	2.5	0.5
Serum sodium mmol/l	161	146	151	158	139	146	132
Serum potassium mmol/l	7.0	7.5	5.4	4.6	2.6	2.9	3.7
Serum chloridine mmol/l	115	—	—	110	86	—	92
Astrup							
pH	6.86	7.12	7.13	7.26	7.31	7.31	7.34
pCO_2 mmHg	29.6	32.6	41.1	36.1	36.9	37.9	30.1
pO_2 mmHg	77.1	54.4	50.0	44.1	45.9	72.3	58.4
BE mmol/l	−28.8	−21.3	−15.2	−9.4	−7.1	−6.7	−3.2
SBE mmol/l	−26.0	−20.3	−14.5	−9.3	−7.1	−6.6	−3.3
Serum total protein g/dl	6.0	—	—	5.8	5.5	5.6	5.8

glucose solution. During the first 30 min 100 ml fluid was given. Acid-base values in capillary blood exhibited severe metabolic acidosis with a pH of 6.86 (Table I).

Intravenous sodium bicarbonate was given hourly and simultaneously 20 ml/kg of 3% plasma protein in drip, and the sodium-glucose infusion was continued. Table I demonstrates the laboratory values during the following hours and days.

Ten hours after admission the respiratory rate decreased to 60/min and the pulse rate to 140/min. The severe acidosis and hypernatraemia were kept under control, but despite adequate rehydration only a few drops of urine could be obtained by catheterization.

Twenty-four hours after admission the patient was still unconscious. The acidosis improved on alkali therapy but the BUN value remained constantly high and the serum creatinine tended to increase. After the first 24 hours the urine volume was only 10 ml. It contained protein but the sediment was normal. Platelet count in the blood was 180 000/mm^3, the sedimentation rate 8 mm/h. Intravenous fluid therapy was continued during the next twenty-four hours. In this period the stools increased in number; they contained a considerable amount of mucus and streaks of blood and pus. For this reason, gentamycin therapy was prescribed.

Forty-eight hours after admission the infant's general condition was satisfactory but oliguria persisted (30 ml urine per 24 h). The serum creatinine value showed a rising tendency. The mannitol test con-

firmed the renal origin of oliguria, as 3 h after a mannitol load only 8 ml urine could be collected. In view of the situation haemodialysis was performed. A 0.25 square meter Gambro AK-5 HMD apparatus (A. B. Gambro, Lund, Sweden) was used with blood lines for infants. The saphenous vein was cannulated and the procedure was continued during the next 3 h at 3 ml/min/kg blood flow rate by the single needle technique. Mannitol and matched blood were also given to maintain the equilibrium. No complication occurred in the course of treatment and one hour after dialysis the BUN and the serum creatinine values fell significantly. Serum potassium on the second day of admission was in the normal range, but the tissue levels remained low. Therefore, in the first hour of dialysis the potassium concentration was elevated to 4 mmol/l in the bath. After dialysis, 2 to 3 mmol/kg body weight potassium was supplemented daily.

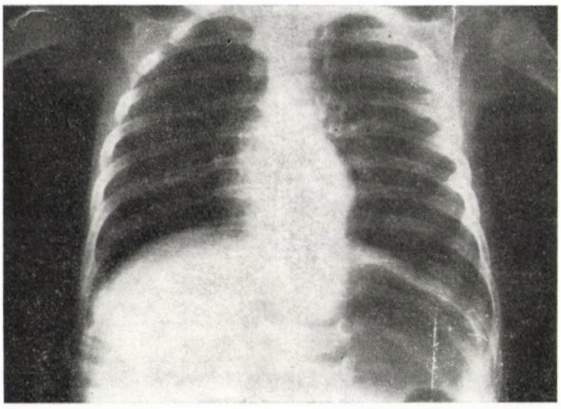

FIG. 1

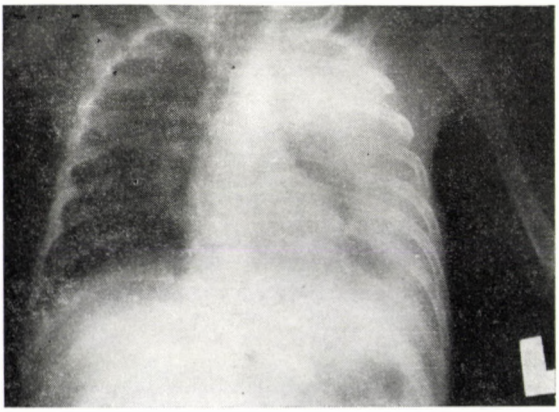

FIG. 2

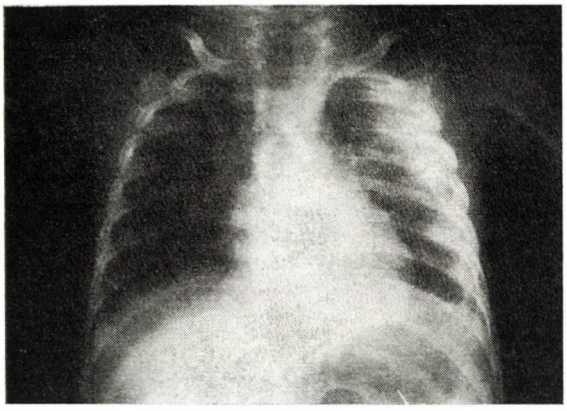

FIG. 3

Twenty-two hours after dialysis a rebound was observed in the BUN value, while the serum creatinine and acid-base values remained constant. The general condition improved gradually, and consciousness was regained. Urinary output increased to 40 ml/12 h. On the third day after dialysis, we began to feed the infant by nasogastric tube. On the sixth day the patient had dyspnoea, and on the left side of the chest the presence of a considerable amount of fluid was established. At admission the chest radiograph revealed a heart configuration characteristic of hypovolaemic shock (Fig. 1), but on the sixth day the chest X-rays showed pleurisy (Fig. 2). By thoracocentesis 20 ml of blood-tinged transudate was drained. Then the infant was anaemic, with Hb 5.3 g/dl, Ht 16%, WBC 26 000/mm^3, and a platelet count of 200 000/mm^3. Bleeding and clotting times were normal. Matched "O" Rh positive blood was transfused on two consecutive days. Three days later the X-rays indicated partial recovery (Fig. 3), and on the 10th day after admission the laboratory examinations gave normal values (see Table I). In this period the patient was fed 20 g milk and vegetables ten times daily. The stools were normal in consistency and urinary volume increased to normal. Giardiasis and pyogenic skin disease were treated during the next ten days. After 22 days of hospitalization the infant was discharged with a body weight of 3650 g.

DISCUSSION

Hypernatraemic dehydration (HD) in infancy has a mean mortality rate of 11.5%, as calculated from the reports of eight authors (13). Brain damage is a frequent sequel, thus adequate treatment has a special importance (12). When HD appears in consequence of gastroenteritis,

diarrhoea with fever, vomiting and anorexia as well as hyperventilation are the main pathogenetic factors (11). Inappropriate feeding of concentrated milk formulas may also cause hypernatraemia (9). If the oral rehydration fluid contains glucose at high concentration (>3%) the osmotic load may intensify the fluid deficit (1). In our material in Lybia, North-Africa, among dehydrated patients the 6% frequency of HD was the same as in South-Africa (2). On the contrary, HD seems to occur more frequently in the USA and England than in the developing countries (13). The difference may be connected with the different feeding habits: in the industrial countries the high protein intake shifts the homeostasis in the direction of hypernatraemia (4), while marasmic infants usually exhibit hyponatraemia and salt depletion. On the other hand, circulatory disturbances and a renal impairment are induced more easily in marasmic children. When the symptoms of HD appear, acute renal failure may set in. In our cases the BUN values were regularly elevated, as experienced by other authors as well (3). In patients with HD, the serum creatinine value is the best laboratory indicator of kidney function. Dialysis must be introduced if besides the elevated BUN the serum creatinine value, too, shows an increasing tendency despite an adequate fluid intake and the correction of acidosis. The indication of dialysis is supported by the occurrence of a mannitol resistant oliguria as well. Peritoneal or haemodialysis improve favourably the prognosis of acute renal failure (6).

In the post-dialysis period, the control of hypokalaemia and anaemia are the main tasks. After recovery, neurological and nephrological control examinations should be done.

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Dynamics of adrenal steroidogenesis in childhood: steroid excretion in prepubertal and pubertal girls

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C_{19}—$C_{21}O_{2-3}$ steroid spectrum and $C_{21}O_5$ corticoids have been studied in the urine collected from 29 girls aged 2–14 years. A total of 34 investigations was carried out by means of simultaneous two-column gas chromatography. The values of steroid excretion have been expressed in terms related to body weight and body surface, respectively.

Before the age of seven the excretion of $C_{19}O_2$ steroids representing adrenal androgens and their metabolites did not exceed 7 μg/kg/day or 170 μg/m²/day ("infantile type androgen excretion"). Then it began to increase between the 7th and 9th years of life and preceded the appearance of the physical signs of adrenarche. The increase was accelerated during puberty and no value under 18 μg/kg/day, or 600 μg/m²/day, respectively, was found when the physical signs of adrenarche were present ("puberty type androgen excretion"). Excretion of 11-oxygenated C_{19} steroids, $C_{21}O_5$ corticoids and pregnenediol was also augmented during puberty; however, when compared with the increase in $C_{19}O_2$ excretion this augmentation was moderate. As a consequence of the marked elevation in $C_{19}O_2$ excretion, the ratio of $C_{19}O_2$ to $C_{21}O_5$, too, increased during puberty, indicating that the cortisol-androgen dissociation, otherwise characteristic of childhood, had ceased.

It is concluded that the most plausible explanation of the increased androgen production is the gradual development of the zona reticularis during puberty.

In addition to cortisol and aldosterone, the adrenal cortex of adults secretes significant amounts of cortical androgens, such as dehydroepiandrosterone, dehydroepiandrosterone sulphate and androstenedione. The production of dehydroepiandrosterone (DHA) and dehydroepiandrosterone sulphate (DHA–S) is very low in children under eight, and activation of adrenal steroid secretion sets in only during puberty. These adrenal androgens are responsible for the development of pubic and axillary hair in females. This effect of adrenal androgens on secondary sex character and the developmental process itself were recognized and termed adrenarche some 40 years ago (2).

Since that time much has been learnt about adrenarche. It has been demonstrated that in children cortisol secretion and also its secretion ratio are identical with those of adults; when calculated for body surface or body weight they do not show any significant elevation even during puberty (12, 14). Complex radioimmune assays have revealed the increase of DHA and DHA–S secretion to begin as early as around the 8th year of life; it precedes by

2–3 years the elevation of the serum level of gonadotropins and the appearance of the first physical signs of puberty (18, 19).

There is convincing evidence available that, in adults, the secretion of DHA is regulated by ACTH and that, under physiological conditions, the secretion of cortisol and that of DHA run parallel (17).

In contrast, another specific form of steroidogenesis occurs in childhood when cortisol and cortical androgens undergo dissociation. There is no generally accepted explanation of either these childhood characteristics of androgen relations or of their gradual disappearance during puberty. The increased serum pregnenolone level (3) and the increased urinary pregnenediol excretion (10) during puberty point to the importance of intraglandular factors, i.e. to an increased biosynthesising activity of the adrenals in the years preceding sexual maturation. Functional effects based on morphological factors may also be of importance since, as demonstrated in obduction material (5), development of the adrenal zona reticularis sets in around the 8th year of life and ceases at the end of puberty. Recently, the existence of a pituitary factor called cortical androgen stimulating hormone (16) has been put forward; the hormone might act jointly with ACTH (19).

In the present study, the steroid excretion of healthy girls has been studied. Based on the investigation of urinary steroid excretion, an attempt has been made to shed light on the characteristics of adrenal steroidogenesis and metabolism in childhood, and on their changes during puberty.

As a rule, gas chromatography does not determine the active hormones but their urinary metabolites. In spite of this fact gas chromatography should be regarded as a full-value method to explore the functioning of the zona reticularis and zona fasciculata, since the amount of metabolites is in direct proportion to the production rate of active hormones.

Gas chromatographic steroid spectrum analysis is one of the most up-to-date methods for determination of urinary steroids. It gives information on the excretion of steroid intermediates, too; thus it is a diagnostic procedure suitable to identify pathologic steroids in enzyme deficiency or in the case of suspicion of hormonally active tumours. The method is quick, with a sensitivity superior to that of the routine photometric methods used for determination of 17-ketosteroids and 17-hydroxycorticoids.

When hepatic or renal damage is associated with the endocrine disease, a steroid value below the physiological level must not in itself be considered a definite sign of endocrine hypofunction; the low excretion may be due to disturbed metabolism, an event leading to increased half life of the active hormone. Thus, when renal or hepatic damage is present, preference should be given to the determination of steroid levels in plasma.

Materials and Methods

Reference steroids were made available by the generous gift of Prof. D. N. Kirk (Medical Council Steroid Reference Collection, London).

Material

Twenty-four-hour urine samples were collected from 29 healthy girls on 34 occasions. No liver or kidney disease was mentioned in their history. The age distribution was, 12 girls between 2 and 7 years, 9 girls between 7 and 10, and 13 girls between 10 and 14 years of age. According to the puberty stage, of the girls between 10 and 14 years, 4 girls were in stage P-2, five in P-3 and four in P-4.

Analytic methods. Extraction, derivate formation and gas chromatographic analysis of C_{19}—$C_{21}O_{2-3}$ steroids were carried out according to the method previously described (9), with a slight modification allowing to investigate 16-oxysteroids (11). The steroids were identified after separation of their acetylated derivatives; the retention times refer to the retention time of 5-α-cholestane (RRT_{ch}).

Gas chromatographic determination of $C_{21}O_5$ corticoids. The metabolites of cortisol were analysed as a common component, after reduction of the ketonic groups by sodium borohydrate and after splitting off the C_{17-21} side chain by periodate (22). Removal of the side chain makes hydrolysis unnecessary, for the conjugating glucuronide will be eliminated together with the side chain. The method of Trafford and Makin (22) applied for derivative formation and gas chromatographic analysis had been modified in the sense that acetylation was used instead of formylation. Briefly, 10% of the daily urine is analysed under the age of four, 5% in the age group 4–10 years, and 2.5% above ten years. Two ml of 10% sodium borohydrate dissolved in 0.01 N sodium hydroxide was added to each 10 ml of the urine sample; the mixture was kept at 50°C for 15 min. The surplus of the reagent was decomposed by addition of 0.5 ml of glacial acetic acid; then, the incubation was continued for 15 further min. Thereafter, the pH of the urine was adjusted to 6.5 by addition of 8 ml of 10% sodium metaperiodate, a procedure followed by incubation anew at 50°C for 20 min. The steroids were extracted by two volumes of dichloromethane. The original extract was then washed with 1 N sodium hydroxide, 1% acetic acid and, finally, with distilled water. This was followed by dehydration by sodium sulphate; the residue was concentrated by means of a rotating evaporator. The concentrates were transferred quantitatively (by washing) into conic centrifuge tubes and evaporated under CO_2. Acetylation of the dry extract was performed at room temperature for 16 h.

A Packard 7300 two-channel gas chromatograph was used for analysis. The extract was applied simulteneously on two 1.82×2 mm columns. Column A with combined filling contained at the inlet end, 3% SP 2250 stationary-phase 78 cm in length on 100/120 mesh Supelcoport support, and at the detector end, 3% SP 2100 stationary-phase 102 cm in length on 80/100 mesh Supelcoport support. Column B contained 3% SP 2100 stationary-phase on 80/100 mesh Supelcoport support. Double flame ionization detector (temperature: 270°C) and as carrier gas, highly purified N_2 were used at 40 ml/min flow rate.

Analytic circumstances: On-column injection technique, heated inlet (270°C). Isothermic period at 250°C with a duration of three times the retention time of the previously injected 5α-cholestane; then the temperature was elevated at a rate of 1°C/min. Prior to analysis, 50 μg of cholesteryl propionate dissolved in 50 μl of ethanol-benzene was added to the extract.

This served as internal standard. On some occasions, after performing the whole procedure, we took linear calibration curves by adding to the urine 50, 100 and

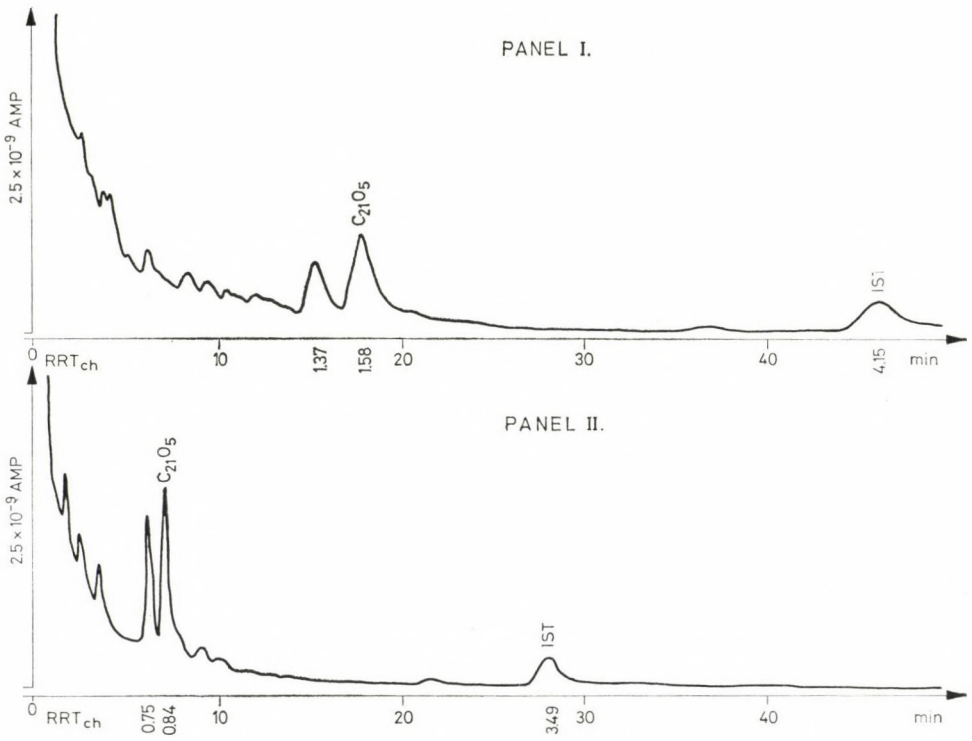

FIG. 1. Simultaneous gas chromatographic analysis of acetylated $C_{21}O_5$ corticoids obtained from the urine of a 9-year-old girl with adrenarche. Before extraction, the sample was reduced with sodium borohydrate and oxydized with periodate. Panel I: SP 2250/SP 2100 combined column; Panel II: SP 2100 column

200 μg of tetrahydrocortisol, tetrahydrocortisone and 20α-cortol, respectively. The slope of the curve was 0.46 ± 0.04 (S.D.). The reciprocal of this value was used as response factor during the analysis of $C_{21}O_5$ steroids.

$C_{21}O_5$ content of the urine in mg/day:

$$\frac{\text{area of } C_{21}O_5 \text{ peak}}{\text{area of the cholesteryl propionate peak}} \times \times \frac{50^*}{1000} \times \text{response factor} \times \times \frac{\text{24-h urine (ml)}}{\text{urine processed (ml)}}$$

Each determination was performed on two columns and the mean of the values obtained was used for further calculation. Of the $C_{21}O_5$ corticoids, 11-β-hydroxy-etiocholanolone (11-OH-E) and 11-β-hydroxy-androsterone (11-OH-A), both originating in the chemical procedures, were evaluated in one common peak (Fig. 1). They were not disturbed by 11-oxy-C_{19}-steroids of urine, since the latter are conjugated at the third carbon atom, and the 11-β-17-β-dihydroxy-5-α-/β-androstane-3-one, produced in the course of oxidation, has a retention time shorter than that of either 11-OH-E or 11-OH-A.

The accuracy of the method was checked

* Amount in μg of added cholesteryl proprionate.

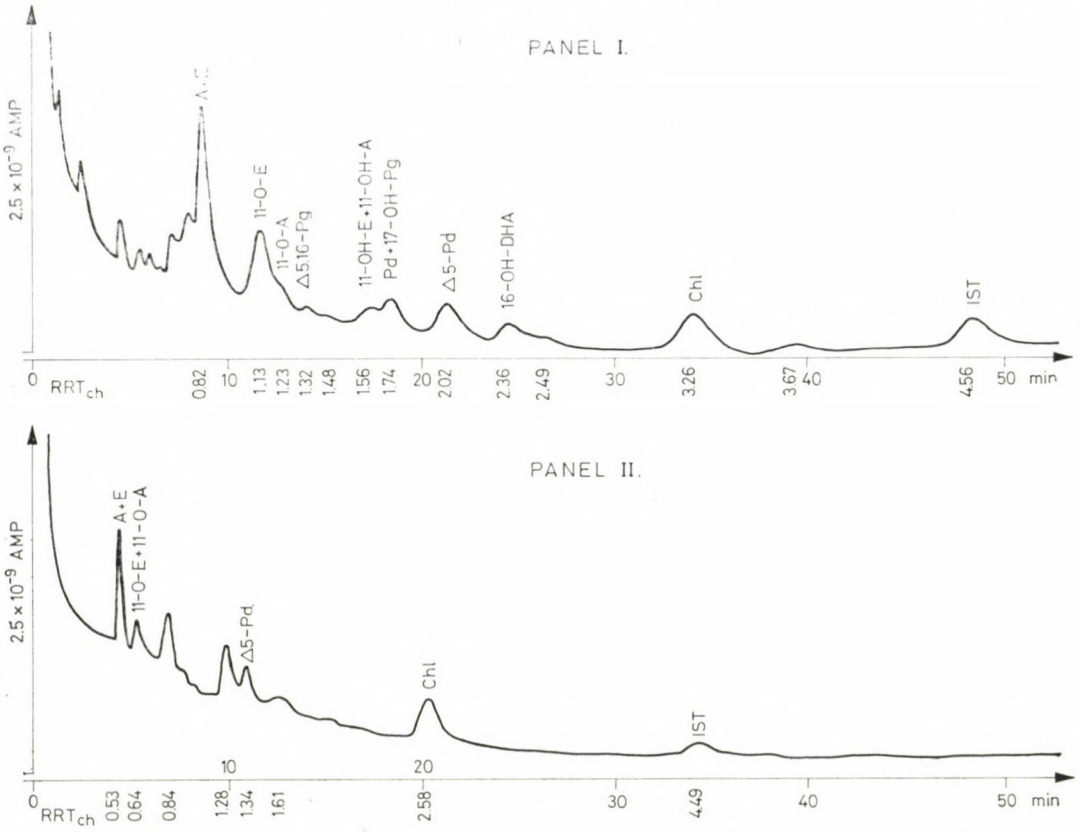

FIG. 2. Simultaneous gas chromatographic analysis of $C_{19}C_{21}O_{2-3}$ steroids obtained from the urine of a 9-year-old girl with adrenarche. Conditions of analysis: acidic hydrolysis, toluene extraction and formation of acetyl derivatives. Panel I: 2250/SP 2100 combined column; Panel II: SP 2100 column

on the basis of the percentage difference between the results of duplicate analysis of the urine of six children. Here, the formula $\sqrt{\frac{d^2}{2n}}$ was applied, where d means the difference in per cent between, and n the number of, the duplicate determinations. The difference amounted to 9.4%, a value to be considered statisfactory as to the reproducibility of the procedure used.

RESULTS

Figure 2 shows the urinary steroid spectrum of a 9-year-old girl with "adrenarche". The panel on the top was obtained from SP 2250/SP 2100 combined column, while the bottom one from SP 2100 column. The dominant peak on both columns was the common peak of androsterone (A) and etiocholanolone (E). Some further peaks were the common peak of 11-keto-etiocholanolone (11-O-E) and 11-keto-androsterone (11-O-A); that of pregnanediol (Pd), 17-hydroxy-pregnanolone (17-OH-Pg), pregnenediol (Δ-5-Pd), 16-α-hydroxy-DHA (16-OH-DHA) and 16-β-hydroxy-DHA, as well as the peak of cholesterol (Chl). The chromatogram also

TABLE I

Simplified scheme showing pathways of adrenocortical steroidogenesis and urinary steroids analysed by gas chromatography. The dotted line indicates a small fraction of pregnenolone

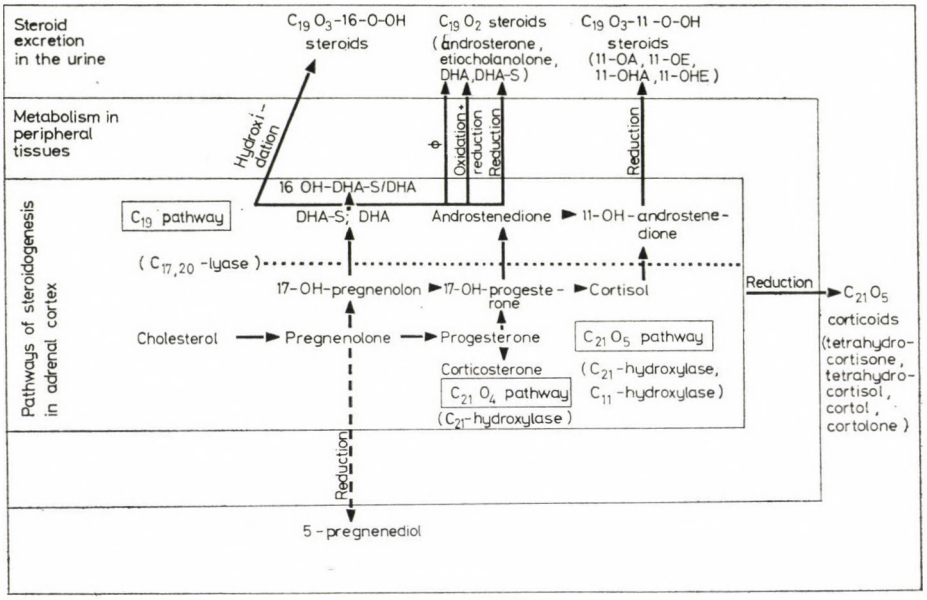

TABLE II

Excretion of C_{19} steroids, $C_{21}O_5$ corticoids and pregnenediol

A

Steroid / Age (year)	$C_{19}O_2$ Mean±S.D.	$C_{19}O_2$ Range	p	$C_{19}O_3$ (11-O-OH) Mean±S.D.	$C_{19}O_3$ (11-O-OH) Range	p	Mean±S.D.
2– 6	4.0± 1.7	2.1– 6.9	<0.02	5.5± 3.1	1.5– 9.3	<0.02	2.4± 2.7
7– 9	8.9± 4.9	3.1–13.8	<0.001	9.9± 8.0	2.2–22.8		3.2± 4.3
10–14	32.4±16.5	7.6–66.9		13.7±10.4	2.0–32.8		6.9±10.3

B

Steroid / Age (year)	$C_{19}O_2$ Mean±S.D.	$C_{19}O_2$ Range	p	$C_{19}O_3$ (11-O-OH) Mean±S.D.	$C_{19}O_3$ (11-O-OH) Range	p	Mean±S.D.
2– 6	97± 43	48– 167	<0.01	134± 80	36–265	<0.01	59± 69
7– 9	241±130	81– 476	<0.001	269±210	59–611		86±116
10–14	1048±597	231–2260		435±338	73–944		217±321

A = values in μg/kg/24 h;

allowed the identification of 11-β-hydroxy-etiocholanolone (11-OH-E) and the dehydrated form (Δ-5,16-Pg) of 16-α-hydroxy-5-pregnenolone. The results shown in Table I according to age group distribution were calculated for kg body weight and also for body surface; they were evaluated by biostatistical methods.

For the sake of an easier survey the steroids investigated had been divided in just four groups, according to the number of carbon atoms and of the functional groupings, respectively. Classification of urinary steroids as well as their connection to the pathways of adrenal steroidogenesis are summarized in Table I.

Group $C_{19}O_2$ contains DHA, androsterone and etiocholanolone. The two latter steroids are the metabolites of DHA, DHA-S and androstenedione. $C_{19}O_3$ steroids were divided in two different groups, based on the unlike position of the third functional group.

11-β-hydroxylation of C_{19} steroids takes place in the adrenals. These 11-β-hydroxy derivatives are the metabolites of androstenedione and, to a lesser extent, of cortisol and/or cortisone. The 16-oxy-C_{19}-steroids are produced not only in the adrenals, but also extraglandularly; their precursor is DHA. The $C_{21}O_5$ corticoids are the metabolites of cortisol and cortisone; their amount corresponds to about 80% of the total corticoid excretion. Pregnenediol represents the first steroid intermediate originating from cholesterol; it is the metabolite of pregnenolone.

The values for steroid excretion have been expressed in μg/kg/day and μg/m^2/day. In addition to the

in healthy girls of different age groups

$C_{19}O_3$(16-O-OH)		$C_{21}O_5$			Δ-5-Pd			No. of cases
Range	p	Mean±S.D.	Range	p	Mean±S.D.	Range	p	
0.3– 6.8		66.4±20.4	39.9– 94.4		3.4±1.8	0.9– 7.2		12
0.2–12.0	n.s.	56.3±23.0	34.4–100.0	<0.01	6.4±5.4	2.3–19.0	<0.01	9
0.1–30.4		91.4±32.8	48.9–159.5		9.0±6.1	0.5–21.7		13

$C_{19}O_3$(16-O-OH)		$C_{21}O_5$			Δ-5-Pd			No. of cases
Range	p	Mean±S.D.	Range	p	Mean±S.D.	Range	p	
6–172		1599±482	1022–2400		84± 50	22–205		12
5–327	n.s.	1536± 602	899–2680	<0.01	174±174	67–509	<0.01	9
4–961		2860±1015	1560–5154		280±201	314–626		13

B = values in μg/m^2/24 h.

mean values, standard deviations as well as upper and lower limits are given in Table II. Of the C_{19}-steroids, the metabolite occurring in the highest amount was 11-oxy-C_{19} in the 2 to 6 year age group. Here, the ratio of $C_{19}O_2$ to $C_{19}O_3$ was 0.72. This ratio increased to about 1.0 in the 7–9 age group and to 2.7 in puberty, due to increasing $C_{19}O_2$ steroid excretion.

Significant differences were found between the amounts of $C_{19}O_2$ steroids excreted in the three age groups. An increasing tendency was seen also in the amount of 11-oxy-C_{19} steroids, even though the difference from the age group of 2–6 years became significant in age group of 10–14 years only. Excretion of 16-oxy-C_{19}-steroids did not differ significantly in the three age groups.

The amount of $C_{21}O_5$ corticoids was increased in the age group of 10–14 years; the difference from the age group of 7–9 years was significant

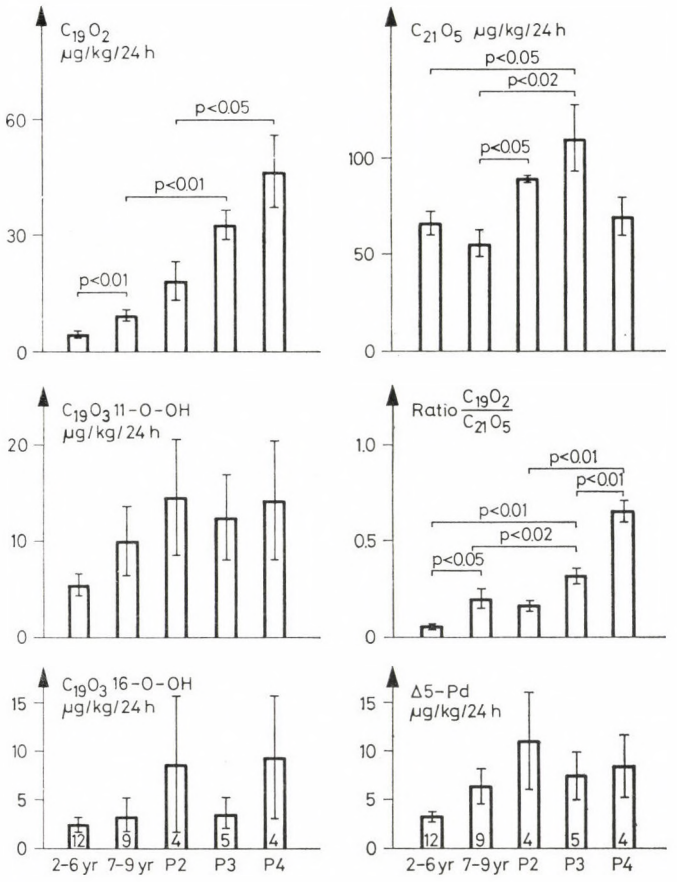

FIG. 3. Excretion values in µg/kg/day of $C_{19}O_2$, 11-oxy-C_{19}, 16-oxy-C_{19} steroids, $C_{21}O_5$ corticoids and pregnenediol according to age group distribution and puberty stages

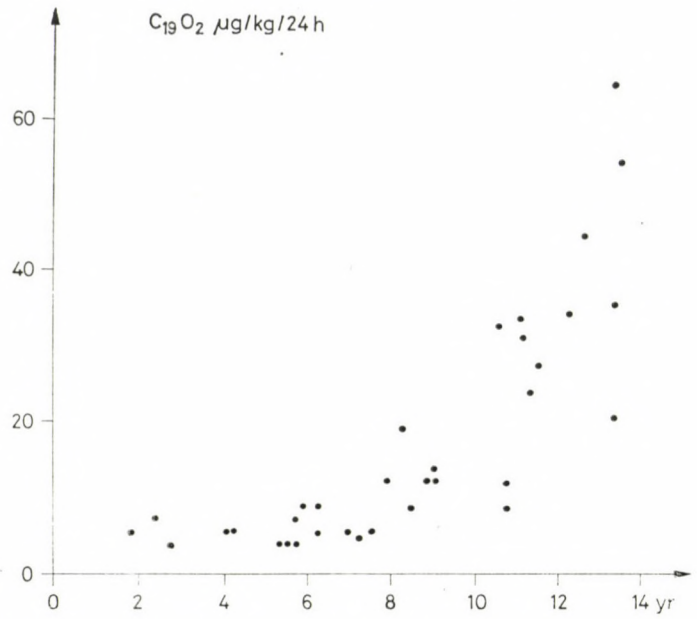

FIG. 4. Excretion in µg/kg/day of $C_{19}O_2$ steroids in 29 girls 2 to 14 years of age

statistically. Pregnenediol excretion had a tendency to increase in the age group of 7–10 years only; however, when compared with the age group of 2–6 years, the increase reached statistical significance in the 10–14 year age group.

All members of the age group of 10–14 years showed the physical signs of puberty. Therefore, this group was also evaluated according to the stage of puberty (21). These values, together with the S.E.M. and the probability level of the significant differences are shown in Fig. 3. As seen, the excretion of $C_{19}O_2$ steroids increased significantly already in the 7–9 age group, when compared with the age group of 2–6 years, even though the former group did not exhibit any physical sign of puberty. The increase continued also during puberty; the differences were significant between age group 7–9 and P-3, as well as between P-2 and P-4.

Excretion of $C_{19}O_2$ steroids in µg/kg/day is also expressed as a function of age (Fig. 4). Values over 7 µg/kg/day never occurred in the age group of 2–6 years ("infantile type adrenal androgen excretion"). The values in the age group of 7–9 years varied between 4 and 18 µg/kg/day. All the values below 10 µg/kg/day occurred in the 7th and 8th year of life. A $C_{19}O_2$ steroid excretion over 18 µg/kg/day was considered "puberty-type androgen excretion". In the age group of 10–14 years, the excretion of $C_{19}O_2$ steroids was below 18 µg/kg/day in two cases only. In these two girls the pubic hair was missing; the patients had been classi-

TABLE III

Quotient of $C_{19}O_2/C_{21}O_5$ excretion in healthy girls of different age groups

Ratio $C_{19}O_2/C_{21}O_5$ / Age (year)	Mean ± S.D.	Range	p	No. of cases
2– 6	0.06 ± 0.03	0.02–0.12		12
7– 9	0.17 ± 0.09	0.06–0.34	< 0.01	9
10–14	0.38 ± 0.22	0.10–0.85	< 0.01	13

fied in stage P-2 on the basis of breast development.

Excretion of 11-oxy-C_{19} steroids seemed to increase continuously till the second stage of puberty, but this increase was not significant statistically. There was an increase in the second and fourth stages of puberty, which, however, was not significant statistically (Fig. 3).

When compared with the age group of 7–9 years, a statistically significant elevation in $C_{21}O_5$ excretion was found in the second and third stages of puberty, but not in the fourth.

Pregnenediol excretion, though exhibiting a slight increase in stage P-2, did not significantly differ from the values found in the two age groups or in the different puberty stages.

In order to follow the corticoid-androgen dissociation we calculated and evaluated the ratio of $C_{19}O_2$

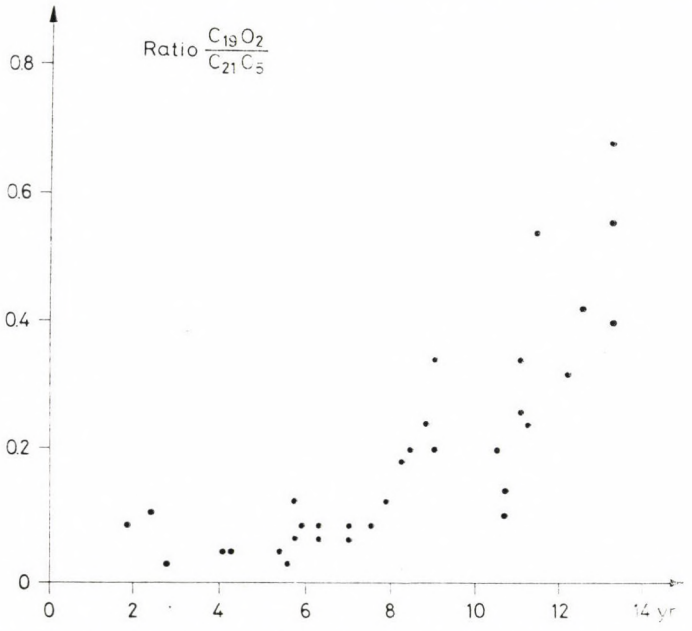

FIG. 5. Ratio of $C_{19}O_2$ to $C_{21}O_5$ corticoids in 29 girls 2 to 14 years of age

to $C_{21}O_5$ (Table III). The average ratio was 0.06 in the age group of 2–6 years. This means that at that age $C_{19}O_2$ steroid excretion only amounts to 6% of cortisol excretion. This ratio gradually increased and reached a value of 0.66 at the fourth stage of puberty, a finding indicative of a decreased androgen-cortisol dissociation during puberty. The distribution of individual values for the ratio was similar to those for $C_{19}O_2$ steroid excretion (Fig. 5).

The correlation between the excretion of pregnenediol and of the other steroid groups was also investigated. Pregnenediol showed a positive correlation with all steroids except $C_{21}O_5$

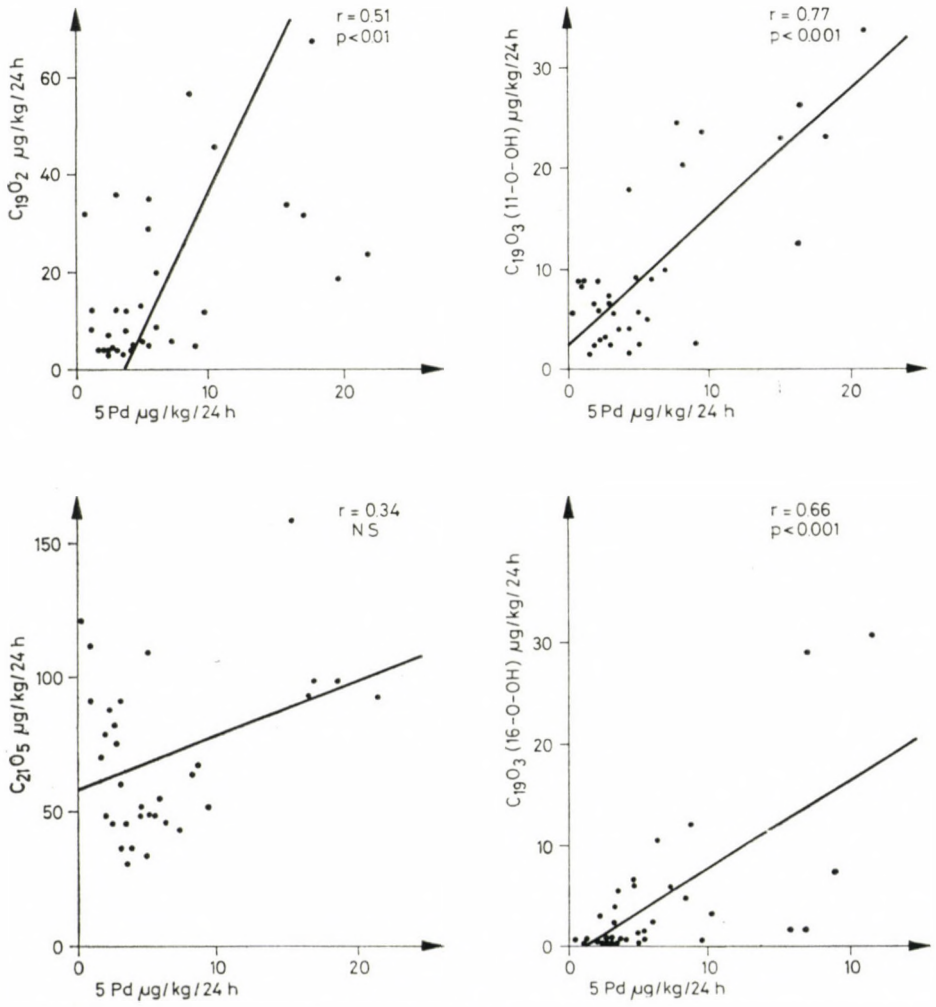

FIG. 6. Correlation between the excretion of C_{19}-steroids, $C_{21}O_5$ corticoids and pregnenediol in 29 girls on 34 occasions. r = correlation coefficient; p = statistical significance of the correlation

corticoids (Fig. 6). The best correlation was found with the 11-oxy-C_{19} and the 16-oxy-C_{19} steroid groups.

The 16-α-hydroxy metabolites of pregnenediol (i.e. 16-α-hydroxy-pregnenolone and 5-pregnene-3-β, 16-α, 20-α-triol) could be demonstrated in 20 out of the 34 cases. Thus, the total amount of the two steroids was between 0.5 and 7.4 μg/kg/day in the age group of 2–6 years (seven cases), 0.8 and 16 μg/kg/day in the age group of 7–9 years (eight cases) and 1.6 and 6.5 μg/kg/day in the age group of 10–14 years (eight cases).

Discussion

To obtain reliable results the amounts of steroids excreted were calculated for kg body weight and for sq.m. body surface. Supposing a physiological development, both procedures of calculation should eliminate any inhomogeneity due to differences in either body weight or body surface. A factor causing inhomogeneity in the 7–9 year age group was the onset of adrenarche the early stage of which, still free of physical signs, was first observed in girls over eight. Four out of the nine members of this group were under eight years at the time of the investigation (Fig. 4). In these girls the daily excretion in μg/kg of $C_{19}O_2$ steroids did not significantly differ from that of the 5–6-year-old children which means that these girls were still before the adrenarche. From the age of eight on, $C_{19}O_2$ steroid excretion surpassed the value of 10 μg/kg/day. This finding can be regarded as the sign of the physically still non-observable adrenarche. $C_{19}O_2$ steroid values between 10 and 18 μg/kg/day are regarded as prepubertal-type androgen excretion. Gonadarche and adrenarche do not always run parallel (18, 19); this was seen in two pubertal girls between 10 and 11 years of age (Fig 4) who on the basis of breast development were classified into the second stage of puberty. Their $C_{19}O_2$ steroid excretion was 7.6 and 14 μg/kg/day, respectively, and, on the basis of the absence of pubic hair they ought to have belonged to stage one.

Our observations on the onset of adrenarche are in agreement with both the classical findings based on investigation of 17-ketosteroids (7, 20) and the most recent results obtained by DHA and DHA-S determination (1, 6, 13, 15, 18, 19).

The augmentation of 11-oxy-C_{19} steroids is less marked than that of the $C_{19}O_2$ steroids. This difference, increasing with the progression of puberty, had previously been expressed by the ratio of 11-deoxy-17-KS to 11-oxy-17-KS, a value obtained during the evaluation of 17-ketosteroid fractionation. The value is 0.7–0.8 in childhood and increases in girls to 1.8–2.7 by the end of puberty (8, 23).

In contrast with data in the literature we observed an increased $C_{21}O_5$ steroid excretion during puberty when the values were calculated on body weight basis. This increase seemed

to be transient if the girls were divided into puberty subgroups. In view of the few cases studied, no final conclusion could be drawn from this observation.

There are few data on 16-hydroxy steroid excretion during childhood. Its excretion, though displaying a tendency to increase, did not significantly differ in the various age groups.

The increase of pregnenediol excretion was near the significance level between the age groups of 2–6 and 7–9 years; it became statistically significant during puberty. Here, however, the pregnenediol originating from the ovaries should also be taken into account. Most probably, the increase in pregnenediol excretion would have been significant in the prepubertal group, too, had the lower limit been set at the eighth instead of the seventh year of life.

ACTH is known to stimulate steroidogenesis by acting on cholesterol-pregnenolone transformation. Investigation of the correlation between pregnenediol and other steroids revealed a positive correlation between the elevation of the pregnenediol level and that of all other steroids except $C_{21}O_5$ corticoids. Pregnenediol is the immediate metabolite of pregnenolone; thus, the elevation in pregnenediol excretion allows the conclusion that the pregnenolone pool is increased also in the adrenals. We do not, however, regard this increased pregnenolone pool as a decisive factor in the augmentation of $C_{19}O_2$ steroids, for the correlation with pregnenediol excretion is lower than that of the slightly increasing 11-oxy-C_{19} steroids.

A prominent sign of steroidogenesis during puberty is the great increase in urinary $C_{19}O_2$ steroid excretion, an event causing the childhood cortisol-androgen dissociation to disappear. The augmentation in $C_{19}O_2$ secretion indicates the opening of a new way of steroidogenesis. This event can be brought into connection with an intraglandular factor, i.e. with the development of the zona reticularis.

The significance of intraglandular factors was pointed out also by Dhom (5) who observed in a large obduction material obtained from unexpectedly died subjects that the first signs of the formation of a continuous zona reticularis appeared in girls as early as at 6–8-years of age, even though full development of the zone was reached after the 13th year of life.

A zona reticularis focal in character was found in half of the subjects at the age of five; an even lower incidence could be observed in children under five. Other authors also regard the zona reticularis as rudimentary between the 3rd and 10th years of life; it reaches full development during puberty (4).

The role of extraglandular factors (FSH, LH, STH and oestrogens) in the activation of adrenal androgens during puberty was investigated by several authors (16). It was found that the above factors do not affect the adrenal. On the other hand, bovine pituitary extract possessed an androgen activating effect higher than that expected on the basis of the ACTH

content. Parker (16) ascribed this effect to an unknown pituitary factor. The existence of an "androgen activating hormone" acting together with ACTH was suggested also by other investigators (19) in connection with adrenarche.

On the other hand, the possibility of an intraglandular inhibition of adrenal androgen steroidogenesis has scarcely been dealt with, even though the existence of such a functional mechanism may be assumed. C_{21}-hydroxylase-deficient patients on corticoid substitution excrete C_{19}-steroids in amounts which is several-fold the physiological level, a fact indicating that the reticular zone of the hyperplastic adrenal cortex is active. In spite of this, there is no elevation in the excretion of $C_{19}O_2$ (otherwise characteristic of puberty) before the sixth year of life, nor are any physical signs of puberty present (11). Probably both phenomena are due to the inactivating effect of the relatively more intensive 16-α- and 11-β-hydroxylations. Another observation (19) has, in accordance with this assumption, shown that an early sexual development starting before the age of six is not accompanied by a change in the serum DHA–S level that would result in values differing significantly from the physiological ones.

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Skin haemangioma: Treatment by cryosurgery

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Cryosurgery has been applied for the treatment of planotuberous haemangiomas. In 270 patients, the procedure proved entirely successful and no complications were observed in any of the cases. The use of cryosurgery is recommended for the treatment of planotuberous lesions of the head and neck.

Haemangiomas are tumours of the blood vessels. The blood vessels proliferate by producing a vascular network separated from the surroundings. The majority of vascular tumours manifest at birth or during the first months of life. Their development is attributed to histogenetic disorders and they are mostly observed at the closure lines of fetal clefts, the majority in the innervation area of the fifth cranial nerve, but they may appear all over the body. In practice, the clinical classification of Schnyder (7) has been generally accepted.

1A. Haemangioma plano-tuberosum (strawberry mark)

1B. Haemangioma tubero-nodosum (cavernosum)

2. Angioma senile (De Morgan spot)

3. Haemangioma-pericytoma

4. Multiple haemangiomatosis (infantile)

5. Blue rubber bleb naevus

6. Haemangioma associated with different syndromes (Kasabach–Merritt syndrome, Mafucci–Kast syndrome, Sturge–Weber syndrome).

In our material, all these forms have been observed but the present paper is devoted only to the most common of the vascular naevi, the plano-tuberous haemangioma.

Plano-tuberous haemangioma or strawberry mark is a red or purplish-blue sharply demarcated tumour of the skin or the mucosa (Fig. 1). It is composed histologically of tightly adjacent capillaries filled with blood and divided into islets by interstitial septa. As to their localization, more than 50% occur in the head and neck regions (7). In a material of 1446 patients with haemangioma the tumour was on the skin or the mucous membranes of the head and neck in 55% of the cases. In our material, 65% of the strawberry marks were on the head, the face or the mucous membranes of these regions.

The growth pattern of plano-tuberous haemangiomas during the first 6 to 8 months of life is variable. After that time, growth usually ceases, the capillaries in the tumour become fibrotic, the tumour shrinks and in 80–90% of the cases disappears by the age of 10 years.

Therapy

There is no generally accepted treatment of haemangiomas. In evaluating the results of treatment and in weighing the indications for it, many authors are of the opinion that one should wait and see whether or not the tumour will tend to recede or even disappear entirely in the course of years (6). In the case of haemangiomas of the head and neck, this attitude is inacceptable in view of the disfiguration the young child has to tolerate.

Traditional Methods

For long decades the traditional methods of treatment used to be surgery or radiotherapy or their combination.

Surgical therapy of haemangiomas is done by radical extirpation or electrocoagulation, by injection into the tumour of some sclerosing agent, or, in suitable cases, by ligation of the growth. In the case of giant haemangiomas with arterio-venous shunt, surgery is the only solution, although it may present difficulties. Giant haematomas are not amenable to cryotherapy.

Radiotherapy. X-ray irradiation may have a favourable effect on planotuberous haemangiomas. In certain head and neck tumours, however, X-ray therapy is contraindicated due partly to technical and partly to developmental reasons.

Cryotherapy

Cryotherapy denotes an intervention which, by local cooling, results in a controllable destruction of living tissue.

The use of cold in medicine goes back to ancient Egypt (8) where it was applied for local treatment of injuries and inflammations. In modern times, some 60 years ago, dry ice was recommended for the therapy of some skin diseases (10). The year 1961 was a milestone in the history of cryotherapy when an efficient cryosurgical apparatus working with liquid nitrogen was constructed (1, 2). It was first used for stereotactic thalamectomy and then for the destruction of benign and malignant brain tumours. Since then, successful and often spectacular results have been achieved by cryotherapy in diverse fields (4, 5, 9, 11).

The direct effect of cryotherapy consists in destruction and dehydration of cells, in changing the concentration of electrolytes, inhibition of cellular enzymes, and denaturation of lipoproteins (3, 9). In addition, cryotherapy has certain indirect effects such as inducing congestion, stasis, thrombosis and occlusion of vessels, further certain immunological actions such as formation of antitumour antibodies (9).

Cryotherapy offers many advantages over surgical and other methods of tissue destruction. It causes no inconvenience or pain to the patients and, therefore, can be performed without anaesthesia; it can be done







FIG. 1. Haemangioma plano-tuberosum
FIG. 2. Haemangioma on the tip of the nose
FIG. 3. Same patient after treatment

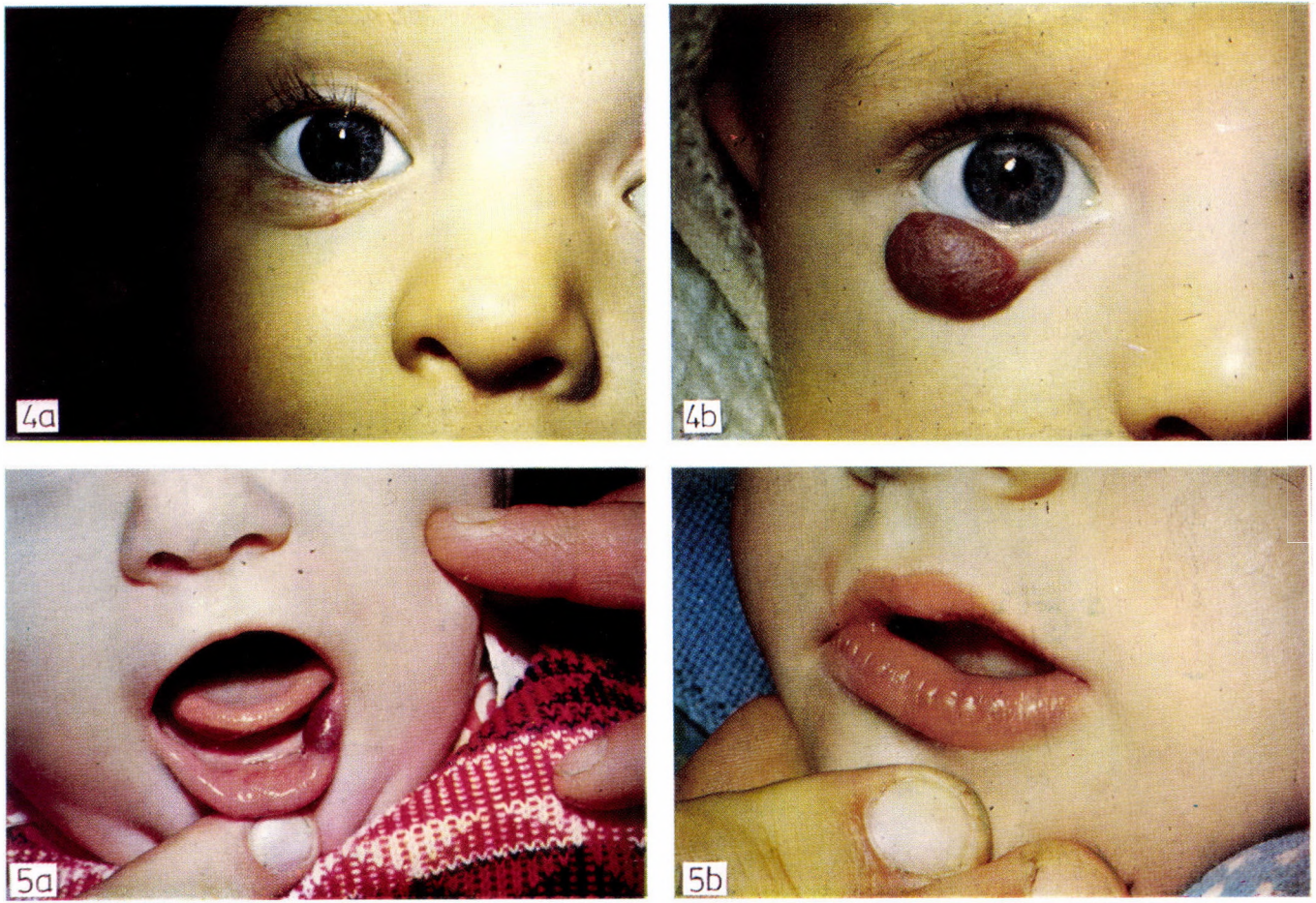

FIG. 4. Haemangioma on the right lower eyelid, and same patient after treatment
FIG. 5. Haemangioma on the lip, and same patient after treatment

on out-patient basis; there is no age limit and there are hardly any complications; the extent of tissue destruction induced is precisely predictable; it can be repeated according to necessity, and the wound caused by it heals without any scar.

Own Results

For treatment, the Cryo-P or the high-power Cryo-Amoils 40A (Erbo, Tuttlingen, GFR) has been used. In our experience, to reach the best results, a cooling rate of 5°C per sec and a final temperature of between −20 and −38°C had to be observed. The treatment was repeated several (4 to 8) times depending on the size and localization of the haemangioma, in intervels of 15 days when the hyperaemia and bullae caused by the intervention had already healed.

The original haemangiomas and the results of the therapeutic procedure are shown in Fig. 2 to 5.

Discussion

As seen from the figures, cryotherapy resulted in an unequivocal and definite recovery, i.e. the total disappearance of haemangiomas. We have not observed any complication in any of our 270 cases. The intervention caused no haematoma or bleeding that is unavoidable with the traditional surgical techniques. From the cosmetic point of view, the result was always fully satisfactory. There were no pathological consequences of the cooling even at those sites (lower and upper eyelids, lips, tip of the nose, nasal meatus, fontanelles) which are inaccessible to any kind of traditional treatment. In our opinion,

Table I

Distribution of 270 patients with haemangioma localization

Child	237 (86%)	Head and neck	192 (81%)
		Other	45 (19%)
Adult	33 (14%)	Head and neck	22 (67%)
		Other	11 (33%)

Table II

Age distribution of 237 children with haemangioma

Months	1	2	3	4	5	6	7	8	9	10	11	12
No. of patients	10	26	30	24	24	25	16	14	10	8	7	8
Years	15	2		3	5							
No. of patients	18	12		4	1							

cryotherapy has great advantages and is more effective in the case of haematomas than in any other treatment applied so far.

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Lymphocyte markers in childhood ALL: The lack of correlation with prognosis

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Presentation blast cells from 39 children with acute lymphoblastic leukaemia were studied for T- or B-lymphocyte characteristics. Eleven patients had blasts with T-cell phenotype and one child had B-cell leukaemia. The rest belonged to the non-T, non-B group. Clinical features were similar in the T-cell and non-T, non-B cell group, including the initial WBC count and the presence of mediastinal mass. Although patients with T-cell leukaemia fared worse than those in the non-T, non-B group, the difference in survival was not statistically significant. These data are not in accordance with the findings of most other groups and are thought to stem from the chance occurrence of relatively low initial WBC counts in the T-cell group.

Twenty years ago childhood leukaemia almost invariably had a fatal outcome. With the introduction of effective cytostatic drugs an increasing proportion of patients achieved full remission. Combination chemotherapy and preventive central nervous system irradiation have led to the promising current situation, which means that 90–95% of the children achieve complete remission. Although 30–40% relapse while still on maintenance therapy, 50–60% of the patients continue in first remission. Some of the best centres report on a 50–65% 5 year relapse-free survival in their ALL patients.

Improving therapeutic results, however, impose the question why some patients relapse early in the course of their disease. The recognition of initial prognostic factors has been a focal point of interest during the past decade. The list of factors known to be associated with a poor clinical response is quite considerable. Some of these prognostic features are listed in Table I.

As the great majority of childhood leukaemias is of lymphoid origin, the introduction of lymphocyte markers in the 1970-s was almost instantly followed by marker studies in ALL. Leukaemic blasts showing T-lymphocyte features characterized a subset of the leukaemias (1). It was recognized early that patients with T-cell leukaemia tend to have

Abbreviations

ALL: acute lymphoblastic leukaemia
WBC: white blood cell
CNS: central nervous system

TABLE I

Factors indicating poor prognosis in ALL

High WBC count (over 50 G/l)
Massive organomegaly
Mediastinal mass
Initial CNS involvement
Male sex
Age: less than 2 years
over 12 years?
T-cell character?
B-cell character
Normal initial Hgb
Black race

a high initial WBC count, mediastinal mass, hepatosplenomegaly and are more often boys. T-cell ALL constitutes some 20% of the acute lymphoid leukaemias.

ALL blasts sometimes derive from the B-lymphocyte axis. These cells carry membrane immunoglobulins, and often present with a characteristic morphologic picture called Burkitt-cell leukaemia (3).

Recently other membrane markers have been introduced in the diagnosis of childhood leukaemia. Some of these define T- or B-lymphocyte subclasses and thus indicate the level of maturation at which the leukaemic cells are 'frozen'. Others demonstrate early fetal antigens that, by virtue of their expression on certain leukaemias, point to the haemopoietic origin of the leukaemic process.

In this paper we analyse the clinical data of 39 children with ALL, whose blasts at presentation were examined for T- and B-lymphocyte markers. Contrary to most literary data we failed to demonstrate a truly significant difference between the patients with T-cell, and those with non-T, non-B ALL.

PATIENTS

Thirty-nine children with previously untreated ALL were analysed. They were diagnosed between 1975 and 1980, and were followed up for 9 to 57 months at the time of analysis. Diagnosis was based on bone-marrow examinations. Smears were stained with May–Grunwald–Giemsa. The FAB criteria were used when establishing the morphological diagnosis. PAS and Sudan-black cytochemical reactions were used routinely and if necessary, complemented by non-specific esterase reactions.

ALL patients had chest X-rays at the time of admission. Hepato-splenomegaly was considered severe, when one of these organs was palpable below the umbilical line. Patients were treated according to uniform protocols. For remission induction, vincristine, daunorubicin and prednisolone were used and in about half of the cases asparaginase. CNS prophylaxis consisted of cranial irradiation and intrathecal methotrexate. 6-mercaptopurine and methotrexate were used for maintenance

chemotherapy. Treatment was continued for a total of 3 years and stopped thereafter, provided that the child was still in full remission.

Methods

Peripheral blood mononuclear cells were separated by simple or density gradient centrifugation (2). T-cells were counted on the basis of their capacity to form E-rosettes as described by Jondal et al. (7). B-cells were identified by immunofluorescence using polyvalent rabbit anti-human Ig antibody. Cells were pre-incubated at 37 °C for 30 min prior to staining in order to eliminate cytophilic immunoglobulins (12). In each technique, 200 cells were counted. In some cases anti-T and anti-B lymphocyte sera were used for checking the results obtained with the above methods. The single case with B-type blasts was analysed in more detail as to the nature of the immunoglobulin on the cells' surface.

A patient was considered to have T- or B-cell disease if 30% or more of the blasts were E-rosette or Ig positive, otherwise the case was classified as non-T, non-B.

Remission and survival data of the patients was analysed using the logrank test (10).

Results

Surface marker studies indicated a T-cell origin in 11 of the 39 newly diagnosed ALL patients (28%). One child presented with immunoglobulin-bearing blasts, while the rest of the patients (69%) had non-T, non-B ALL. Morphological analysis of the presentation bone-marrow smears showed a typical 'Burkitt-like' picture (L_3) in the child with B-cell disease. All the other patients had L_1 or L_2 morphology and there was no difference in the distribution of L_1-s and L_2-s between the T-cell and the non-T, non-B group.

Table II

Initial clinical features in childhood ALL

	Non-T Non-B ALL	T-ALL	B-ALL
No. of patients	27	11	1
Median age, years	5.0	5.5	12.5
Male/female ratio	1.3:1	10:1	Male
Hepatomegaly	13	7	0
Splenomegaly	15	7	0
Lymphadenopathy	13	8	1
Mediastinal mass	1	1	0
Initial WBC count $\leq$ 50 G/l	24	8	1
$>$ 50 G/l	3	3	0
Initial Hb $\leq$ 4.0 mmol/l	14	4	
$\geq$ 4.0 mmol/l	13	7	1

Initial clinical features of the patients are shown in Table II. Median age and the extent of organomegaly were approximately the same in the T and non-T groups. In contrast with data in the literature, the initial WBC count was elevated only in a quarter of the patients with T-ALL. The ratio of boys to girls, however, was much higher in this group.

All patients in the T-cell group achieved remission, while one child in the non-T, non-B group, and the child with B-ALL failed to remit. Analysis of the response to therapy was carried out using the life-table method and logrank analysis. Although patients with T-ALL had a more steeply declining survival curve, the difference between the two groups was not significant (Fig. 1). Analysis of the remission curves (Fig. 2) showed the T-cell group to have inferior results but the difference — at the time of analysis — was not significant statistically.

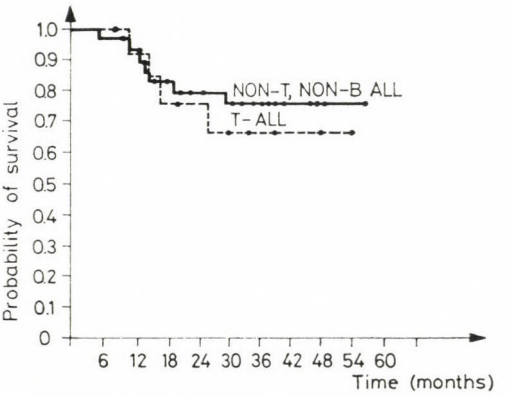

FIG. 1. Survival in ALL, according to cell-surface characteristics. No. of patients: Non-T, non-B ALL: 27, T-ALL: 11, $\chi^2 = 0.28$, $P > 0.1$

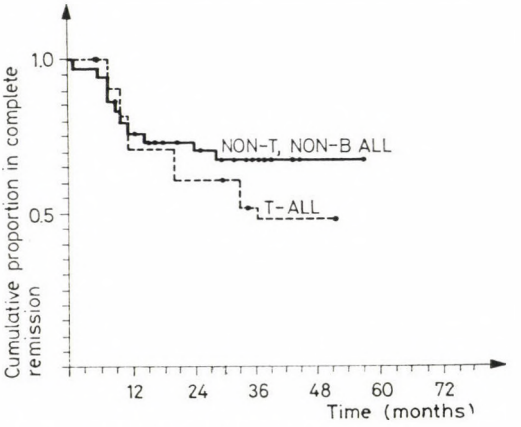

FIG. 2. Cumulative complete remission in ALL according to cell surface characteristics. No. of patients: Non-T, non-B ALL: 27, T-ALL: 11, $\chi^2 = 0.785$, $P > 0.1$

TABLE III

Rate and site of relapse in ALL

	Non-T, non-B ALL	T-ALL
No. of patients	27	11
Site of first relapse:		
Bone marrow	6	3
Meninges	2	2
Testes	—	2
Combined	1	—

The patient with B-cell ALL did not achieve remission and died while still in relapse after 2 months of treatment.

The relapse rate seemed to be higher in the T-cell group (Table III) and although 2nd and 3rd remissions could be achieved, they were short-lived.

DISCUSSION

The rapid development of immunological methods during the past decade expanded the field where they can be employed for the detection of pathological processes. Lymphoid leukaemias were among the first malignancies to be characterized by immunological approaches. The analysis of cell surface antigens and other biochemical characteristics provides a phenotypic pattern unique for the given cell proliferation (4). T and B-cell markers allowed the identification of the target cell of the leukaemic process in 20–25% of the childhood leukaemia cases.

In our material 28% of the patients presented with T-cell leukaemia, i.e. more than 30% of the blasts formed E-rosettes and stained positively with anti-T-cell serum. These patients were reported to have a worse prognosis with high initial WBC count, expressed organomegaly and mediastinal mass. We found the same tendency but have failed to demonstrate a significant difference in these factors between the T-cell group and that of the non-T, non-B variety (Table II). Analysis of their remission and survival curves showed the T-cell group to fare worse, but the difference at present is not significant statistically. Recently it has been suggested that the worse prognosis in the T-cell group is not linked to the cells' surface phenotype but simply to the higher initial WBC in this group (9). If patients with T-cell leukaemia are analysed according to their initial leukocyte count, those with lower counts fare equal to the non-T, non-B group. The numbers in our series do not allow for such an analysis, especially since

only 3 of the 11 patients with T-cell disease had WBC counts over 50 G/1. This seems to be the reason why we failed to show a statistically significant difference between the two groups at the time of analysis. The long-term results, however, are most likely to be inferior in the T-cell group, since these patients had more relapses than those in the other group (Table II).

The single patient with B-cell leukaemia was found to have blasts which all carried a monoclonal immunoglobulin on their surface. After trypsinization nearly all the cells resynthetized the antibody molecules. This patient did not achieve remission and in spite of intensive chemotherapy died 2 months after the diagnosis. B-cell leukaemia seems to be a particularly aggressive proliferation and its treatment remains completely unsolved (8).

Lymphoid leukaemias can further be characterized by the use of xenogeneic antisera directed against 'leukaemia-associated antigens'. Greaves et al. (5) were the first to demonstrate the presence of the so-called common-ALL antigen (cALLa) on the majority of ALL cells. This antigen, however, may very well turn out to be an early differentiation antigen, normally found on early haemoprecursors. Nevertheless, this antibody proved to be an operationally most useful reagent not only in differential diagnosis but also in throwing light on the association of various leukaemias (6).

The latest development in immunodiagnosis employs a wide variety of monoclonal antibodies. Reinherz and Schlossman (11) produced a series of anti-T cell antibodies that characterize T-cells in different stages of development and an antibody seemingly detecting the same cALL antigen as Greaves' xenogeneic antisera (12). These antibodies can be produced in bulk which makes them more accessible for diagnostic purposes and should provide valuable information in the next few years concerning the origin and development of various leukaemias.

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The cerebrospinal fluid of newborn infants with perinatal hypoxia

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Concentration of glucose, protein and amino acids in plasma and CSF was studied in 22 newborn infants suffering from perinatal hypoxia of various severity. A wide range of individual values characterized all parameters both in plasma and CSF, but a statistically significant correlation was found between gestational age and CSF protein, gestational age and CSF/plasma protein ratio, CSF and plasma protein and glucose concentration, and CSF and the plasma amino acid level. The possible explanation and clinical implications of the findings are discussed.

Despite extensive studies, the function and role of cerebrospinal fluid (CSF) are not quite clear. It seems that in addition to providing protection against mechanical trauma, it serves as a source of metabolites and various nutrients for the CNS (8, 17).

Under normal conditions, the composition of CSF is regulated and kept fairly unchanged by subtly coordinated mechanisms. The concentration of various substances in CSF depends on the rate of their penetration from blood and brain tissue, on CSF utilization by the surrounding nervous tissue, and on clearance mechanisms. Depending on the substance, one, two or perhaps all three mechanisms are operating. If any one is disturbed, remarkable distortions in CSF composition result, which may be useful in diagnostics and prognostics.

When studying pathological changes in CSF, the reliable knowledge of normal values is a fundamental requirement. Unfortunately, this is the main difficulty in CSF studies performed on newborn infants, since normal values are, in general ill-defined. For this reason we have examined the relationships between plasma and CSF glucose, protein and amino acids in newborn infants suffering from perinatal hypoxia. The question, particularly in context with amino acids, is justified by the increasing use of various regimes of parenteral nutrition for newborn infants with increased metabolic vulnerability of their damaged central nervous system and blood-brain-CSF barrier.

Patients and Methods

Twenty-two newborn infants were studied. All of them had an Apgar score <6 at 5 min and needed resuscitation at birth.

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TABLE I

Clinical data of the 22 newborn infants studied

	Mean	±S.D.	Range
Birth weight, g	2544	214	750–4150
Gestational age, weeks	35.6	1.1	26– 42
Postnatal age, hours	18.0	3.5	1– 69

As shown in Table I, mean (±S.E.) birth weight, gestational age and postnatal age of the infants were 2544±214 g, 35.6±1.1 weeks, and 18.0±3.5 hours, respectively, with a wide range of individual values in all three parameters.

Eleven of the 22 infants were preterm (gestational age $\leq$ 37 weeks), the rest were full-term (gestational age $>$ 37 weeks). Four of the 8 female and 4 of the 14 male newborn infants died, all with hyaline membrane disease associated in 5 cases with subependymal or intraventricular haemorrhage. All patients needed oxygen therapy via head-box or by CPAP or IPPV ventilation and 5–10% glucose + 4.2% bicarbonate drip infusion, right from the time of their admission to the SCBU, and were on this therapy at the time of the collection of specimens for study.

From all patients an umbilical venous blood sample was taken for measuring plasma glucose and protein concentration and performing amino acid analysis. Simultaneously, a lumbar tap was performed in order to detect or exclude intracranial haemorrhage. CSF samples were analysed for glucose, protein and amino acids, furthermore for routine laboratory tests, culturing included. In case of blood stained CSF, the specimen was centrifuged immediately and the supernatant was used for further analysis. If the possibility of inflammation could not be excluded with certainty, the patient was excluded from the study.

Plasma glucose and CSF glucose concentration was measured by the o-toluidine method. CSF and plasma protein level was determined with turbidimetry (32) and the biuret reaction, respectively.

Blood specimens for amino acid analysis were collected in heparinized tubes and promptly centrifuged. Plasma was deproteinized by addition of four volumes of 5% sulphosalicylic acid. Protein-free supernatant corresponding to 0.5 ml plasma was immediately frozen and stored at −20 °C until assayed. Amino acid measurement was performed by an automatic Beckman Multichrom Liquid Column Chromatograph, using norleucine as an internal reference standard.

Regression equations were calculated by the method of least squares.

Results

Table II shows the mean (±S.E.) CSF and plasma concentration of total protein and glucose in the 22 subjects, furthermore the CSF/plasma ratios calculated for both substances. A wide range of individual values characterized all parameters measured, in both plasma and CSF. On further analysis, however, a statistically significant negative correlation was found between the following parameter-pairs: gestational age–CSF protein ($r = -0.5594$, $p < 0.01$), gestational age–CSF/plasma protein ratio ($r = -0.6314$, $p < 0.01$), birth weight–CSF protein ($r = -0.6218$, $p < 0.01$), birth weight–CSF/plasma protein ratio ($r = -0.6519$, $p < 0.001$). On the other hand, no remarkable relation could be observed between either CSF glucose or CSF/plasma glucose ratio and the gestational age or birth weight of the newborn infants. A highly significant reverse correlation was detected between plasma and CSF protein level ($r = -0.6529$, $p < 0.001$), whereas a positive relation was found between plasma and CSF glucose concentration ($r = 0.8970$, $p < 0.001$). A significant direct relation was observed between gestational age and plasma protein concentration ($r = 0.6319$, $p < 0.01$), but not between postnatal age and CSF protein or glucose level.

The mean (±S.E.) plasma concentrations of the free amino acids measured are shown in Fig. 1. It is seen that the plasma level of all individual amino acids measured in the hypoxic newborn infants, were closely similar to that of normal full term and preterm infants (19, 29). Figure 2 shows CSF amino acid concentration of 11 newborn infants with perinatal hypoxia, compared with that of control subjects. It has, however, to be emphasized, that in lack of normal values for CSF amino acid concentration in healthy newborn infants, values of normal subjects of various ages published in

Table II

Plasma and CSF concentration and CSF/plasma concentration ratio of protein and glucose in the 22 newborn infants studied

	Mean	±S.E.	Range
Protein			
plasma, g/l	46.6	1.9	25.6–62.0
CSF, mg/l	1829	396	350–7600
CSF/plasma ratio	0.048	0.014	0.006–0.271
Glucose			
plasma, μmol/l	4.4	0.6	0.7–12.7
CSF, μmol/l	2.9	0.3	0.3– 6.7
CSF/plasma ratio	0.60	0.04	0.2– 1.0

the literature (9, 12, 18, 24, 27) have been used for comparison. Figure 1. shows clearly the great variation found in CSF concentrations of individual amino acids in hypoxic newborn infants. It is seen in Fig. 2 and in more detail in Table III that in hypoxic babies the CSF concentration

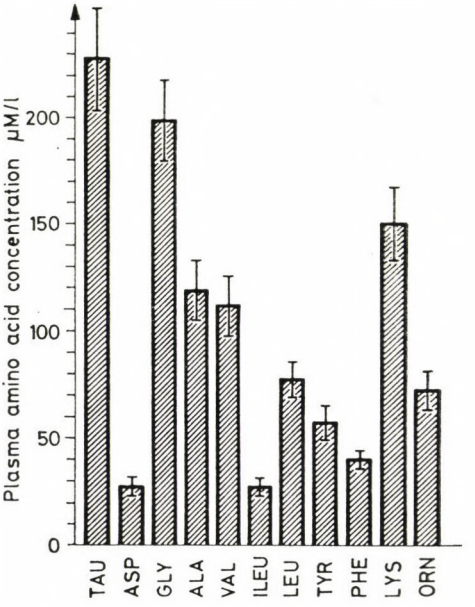

FIG. 1. Plasma concentration of individual amino acids in newborn infants with perinatal hypoxia (mean ± S.E.)

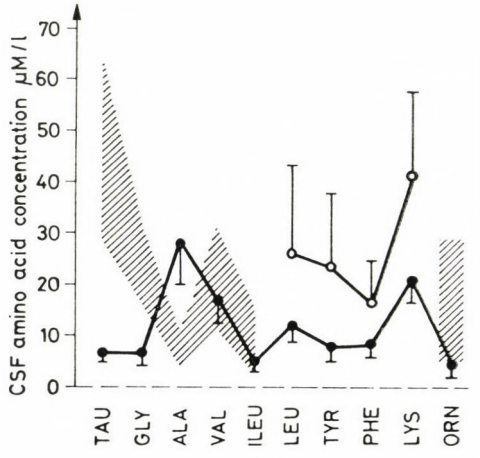

FIG. 2. CSF amino acid concentration in newborn infants with perinatal hypoxia and in control subjects (mean ± S.D.). Empty circles, mean values of newborn infants; shaded area, range of values in newborn infants; full circles, mean of summarized control values from references Nos 9,12,18,24,27

TABLE III

Concentration of some amino acids in CSF, mean ± S.D. μmol/l

	TAU	GLY	ALA	VAL	ILEU
I	7.6±2.1	5.9±1.8	34.8±8.2	19.9±4.1	6.2±1.4
II	6.8±1.7	4.7±1.5	23.2±5.1	15.0±2.8	3.9±1.0
III	6.4±1.5	5.8±0.9	32.7±6.3	20.9±4.8	5.3±1.5
IV	6.3±1.8	6.6±1.8	23.2±9.4	14.6±5.5	4.4±1.3
V	5.3±1.4	8.5±2.5	27.9±9.9	14.3±4.0	5.0±0.9
VI	29.2–63.5	16.5–31.3	3.8–11.2	10.8–30.7	2.5–15.2
	(r)	(r)	(r)	(r)	(r)

	LEU	TYR	PHE	LYS	ORN
I	14.8±3.9	9.5±2.6	9.9±2.0	20.8±4.0	3.8±0.9
II	10.1±2.1	6.4±1.5	6.5±1.2	21.7±3.7	3.7±1.0
III	14.9±3.3	9.0±2.4	9.5±2.1	29.1±4.8	4.9±1.8
IV	10.9±3.6	9.1±5.0	9.2±5.8	18.7±6.6	5.7±1.8
V	11.6±2.4	7.9±2.3	7.5±2.2	18.6±6.4	5.7±1.8
VI	26.1±18.3	24.1±14.5	17.0±8.2	41.8±18.7	5.1–29.3
					(r)

I = McGale et al [18]
II = Gjessing et al [12]
III = Perry et al [24]
IV = Dickinson et al [9]
V = van Sande et al [27]
VI = Present study
r = range

of taurine, glycine, leucine, tyrosine, phenylalanine and lysine was much higher than in control subjects. Figure 3 demonstrates the remarkable variations of CSF/plasma amino acid ratios, concerning each amino acid measured. It should be noted that the greater the number of calculated ratios, the wider was the range of values.

Statistical analysis revealed no significant correlation between CSF/plasma amino acid ratio and gestational age, birth weight, postnatal age, furthermore the CSF/plasma ratio of glucose and protein. At the same time, a highly significant ($r = 0.5414$, $p < 0.001$) direct relation was observed between the plasma and CSF concentration of all individual amino acids measured.

DISCUSSION

The composition of CSF is kept more stable than that of plasma (2–5, 8, 15, 20, 26). Pathological distor-

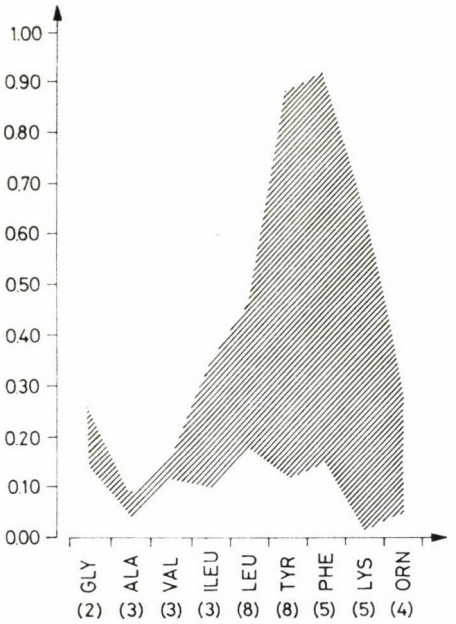

FIG. 3. CSF/plasma amino acid ratios in newborn infants with perinatal hypoxia (ranges). Figures in parentheses indicate the number of values

tions in the composition of CSF may result from changes in the permeability of the blood-brain-CSF barrier, or the metabolism of brain tissue and the clearance of various substances from CSF. All three mechanisms are certainly maturity-dependent, but may be easily damaged in newborn infants suffering from perinatal hypoxia. Consequently, studies on the composition of CSF of pathological newborn infants may provide useful information for the clinician in the diagnosis, treatment and prognostication of ill newborn babies.

Despite the relatively small number of studies on cerebrospinal fluid of normal and pathological newborn infants, it seems to be firmly established that CSF protein concentration is remarkably higher in preterm and term newborn infants than in later life (1, 10, 14, 20, 23). The high protein content of CSF may result from intracranial haemorrhage or from the increased permeability of the blood-brain-CSF barrier, but the real cause is still disputed. We found a significant negative correlation between gestational age, birth weight and CSF protein level and CSF/plasma protein ratio. The rise in CSF protein level in the newborn infants must be independent of the serum protein concentration, since a strong reverse correlation was observed between the two parameters. The finding that plasma protein concentration correlated directly with gestational age suggests that both plasma and CSF protein concentration is the function of maturity, but in an inverse way. We conclude therefore that the higher protein concentration in

the CSF of preterm infants is probably due to the increased permeability of the barrier and has nothing to do with plasma protein concentration. Since the correlations could be found after perinatal hypoxia of various severity, the CSF protein concentration is probably much more dependent on the functional maturity of the newborn infant than on the hypoxic insult suffered perinatally.

CSF and plasma concentration of glucose has been known to be lower in full term and even more so in preterm newborn infants than in older infants or children (10, 21, 23, 28). In view of the uncertainty in the diagnostic evaluation of a low CSF glucose level in newborn infants, simultaneous measurement of blood glucose and calculation of CSF/plasma glucose ratio has been proposed (10, 21). This is especially important in pathological newborn infants since bidirectional blood glucose and secondary CSF glucose alterations may occur due to the pathologic changes but also to the therapeutic procedures. The CSF and plasma glucose levels of our patients corresponded well to the normal values reported by others. In newborn infants with perinatal hypoxia we found the CSF/plasma glucose ratio to be similar to that observed by Sarff et al. (28) and Monaco et al. (21). No relation could be detected between gestational age, birth weight, postnatal age and CSF glucose level or the CSF/plasma glucose ratio. Still, no conclusion can be drawn as to the spontaneous connections and interrelationships between the two parameters as all patients received glucose drip infusion before and during the time of sample collection. Nevertheless, the fact that no significant correlation was found between gestational age and actual CSF/plasma glucose ratio, furthermore that a highly significant positive correlation was observed between plasma and CSF glucose concentration, deserves some consideration. It is not unrealistic to suggest on the basis of these observations that the blood-brain-CSF barrier for glucose is either not maturity-dependent, or that the permeability for glucose increases remarkably due to the hypoxic insult and the CSF glucose level becomes dependent on the blood glucose concentration. If this conclusion is correct, then apart from other reasons, special care must be taken in order to avoid iatrogenic hyperglycaemia in newborn infants with perinatal hypoxia, since the effects of a secondary rise of the CSF glucose concentration and CSF hyperosmolality are unknown at that time but may well be dangerous.

An increasing body of knowledge has been accumulated on the amino acid concentration of CSF (6, 7, 9, 11–13, 16–18, 24, 25, 27, 31) in children and adults with various pathological conditions, but data are lacking for the human newborn. It is known that the concentration of most amino acids in the CSF is usually lower than their equivalent levels in plasma, but remarkable inter-individual variations character-

ize the CSF/plasma concentration ratios of different individual amino acids. It has also been pointed out that a raised CSF amino acid concentration or CSF/plasma amino acid ratio may reflect a disturbed cerebral metabolism, but may also be due to damage to the blood-brain-CSF barrier or the amino acid clearance mechanism. The concentration of the 10 amino acids measured in the CSF was raised in the 11 newborn infants with perinatal hypoxia (Fig. 2 and Table III) in comparison with the controls. In evaluating this observation, care must be taken because of the small number of samples and the wide range of individual values. Furthermore, the question remains unanswered whether or not the increased CSF amino acid concentration was related with gestational or postnatal age, or with the perinatal hypoxia suffered by the infants.

Similarly to other authors (18) we observed a wide range of CSF/ /plasma amino acid ratios and of each individual amino acid (Fig. 3). If the postulation is accepted that the CSF/plasma ratio reflects amino acid transport into and out of CSF directly or indirectly from or to plasma and brain tissue, then this transport is not related to gestational age in hypoxic newborn infants.

Settergren et al. (30) studied 12 infants and 7 children aged 5 months and 12 years, respectively, and found a significant correlation between the arterial concentration and the cerebral exchange of 7 individual amino acids. McGale et al. (18) observed a direct relationship between venous plasma and CSF concentration of 13 individual amino acids in normal adult humans. Our finding of a significant correlation between the plasma and CSF concentration of amino acids in newborn infants with perinatal hypoxia might have some practical clinical implications. The close relationship of plasma and CSF amino acid level indicates an unrestricted transport, and so a non-physiological rise in plasma amino acid concentration may well result in abnormal amino acid accumulation in the brain and CSF. Considering the fact that birth asphyxia (29) and uncontrolled total or partial parenteral nutrition both affect the plasma amino acid pool, secondary adverse effects on the CNS might be anticipated; the more so as many amino acids are known to have toxic effects on the brain if given in excess, while others are transmitter substances and have neuroexcitatory or inhibitory effects on synaptic transmission (31).

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Thyrotropin and prolactin response to ambient temperature in newborn infants

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Plasma TSH and PRL level was measured by RIA in 8 full-term newborn infants with mean birth weight of 3420 g (range 3000–4100 g) and mean gestational age of 39.0 weeks (range 38–40 weeks) at postnatal age of 3–4 days. Determinations were performed at neutral thermal environment (32°C) and after 1 h exposure to cold (28°C) and warm (37°C) environment.

In response to cold challenge, TSH and PRL increased significantly from 4.17 ± 0.28 mU/l and $4625 \pm$mU/l to 4.76 ± 0.21 mU/l ($p < 0.01$) and 5975 ± 944 mU/l ($p < 0.05$), respectively. When the newborns were placed in warm environment, both TSH and PRL fell significantly to 3.52 ± 0.4 mU/l ($p < 0.05$) and 3318 ± 759 mU/l ($p < 0.05$), respectively.

A weakly significant positive correlation was found between TSH and PRL levels suggesting that thermal stress in the newborn results in similar alterations in TSH and PRL, presumably through the same central mechanism.

Activity of the hypothalamic-pituitary-thyroid axis has been reported to vary considerably with changes in ambient temperature (6). Based on the observation that acute exposure to cold resulted in an increase of TSH and thyroxine (T_4) levels, the initial increase in TSH and the subsequent increase in T_4 observed immediately after birth has been attributed to the cooling effect of the extrauterine environment (1, 6, 7). It has also been claimed that the cold induced rise of TSH is due to the higher rate of hypothalamic TRH secretion (17) because TSH stimulation by cold could be suppressed by antiserum to TRH (26).

Furthermore, evidence has been provided to indicate that TRH may be the prolactin releasing factor of the hypothalamus, since TRH releases prolactin (PRL) by a direct action on the pituitary (4, 9, 11) and administration of TRH antiserum suppressed PRL secretion (12).

On the basis of these observations we have assumed that TSH and PRL may be released similarly in response to various ambient temperatures and their release may be mediated by the same central mechanism. To test this hypothesis the present study was designed to investigate plasma TSH and PRL levels in newborn infants kept in neutral thermal environment and then after exposure to acute cold and heat stress.

Material and Methods

The studies were carried out in 8 healthy, fullterm newborn infants with mean birth weight of 3420 g (range 3000–4100 g) and mean gestational age of 39.0 weeks (range 38–40 weeks).

Gestational age was calculated from the mother's menstrual history and was confirmed by physical examination of the infants. All infants were delivered vaginally with a one-minute Apgar score of 7 or more, and they were healthy during the whole perinatal period.

None of the subjects received anticonvulsant therapy and none had a family history of thyroid disorder. Breast milk was fed to the infants and 5% glucose in water added to ensure an adequate fluid intake.

Determinations were performed at the postnatal age of 3–4 days at 3 different ambient temperatures.

First, the unclothed infants were placed in an incubator providing neutral thermal environment (temperature 31–32°C; relative humidity 40–60%). Next, they were kept in a cool (temperature 28°C, relative humidity 40–60%) and, finally, in a warm environment (temperature 37°C, humidity 40–60%). After one hour exposure to each ambient condition, blood was taken from a scalp vein for TSH and PRL determination. Samples were centrifuged immediately, and the plasma was stored at —20°C until analysed. During the test periods, abdominal skin and rectal temperature were recorded continuously.

Informed parental consent was obtained for the study. Plasma TSH was measured by radioimmunoassay using Amersham kits according to the method of Martin and Landon (15). Plasma PRL measurements were made by radioimmunoassay using commercial kits manufactured by Serono. Results were expressed as mean ± SE and analysed using Student's paired *t*-test. When necessary, the correlation coefficient and the equation of regression were also calculated.

Results

Body temperature

At 32°C ambient temperature, rectal and abdominal skin temperatures were 36.77 ± 0.05 and 36.52 ± 0.05°C, respectively. When the newborn infants were placed in incubators of 28°C, their rectal and skin temperature declined to 36.45 ± 0.07°C and 36.21 ± 0.07°C ($p < 0.01$), respectively. At 37°C, however, both rectal and skin temperatures increased significantly to reach mean values of 37.31 ± 0.12 and 36.65 ± 0.10°C ($p < 0.01$). At the end of the exposure to the warm environment visible sweating occurred in 5 out of the 8 infants.

Hormone levels

Figure 1 shows plasma PRL and TSH levels measured at various ambient conditions. It can be seen that in response to cold challenge, TSH and PRL rose simultaneously from 4.17 ± 0.28 mU/l and 4625 ± 589 mU/l to 4.76 ± 0.21 mU/l ($p < 0.01$) and 5975 ± 944 mU/l ($p < 0.01$), respectively. In newborns placed in warm environment, both TSH and PRL fell significantly to the respective values of 3.52 ± 0.40 mU/l ($p < 0.05$) and 3318 ± 759 mU/l ($p < 0.05$). TSH ($p < 0.05$) and PRL ($p < 0.05$) were found to correlate inversely with the rectal temperature (Fig. 2) whereas TSH showed a weakly significant positive correlation with the PRL level ($p < 0.05$) (Fig. 3).

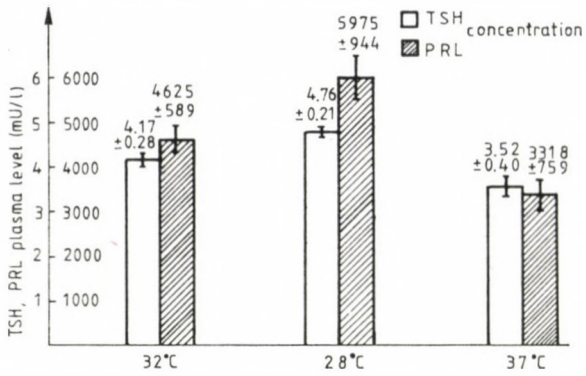

FIG. 1. Plasma TSH and PRL in newborn infants subjected to various ambient conditions

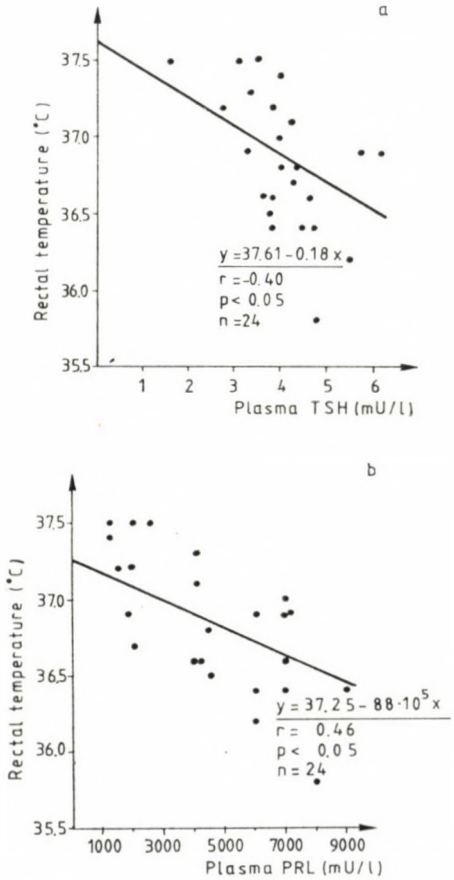

FIG. 2. Rectal temperature and plasma TSH *(a)* and PRL *(b)* in newborn infants subjected to various ambient conditions

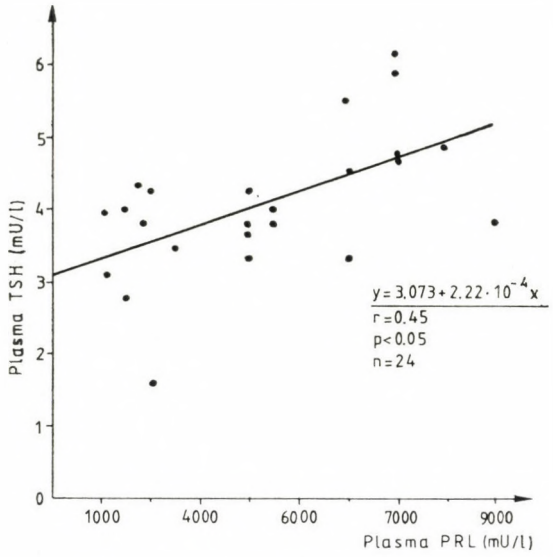

FIG. 3. Plasma TSH and PRL levels in **newborn infants subjected to various** ambient conditions

DISCUSSION

The present results are in good agreement with those previously reported showing that acute exposure of newborn infants to cold results in increased secretion of TSH within 30 min (6, 7, 22). Warm exposure of the newborn infants resulted in a fall of the plasma TSH level, as compared with the value measured in neutral thermal environment.

Few data are available concerning the influence of warm environment on pituitary-thyroid function. It has been suggested that heating produced histological signs of thyroid inactivity in various mammals (3, 8) reduced thyroid ^{131}I uptake (28) and resulted in a fall of the pituitary TSH content (13). O'Malley et al. (19) provided evidence that in patients with primary hypothyroidism heat reduced TSH production, and Sack et al. (21) observed that in newborn lambs delivered into water of 39°C the rise of TSH, T_3 and T_4 level generally seen after normal delivery could be prevented. The reason for the alteration in TSH in response to ambient temperature variation is not clear; changes in the hypothalamic control mechanism have been assumed to be responsible (1, 20).

The physiologic significance of the cold-induced activation of the thyroid is well documented (1, 5, 7, 27). It is involved in the metabolic response to cold of the newborn through the direct effect of thyroid hormones on metabolic heat production, or indirectly by potentiating the catecholamine-mediated non-shivering thermogenesis (14, 25).

Interpretation of the depressed thyroid function in neonates kept in warm environment is not so apparent. The metabolic rate of the newborn infants kept in an environment warmer than neutral is about 20% lower than under neutral conditions (24). This observation lends support to the possibility that the decrease of thyroid function may contribute to the reduction of metabolic heat production when the newborn infants are in a warm environment.

A further important point is that in response to temperature changes the plasma PRL level changed in a way similar to that of TSH (22). This finding is consistent with the observation that TSH and PRL levels increase simultaneously in newborn infants after birth or after TRH administration (4, 22).

The relationship between environmental temperature and the PRL level has not been entirely clarified. Several reports indicate that cold exposure suppresses the circulating PRL level in rats (18) and in cattle (23), but there are no such data available for the human newborn. There is some controversy concerning the effect of warm exposure since it was found that in response to a high ambient temperature plasma PRL either increases (16), or remains unchanged (10).

The clinical significance of our observations needs further studies to define the relationship of ambient temperature to the plasma PRL level in the neonatal period.

In view of the fact that PRL has been shown to enhance renal tubular reabsorption of salt and water it is tempting to speculate that in newborns suffering from neonatal cold injury the high level of cirulating PRL may contribute to œdema formation (2).

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Effect of intravenous alanine on blood glucose, glucagon and growth hormone levels in diabetic children

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To study its glycaemic effect and to evaluate alpha cell function, L-alanine was administered intravenously (0.15 g/kg b.w.) to ketotic diabetic children. Their preinfusion condition was characterized by hyperglycaemia, elevated alanine, glucagon and growth hormone levels. Alanine failed to cause a further increase in the plasma glucagon level and had no glycaemic effect, but induced a consistent rise in plasma growth hormone. It is concluded that, in contrast with adult diabetics, alanine had no glycaemic effect and was not a potent alpha-cell secretagogue in ketotic diabetic children. The demonstrated growth-hormone provoking effect suggests that endogenous hyperalaninaemia may contribute to ketosis via the antiinsulin effect of growth hormone.

Alanine not only serves as a key substrate for gluconeogenesis, but also stimulates the release of the key gluconeogenic hormone, glucagon (7, 12). In diabetic subjects gluconeogenesis is accelerated and alanine carbon is preferentially channelled towards gluconeogenesis (5, 10). In ketotic diabetic children hyperalaninaemia and hyperglucagonaemia have been observed (6). Since alanine is an alpha cell secretagogue, endogenous hyperalaninaemia, apart from its direct glycaemic effect, may contribute to the hyperglucagonaemia and thus to ketosis. It was, therefore, thought to be of interest to determine the effect of L-alanine on glucagon levels in ketotic diabetic children. The effect of alanine on growth hormone, the other important antiinsulin hormone, was also studied.

Materials and Methods

Five diabetic children with frequent or constant ketonuria were chosen for the study. The relevant clinical data are shown in Table I. All subjects had diabetes of long duration with high postabsorptive blood glucose levels (Table II) and probably high mean daily blood glucose as indicated by the high percentage of glycosylated haemoglobin.

The patients were studied in the morning after an overnight (12 h) fast. Insulin was withheld on the morning of the study.

L-alanine was dissolved in sterile distilled water to a 10% solution. The solution was buffered to pH 7.4 and autoclaved in 100 ml dispensing vials. Each batch was

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TABLE I

Clinical data of diabetic children

Subject No.	Age, year	Duration of diabetes, year	Daily insulin dose, U/kg	Weight, kg	Height, cm	Hb_{A1a-c} per cent	Urinary glucose, g/day	Ketonuria
1	16	6	1.4	51	163	15.5	30	*
2	16	9	1.1	59	161	17.9	36	**
3	14	5	1.0	39	150	10.2	40	***
4	16	6	0.9	48	143	14.6	48	***
5	13	9	1.2	40	140	14.0	39	**
Mean	15	7	1.1	47.4	151.4	14.4	38.6	
Range	13–16	5–9	0.9–1.4					

tested for pyrogen and cultured for bacterial contamination before use.

Alanine was administered in a dose of 0.15 g/kg b.w. by infusion in an antecubital vein over a period of 5 min. Venous blood samples were drawn before, and at 10–30 min intervals for 180 min after the completion of the infusion.

Blood glucose was measured by the glucose-oxidase method, plasma glucagon and growth hormone were estimated by radioimmunoassay. Plasma alanine was measured by automatic ion-exchange chromatography using a Beckman Multichrom amino acid analyser.

RESULTS

Fasting blood glucose, growth hormone, glucagon and alanine levels are shown in Table II. As anticipated on the basis of previous data (6),

TABLE II

Blood glucose, plasma glucagon, growth hormone and alanine levels in the basal state

Subject No.	Glucose mM/l	Glucagon pg/ml	Growth hormone pM/l	Alanine μM/l
1	13.7	300	279	479
2	19.4	410	474	610
3	21.2	540	381	648
4	14.3	440	419	459
5	12.2	300	372	—
Mean	16.2	393	385	549
SE	1.75	45.4	32	47
Control (M±SE)	5.2±0.3	102±18	72±34	280±24

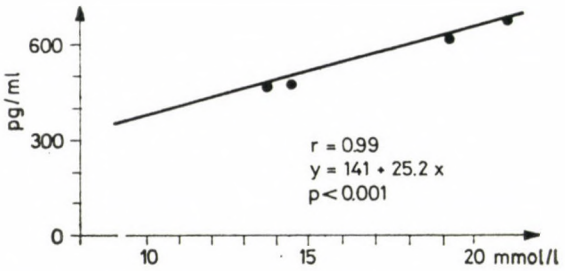

FIG. 1. Relationship between blood glucose and plasma glucagon levels

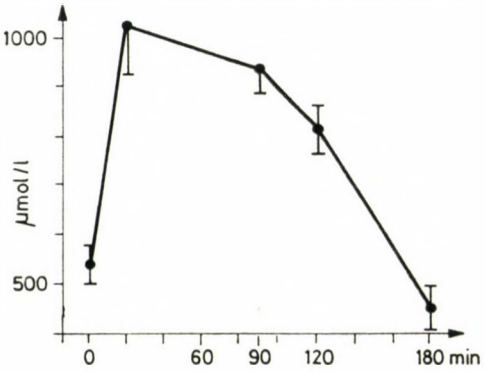

FIG. 2. Plasma alanine level during alanine-load (0.15 g/kg)

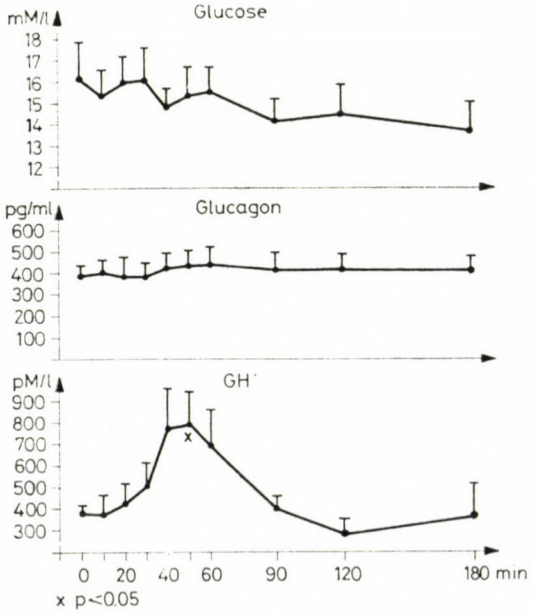

FIG. 3. Blood glucose, plasma glucagon and growth hormone levels during i.v. alanine administration

alanine concentration was increased in the basal state, and glucagon and growth hormone concentrations also increased. There was a positive linear relationship between blood glucose and plasma glucagon (Fig. 1).

Infusion of alanine resulted in very high plasma alanine levels throughout the whole observation period (Fig. 2).

Blood glucose and plasma glucagon levels remained the same, but growth hormone increased under the effect of alanine infusion (Fig. 3).

Discussion

Although the elevation in the branched-chain amino acids in diabetes had been shown in 1953 (9), nearly twenty years had to pass until Felig and Cahill's group employing automatic ion exchange column chromatography described the changes of the individual free amino acids in plasma (3) and in cerebrospinal fluid (1). Recently (8), catheterization techniques were used to study human splanchnic metabolism and gluconeogenesis during ketoacidosis. Adult diabetic patients participated in these studies and to the best of our knowledge there is only one report (6) apart from our observation on the plasma amino acids in diabetic children (10).

Both in adults and in children, ketoacidosis was characterized by hyperaminoacidaemia. This was due mainly to the accumulation of the branched-chain amino acids, but there were two important differences in the aminograms of the two diabetic age groups. A two- to three-fold elevation of leucine, isoleucine and valine was accompanied by a 25 to 40% reduction in the plasma alanine, glycine, threonine and serine levels in the adult diabetics (2, 3). In diabetic children hyperaminoacidaemia was more pronounced. There was a three- to tenfold elevation of the branched-chain amino acids and, in contrast with adults, a 50 to 160% increase in plasma alanine. In the present ketotic, but not ketoacidotic, diabetic children, a more than twofold elevation of plasma alanine was observed. The reason for the difference in the plasma alanine levels of adults and children is not known. Since decreased alanine levels in adult ketoacidosis are generally thought to be due to increased gluconeogenesis (2, 3), this would suggest that gluconeogenic regulatory factors may be different in children and adults. It is also possible that the increased alanine levels in children are due to increased alanine release from muscle or decreased uptake by the liver. The latter possibility was supported by the absence of a glycaemic action of alanine in the present study.

Furthermore, in contrast to adult diabetics, infusion of alanine failed to cause an increase above basal levels in the plasma concentration of glucagon and glucose (11).

The explanation of the difference between the adult and child is not apparent on the basis of the data available. It is important to emphasize that both basal alanine and glucagon

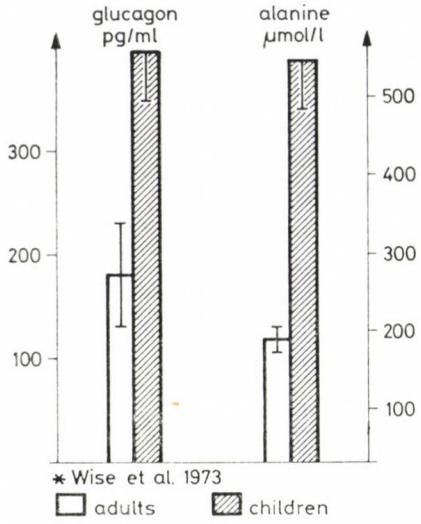

FIG. 4. Comparison of basal alanine and glucagon levels in adult and childhood diabetes

levels were higher in our patients than in those of Wise et al. (11) (Fig. 4). Hyperglycaemia was also more pronounced in our patients. Interestingly, in healthy dogs, the glucagon-provoking effect of alanine could be prevented by hyperglycaemia produced by glucose infusion.

Although the infusion of alanine failed to increase glucagon levels it proved to be an effective stimulus of growth hormone secretion. The elevated growth hormone levels were further increased by alanine (Fig. 3). Oral administration of L-alanine in doses of 0.5 or 1.0 g/kg also induced a delayed release of growth hormone, similar to that found in our patients (4).

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Current issues in the practice of genetic counselling

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Ten problems of genetic counselling based on Hungarian experiences are discussed, *viz.* (1) the competency of counsellees; (2) the high rate of self-referrals; (3) the low rate of most competent couples; (4) 20.2% of probands without definite nosological diagnosis; (5) advantages and disadvantages of the information-guidance type of counselling; (6) the unpleasant role of random risk; (7) 12.9% pregnant consultands; (8) the high rate of counsellees with two or more affected children; (9) combinations of independent disorders in the probands; (10) the so-called mixed pedigrees

The Genetic Counselling Service has been part of the National Mother and Child Care System in Hungary since 1976, thus the genetic counselling clinics are an integral part of the Hungarian health care system. Hungary has 10.7 million inhabitants and there are 14 genetic counselling clinics, 5 in Budapest, and 9 in the provinces, all having an adequate cytogenetic and clinical background. Most of them are in hospitals. Each genetic counselling clinic supplies a defined territory. The number of new counsellees showed an increasing trend until 1976 (Table I). Since that year a nearly constant number of about 3700–3800 couples or persons is registered yearly. In 1976–78, this meant a rate of 2.1–2.2 per 100 livebirths in Hungary, and our genetic counselling clinic sees about 20% of all counsellees.

The purpose of this paper is to summarize the current issues of genetic counselling on the basis of Hungarian experience.

1. The counsellees were divided into 15 categories according to their reason for seeking advice (Table II) (3). This classification was based first of all on the disorder of probands. The percentage values of these categories seem to be interesting as they show that in Hungary the genetic counselling service is an important basis of qualitative family planning in general. The first and third largest categories are sterility and fetal death, disorders rarely mentioned in the textbooks of genetic counselling. In a minority of these cases, genetic examinations and advice may be useful, but for the majority the genetic clinic has little to offer. At present this point causes a number of problems. It is a well-known opinion that "ultimately genetic counselling should become available to everyone who needs it" (6). Still, the

TABLE I

Annual number of new counsellees (couples or persons) in 14 genetic counselling clinics in Hungary, 1973–1978

		1973	1974	1975	1976	1977	1978	Total
Other 13 genetic counselling clinics	No.	1 013	1 386	2 020	3 152	3 071	2 624	13 266
	per cent	94.9	81.3	75.1	80.2	78.6	70.0	77.8
Own genetic counselling (NIH)	No.	54	318	668	780	834	1 124	3 778
	per cent	5.1	18.7	24.9	19.8	21.4	30.0	20.8
Total	No.	1 067	1 704	2 688	3 932	3 905	3 748	17 044
	per cent	100.0	100.0	100.0	100.0	100.0	100.0	100.0
No. of livebirths in Hungary		156 224	186 288	194 240	185 405	177 574	168 096	1 067 827
$\frac{\text{No. of new counsellees}}{\text{No. of livebirths}} \cdot 100$		0.68	0.91	1.38	2.12	2.20	2.23	1.60

TABLE II

Distribution of nosological categories in the genetic counselling material, 1973–1978

Nosological categories	No.	per cent
Nosological diagnosis		
Genetic		
Mendelian conditions	582	15.4
Chromosomal aberrations	254	6.7
Multifactorial-congenital abnormalities	473	12.5
Multifactorial-common diseases	154	4.1
Mutagenic noxae	59	1.6
Consanguinity	44	1.2
Enviromental		
Congenital abnormalities caused by teratogenic or maternal factors	102	2.7
Enviromental factors during pregnancy	266	7.0
Clinical diagnosis		
Sterility	760	20.1
Fetal death	498	13.2
Early death	177	4.7
Defects (mental subnormality, etc.)	104	2.7
Congenital abnormalities of unknown origin	163	4.3
Diseases of non-genetic origin	78	2.1
General information	64	1.7
Total	3778	100.0

cost and benefit are an important argument against unsuitable referrals.

2. The appropriate persons to refer counsellees to genetic counselling clinics would be general practitioners or specialists of the Mother and Child Care System. To our genetic counselling clinic, however, 67.9% of the counsellees came on their own initiative. One cause of this fact may be the effect of two TV programme series in connection with genetic counselling and family planning which directly influenced the number of counsellees (Fig. 1). Physicians referred only 26.2% and the rest was called to report in connection with some special preventive programme such as the prevention of recurrence of neural tube defects, etc., or they were sent by other geneticists.

3. This point is connected with the two former ones. The necessity of genetic counselling has a wide spectrum within the potential consultands. It is well known that only a low percentage of those who would really need it, seek such advice (4). Experience in Hungary has shown that the severity of manifestation immediately after birth, the notoriety of the disease and its genetic background,

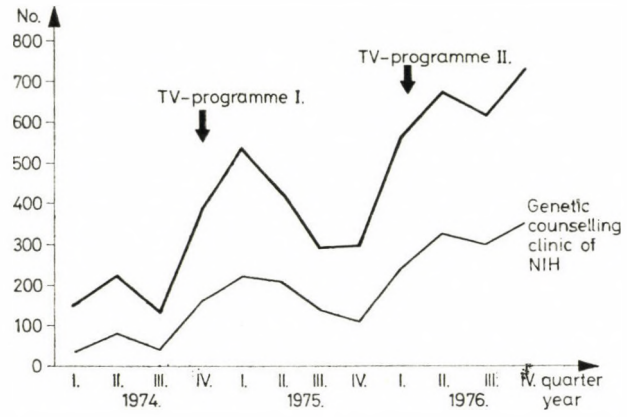

FIG. 1

and the educational level of the couples are the most important factors (3). Mendelian conditions manifesting in adult life have been rare in our material, e.g. hyperlipoproteinaemia type II has not occurred at all. The greatest problem is the absence of couples with low educational and socioeconomic conditions, e.g. the number of gipsy consultands was 6 compared with the figure 150 expected, based on their proportion in the population.

4. According to a well-known rule, the reliability and efficacy of genetic counselling depends on the accuracy of the clinical, and mainly of the nosological diagnosis. However, in a number of couples referred by physicians and mainly in the self-referral counsellees there was only a clinical diagnosis (e.g. cataract, muscular dystrophy, multiple malformation, sterility). Thus, the most difficult task of the genetic counsellor is to try to establish an exact nosological diagnosis. That is why we have a network of specialists. The previous diagnosis was sufficient for genetic counselling in 57.0% of the probands, and the diagnosis had to be changed in 22.8%. Unfortunately, in 20.2% of the consultands genetic counselling had to be done without a definite nosological diagnosis. Of course, in these cases we had to rely on empirical risk figures or subjective estimations. Another important rule of genetic counselling is that "any medical advice is better than no advice". As Fraser wrote: "Useful advice can still be given even in the absence of certainty" (7). Our data have proved the importance of an aetiological diagnosis. A recurrence occurred in 8.9% of the consultands who had previously had a baby with multiple malformation of unknown aetiology. In contrast, this rate was only 1.9% in the Mendelian conditions excluding cases when pregnancy had not been recommended (2).

5. The method of genetic counselling is an important theoretical prob-

lem. The classical English opinion is to give information without any advice (7). Carter (1) developed the method for consultands with low and moderate risk, encouraging the counsellor to say "In your place I would not take a risk of this kind too seriously". Our counsellees, however, needed, as they said, "more help"; they were not satisfied with the explanation of the risk and the perspectives, they usually wanted to hear a definite advice from the counsellor. This demand was the basis of our information-guidance type of counselling. Thus, knowing the history and the pedigree of the proband and having established the diagnosis, our counselling session involves three steps, viz. (i) information and explanation concerning the perspectives; (ii) a summary of the counsellor's advice; (iii) checking whether the explanation and advice had been understood. The information concerning the perspectives of family planning has been summarized in 8 points (Table III): (1) the severity of the expected disorders in the offspring; the possibilities of (2) treatment and (3) prenatal diagnosis; (4) the grade of the specific genetic (or teratogenic) risk; the possible environmental—"familial" hazard regarding the development of the offspring connected with (5) diseases of the parents, (6) the socio-economic status of the family, and (7) the familial structure: the number of healthy and affected sibs; and, finally (8) the potential maternal risk during pregnancy. Although genetic counselling is always individual, the advice given by the genetic counsellor may be considered to fall into five categories:

a) No significant problem (e.g. the specific risk is under 2 and sometimes 5% or the expected disorder is mild or treatable), pregnancy is suggested.

b) Pregnancy is suggested after some preparations, e.g. because the high risk is preventable by some treatment or the risk involves "only" fetal death, as in habitual abortion.

c) Pregnancy is suggested with prenatal examination, provided the latter is feasible and the risk is at least 2%.

d) Family planning deserves consideration for some specific reason, e.g. (i) the specific risk is between 5–19% and the expected disease is severe and there is no effective treatment; (ii) the disease (e.g. alcohol addiction, schizophrenia, mental subnormality) of the parents, mainly of the mother, may affect the offspring's development; (iii) the pregnancy increases the maternal hazard.

e) Pregnancy is not suggested, as the specific risk exceeds 20% in a severe untreatable disorder in the offspring or the pregnancy threatens the life of the mother.

Of course, the decision is the right and responsibility of the consultand, and the counsellor has to help its realization independently of his own previous advice. It is another point that a considerable part of our counsellees expect a directive for their family planning as they prefer to

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TABLE III

The main factors having an influence on genetic counselling (with an increasing hazard from left to right)

I. Specific genetic (or teratogenic) hazard							
1. Severity ("burden")	Minor	Mild	At disadvantage	Semilethal	Semilethal + at a disadvantage	Lethal	Serious long-term handicap
2. Treatment	Very effective	Effective	Low effectivity	No	"Severe"		
3. Prenatal diagnosis	Yes, reliable	Yes, not very reliable	None				
4. Specific risk	Minimal (<2%)	Low (2–4%)	Moderate (5–9%)	High I (10–19%)	High II (20–50%)	High III (>50%)	
II. Environmental-familial factors							
5. Dangerous disease of parents	Father	Mother	Father and mother				
6. Socio-economic status	Low	Very low	Dangerous				
7. Number of sibs	Only affected sib(s)	No sib	Healthy sib(s)				
III. Maternal hazard							
8. Hazard during pregnancy and/or delivery	Low risk	Moderate risk	High risk				

accept the counsellor's decision. This, however, is not permissible.

The advantage of this type of counselling is a high social efficacy as only 4.7% and 9.6% of the consultands did not want to have a further child after an advice of types *a–b* and *c*, respectively (2), as well as the fulfilment of the counsellee's demand. The disadvantages may be an offence against human rights and the hazard to the counsellor. The former point may be negligible because a summary of the counsellor's advice is asked for by the counsellees. Obviously, this kind of genetic counselling increases the counsellor's responsibility and may raise some legal problems. The random risk is a disturbing factor as the outcome of pregnancy may be unfavourable, even after adequate advice. Therefore, it is an obligatory task of the counsellor to draw attention to the unpredictable random risk. This problem is somewhat similar to the activity of general practitioners, and in Hungary the medical geneticists obtain protection against the random risk. From both the practical (efficacy) and the ethical (the interest of counsellees is more important than the interest of counsellors) point of view the information-guidance model of counselling is better than the non-directive model, i.e. information and explanation, because by such an approach we can give more help to our consultands leaving to them the right to make their own decisions. We have not experienced the alleged psychological drawbacks of our more direct approach (5).

6. In spite of the legal protection, the greatest practical problem is the random risk. Genetic counsellors could tell about several such cases. Let us mention here three cases only. In the first one, a woman whose first child was born with anencephaly, amniotic AFP-examination was suggested during her second pregnancy. The value was normal, the baby, however, was born with a complex heart defect. The other example was a male consultand whose two wives had one spontaneous abortion and three livebirths: these children were born with three different congenital abnormalities. The third case was a pregnant woman who allegedly had had rubella-virus exposition during this pregnancy. Because it had been excluded serologically, she was dissuaded from having her pregnancy interrupted. She agreed happily and later she delivered a baby with Down syndrome.

The 1973–1978 material of our genetic clinic included 3778 cases had 76 "clusters" of independent serious congenital abnormalities in sibs. Obviously this 2% frequency fits well with the random risk (2) but in practice it caused much distress.

7. Of the counsultands, 20.4% were pregnant when they reported at the clinic. The number of pregnant women seeking genetic counsel for teratogenic or maternal noxae, as well as of the women referred for prenatal diagnosis has to be deducted from this value. Still, the remaining 12.9% seems to be high. On the one hand, the time is too short to make the

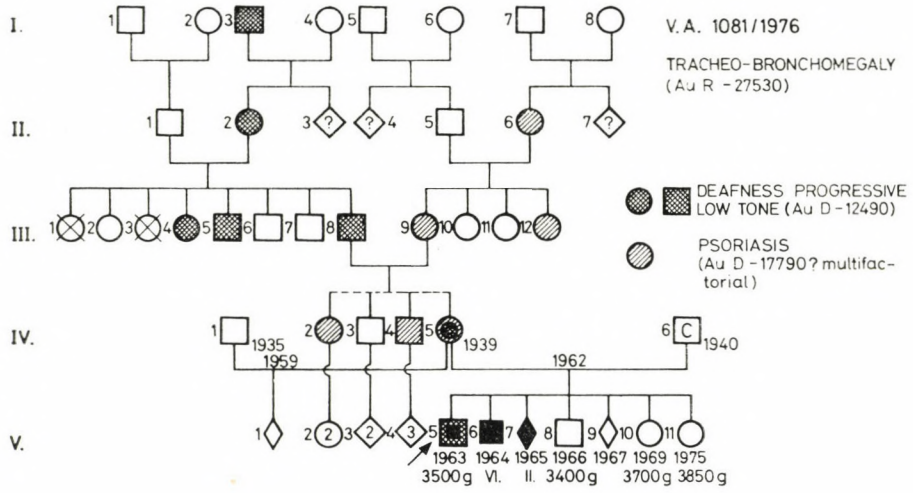

FIG. 2

necessary examinations and, on the other, the range of possible actions is limited.

8. The proportion of consultands who seek counselling after one or more affected children is extremely high. Obviously, fresh mutations and most recessive and multifactorial disorders are recognized only after the affected child was born. Still, 75 consultands, 5.1% of our material of genetic origin came to our genetic clinic after two affected children. The fact that there were 7 new counsellees with three seriously affected children each, is somewhat surprising.

9. The combinations of independent disorders in probands may sometimes raise a problem, e.g. cleft palate associated with coeliac disease; adrenal hyperplasia with myopia and congenital dislocation of the hip; phenylketonuria with spina bifida cystica. The question is whether these are different entities with different recurrence risks (i.e. their risk are additive), or they can modify each other's risk, or else, they represent a special nosological entity with one definite risk.

10. The so-called "mixed" pedigree (Fig. 2), when two or three independent inherited diseases appear in the same family, may also cause problems. Is there only a simple additive risk $(a+b+c-abc)$ or can they modify each other? It would be important to establish the principles of genetic counselling in such cases.

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Über die Pathogenese des Bronze-Baby-Syndroms: Kopronorphyrinentleerung hyperbilirubinämischer Neugeborenen

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Der normale Koproporphyrinwert liegt im Harn der Neugeborenen wesentlich niedriger als bei Erwachsenen. Es wurde nachgewiesen, daß die Hyperbilirubinämie (mit oder ohne Phototherapie) mit keiner Koproporphyrinerhöhung im Harn verbunden ist. Im Zusammenhang mit einer Leberschädigung tritt auch im Neugeborenenalter eine kleingradige, sekundäre Erhöhung des Harn-Koproporphyringehalts auf.

Die Ergebnisse liefern einen Beweis für die Hypothese, laut der beim "Bronze Baby-Syndrom" die Leberläsion eine primäre Veränderung ist.

Anhand von Literaturdaten und mehrjährigen Erfahrungen können wir feststellen, daß sich die Phototherapie bei der Behandlung der neonatalen Hyperbilirubinämie bewährt hat und eine bedeutende Verringerung der Zahl der Austauschtransfusionen mit sich brachte. Die Phototherapie ist im allgemeinen eine komplikationsfreie Methode, mitunter können sich aber auch Nebenwirkungen wie das Bronze-Baby-Syndrom melden.

Dieses Krankheitsbild ist dadurch charakterisiert, daß sich innerhalb von 24–72 Stunden nach dem Beginn der Phototherapie Haut, Harn und Serum gräulich-braun verfärben, der Koproporphyrinspiegel im Harn und Blut ansteigt, sich das normale Melanin in den oberen Hautschichten vermehrt, sich die Konzentration des eine direkte Reaktion ergebenden Bilirubins im Serum bedeutend erhöht und gleichzeitig Anämie auftritt. In unserem Fall (11) fanden wir nebst den pathologischen Leberfunktionsproben sowohl lichtmikroskopisch (Cholostase, Pericholangitis) als auch elektronenmikroskopisch (Destruktion der Membran der Gallenkanälchen, Anhäufung von Gallendeposita in den Leberzellen) schwere morphologische Veränderungen, die sich aber für reversibel erwiesen haben.

Das Koproporphyrin ist kein Photodegradationsprodukt, wie das einige Autoren früher angenommen haben (4, 9), sondern, laut Weitz (16) eine Zwischenstation der Häm-Synthese (es befindet sich also auf der anabolischen Seite): Glycin + Sukzinyl-CoA, Delta-Aminolevulinsäure,

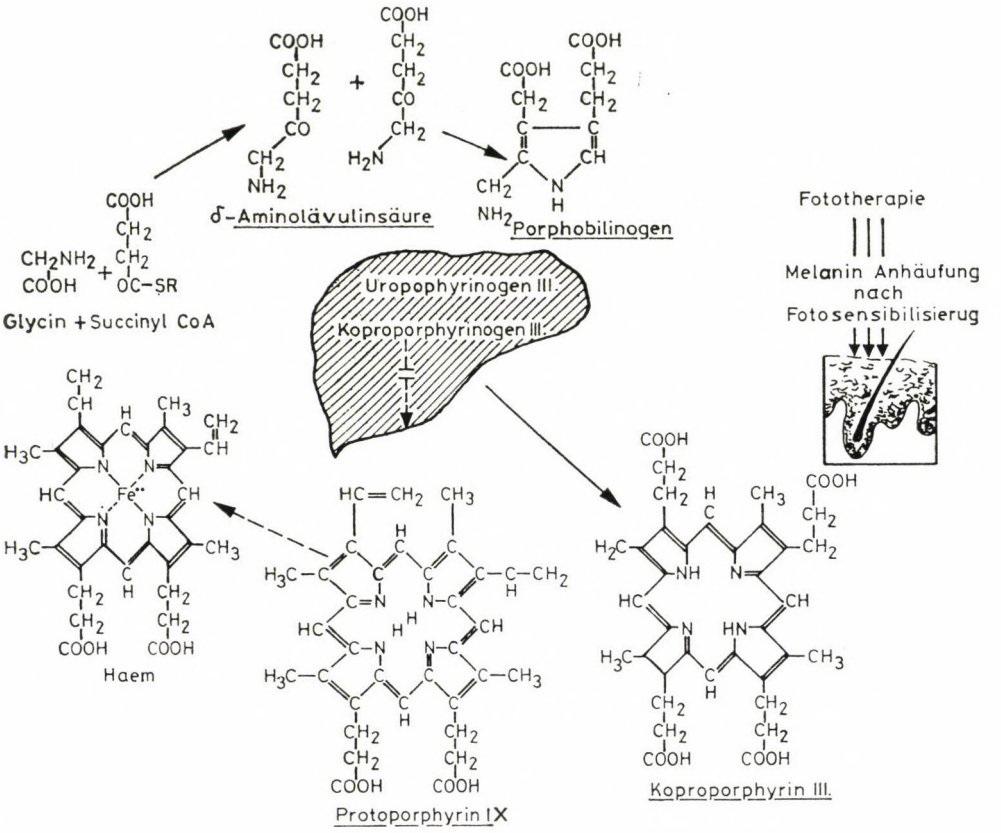

Abb. 1

Porphobilinogen → Uroporphyrinogen III → Koproporphyrinogen III →
→ Protoporphyrin IX → Häm
↘ Koproporphyrin III (s. Abb. 1).

Die Verfärbung der Haut steht mit dem Protoporphyrin im Zusammenhang (16). Durch die Vermehrung dieser bekanntlich photoaktiven Substanz werden die Melanophoren stimuliert, und die dadurch gesteigerte Melaninproduktion führt zur Verfärbung der Haut.

Es muß mit Nachdruck betont werden, daß noch nicht alle Stufen der geschilderten Mechanismen geklärt sind, die Annahme jedoch, daß auch die Leberfunktion in Mitleidenschaft gezogen ist, dürfte als unbedingt berechtigt betrachtet werden. Die mit den Leberschädigungen verbundenen Koproporphyrinurien gehören in die Gruppe der sekundären, sich zu der Grundkrankheit gesellenden Koproporphyrinurien (5, 10).

Da die Vermehrung von Koproporphyrin in der Amnionflüssigkeit, im Harn und Blut sowohl bei unreifen Neugeborenen als auch in sonstigen Krankheitsbildern sogar ohne Phototherapie zu beobachten war, hielten wir die Untersuchung der Harn-Koproporphyrinentleerung von hy-

perbilirubinämischen Neugeborenen für lohnhaft, um auf diese Weise zur Klärung des Pathomechanismus des Bronze-Baby-Syndroms weitere Anhaltspunkte zu erhalten. Betreffs der normalen Koproporphyrin-Harnwerte der Neugeborenen fanden wir keine Literaturdaten, einige Autoren haben indessen darauf hingewiesen, daß die Normalentleerung im Kleinkindesalter wesentlich niedriger als bei Erwachsenen ist (1, 13, 17).

Material und Methodik

Die Harn-Koproporphyrinkonzentration wurde bei 22 hyperbilirubinämischen Neugeborenen (unter denen 15 eine Phototherapie erhielten) sowie in je einem Fall mit intrahepatischer Gallenwegsatresie, hämolytischer Anämie, Bronze-Baby-Syndrom, bei einem septischen Neugeborenen und bei 5 gesunden, nicht ikterischen Neugeborenen mit der quantitativen Extraktionsmethode untersucht. Die Bestimmung der Erythrozyten-Koproporphyrinwerte erfolgte bei 12 hyperbilirubinämischen Neugeborenen mit dem qualitativen Test. Sämtliche erhaltenen Ergebnisse sind in Tabelle I dargestellt.

Ergebnisse

Wegen der wohlbekannten praktischen Schwierigkeiten des Harnsammelns bei Neugeborenen wurde die Untersuchung in 10 hyperbilirubinämischen Fällen aus Harnproben, in allen übrigen Fällen jedoch aus 24-Stunden Harn vorgenommen.

Der Normalwert der Harn-Koproporphyrinentleerung lag bei Neugeborenen unter 10 μmol/24 St und damit wesentlich unter dem, 153 μmol/24 St ausmachenden Wert der Erwachsenen. Etwas höhere Werte waren bei unseren Patienten mit Gallenwegsatresie, hämolytischem Ikterus und bei Bronze-Baby-Syndrom vorzufinden; in allen drei Fällen waren soz. alle Symptome der Leberläsion zu beobachten (Fälle Nr. 26, 27 und 28).

Die Erythrozyten-Koproporphyrinwerte zeigten bei hyperbilirubinämischen Neugeborenen keine pathologischen Abweichungen.

Besprechung

Angesichts der niedrigen Zahl unserer Fälle und der Heterogenität des Materials, wäre es unberechtigt, weitgehende Folgerungen zu ziehen. Folgendes kann aber mit Sicherheit festgestellt werden:

1. Im Neugeborenenalter ist der Koproporphyrin-Gehalt des Harns wesentlich niedriger, als bei Erwachsenen;

2. wie dafür auch die Literaturdaten zeugen, werden durch die Hyperbilirubinämie weder mit noch ohne Phototherapie keine gesteigerte Koproporphyrinentleerung und durch die Phototherapie keine Leberschädigung verursacht (7, 15);

3. bei Erwachsenen ermittelte Beobachtungen führten zur Feststellung, daß sich die Untersuchung der Harn-Koproporphyrinentleerung zur Registrierung der Aktivität der Leberläsion (z.B. bei Zirrhose) eignet, weil gesteigerte Werte mit der Schwere

TABELLE I

Nr.	Gestations-Alter, Woche	Geburts-gewicht g	Serum-Bilirubin µmol/l		Hb µmol/l	Ht	Blutgruppe	
			Direkt	Indirekt			Mutter	Neugebor.
1	31	4000	—	290.7	10.5	0.52	B pos.	B pos.
2	38	3250	—	256.5	10.1	0.42	O pos.	O pos.
3	40	3800	—	265.9	10.3	0.56	O pos.	O neg.
4	40	3900	—	200.0	10.3	0.60	AB pos.	A neg.
5	36	2600	—	259.9	11.9	0.60	A pos.	A pos.
6	40	3100	—	249.9	8.4	0.62	A pos.	B pos.
7	35	2500	—	480.6	10.1	0.50	A pos.	A pos.
8	40	4250	—	359.0	13.6	0.56	B pos.	O pos.
9	40	3200	—	179.5	11.4	0.54	O pos.	O pos.
10	40	3000	—	237.6	10.5	0.52	B pos.	O pos.
11	36	3000	—	369.0	10.2	0.58	A neg.	AB pos.
12	38	2400	—	379.0	11.6	0.58	AB neg.	A pos.
13	40	3150	—	217.0	7.44	0.32	A pos.	B pos.
14	38	2700	—	292.0	10.4	0.51	A pos.	A pos.
15	37	3350	—	406.1	10.5	0.28	B pos.	B pos.
16	36	2800	—	307.0	12.1	0.54	O pos.	O pos.
17	39	2500	—	384.7	13.3	0.60	A pos.	AB pos.
18	41	2800	—	324.0	12.4	0.62	A pos.	A pos.
19	42	2950	—	319.0	11.9	0.60	O pos.	O pos.
20	40	2750	—	236.0	12.5	0.68	O pos.	A pos.
21	41	2300	—	323.0	8.7	0.50	B pos.	
22	37	2850	—	327.0	12.1	0.60	O neg.	O pos.

Gesamt-eiweiß g/l	Koproporphyrin		Therapie			Austausch-transfusion	Diagnose	GT
	Urin	Blut	Licht	Albumin	Transfusion			
66	19 nmol/l	–	+	+	–	–	Hyperbilirubinämie	22 IE
55	113 nmol/l	–	+	+	–	–	Hyperbilirubinämie	20 IE
–	14 nmol/l	–	–	+	–	–	Hyperbilirubinämie	16 IE
77	21 nmol/l	–	+	+	–	–	Hyperbilirubinämie	20 IE
47	35	–	–	+	–	–	Hyperbilirubinämie	22 IE
50	41 nmol/l	–	+	+	–	–	Hyperbilirubinämie	20 IE
53	14 nmol/l	–	+	+	+	+	Hyperbilirubinämie	16 IE
70	113 nmol/l	–	+	+	+	–	Hyperbilirubinämie	10 IE
–	12 nmol/l	–	–	–	–	–	Hyperbilirubinämie	12 IE
–	35 nmol/l	–	–	–	–	–	Hyperbilirubinämie	12 IE
56	9.8 nmol/24 St.	norm.	+	+	–	–	Hyperbilirubinämie	10 IE
55	1.53 nmol/24 St.	norm.	+	+	+	–	Hyperbilirubinämie	15 IE
45	7.8 nmol/24 St.	norm	–	–	+	–	Hyperbilirubinämie	20 IE
43	2.3 nmol/24 St.	norm.	–	+	+	–	Hyperbilirubonämie	12 IE
55	–	–	+	+	+	+	Hyperbilirubinämie	18 IE
50	9.8 nmol/24 St.	norm.	+	+	+	–	Hyperbilirubinämie	18 IE
60	3.1 nmol/24 St.	norm.	–	+	–	–	Hyperbilirubinämie	12 IE
53	0.8 nmol/24 St.	–	–	+	–	–	Hyperbilirubinämie	20 IE
67	7.0 nmol/24 St.	norm.	–	+	–	–	Hyperbilirubinämie	22 IE
67	2.0 nmol/24 St.	norm.	+	+	–	–	Hyperbilirubinämie	10 IE
50	1.4 nmol/24 St.	norm.	+	+	–	–	Hyperbilirubinämie	16 IE
–	6.4 nmol/24 St.	norm.	+	+	–	–	Hyperbilirubinämie	26 IE

TABELLE 1 *(Fortsetzung)*

Nr.	Gestations-Alter, Woche	Geburts-gewicht g	Serum-Bilirubin µmol/l Direkt	Serum-Bilirubin µmol/l Indirekt	Hb µmol/l	Ht	Blutgruppe Mutter	Blutgruppe Neugebor.
23	35	2350	—	279.0	10.2	0.50	A pos.	
24	34	2000	—	333.0	11.1	0.58	O pos.	B pos.
25	40	3100	162.0	179.5	11.4	0.52	O pos.	A neg.
26	32	2150	114.5	124.0	6.8	0.30	A pos.	A pos.
27	36	3200	172.7	—	9.4	0.50		B pos.
28	38	3000	66.6	412.1	7.7	0.30	O neg.	O neg.
29	36	1100	—	—	12.4	0.67	O pos.	A pos.
30	36	1850	—	—	10.3	0.57	O pos.	A pos.
31	35	1900	—	—	11.9	0.57	B pos.	
32	35	1950	—	—	10.4	0.52	B pos.	
33	40	3450	—	—	12.7	0.59	AB pos.	

der Krankheit korrelieren und auch eine prognostische Bedeutung haben (3). Dies scheint auch unsere Beobachtung zu unterstützen, laut der in 3 unserer Fälle mit erhöhtem Harn-Koproporphyringehalt auch die klinischen und morphologischen Symptome der Leberläsion vorhanden waren. Dementsprechend dürfte dieser Untersuchung im Neugeborenenalter bei der Beurteilung der Leberläsion eine wichtige Rolle beigemessen werden.

Wenn bei neonataler Hyperbilirubinämie mit angestiegenen Werten des eine direkte Reaktion ergebenden Serumbilirubins, Phototherapie zur Anwendung kommt, muß unseres Erachtens mit der Entwicklung eine Bronze-Baby-Syndroms gerechnet werden. Es scheint, daß die Leberläsion eine primäre Veränderung ist — d.h., daß sie sich nicht der Therapie zufolge entwickelt — und sich das Syndrom unter Wirkung der Phototherapie manifestiert. Unserer Anschauung nach sollte somit die Phototherapie in solchen Fällen vermieden werden.

Vorliegende Untersuchungen besitzen nur einen Orientierungscharakter, wir betrachten sie jedoch außerdem auch für richtungsweisend, die uns dazu veranlassen die diesbezüglichen Forschungen in der Zukunft auf

Gesamt-eiweiß g/l	Koproporphyrin		Therapie			Austausch transfusion	Diagnose	GT
	Urin	Blut	Licht	Albumin	Transfusion			
—	2.0 nmol/24 St.	norm.	+	—	—	—	Hyperbilirubinämie	22 IE
—	2.9 nmol/24 St.	norm.	+	+	—	—	Hyperbilirubinämie	16 IE
71	12.7 nmol/24 St.	—	—	+	+	+	Sepsis	85 IE/1
56	75.0 nmol/24 St.	—	—	+	+	—	Hämolytische Anämie	110 IE/1
53	21.0 nmol/24 St.	—	—	—	—	—	Gallenwegsatresie	250 IE/1
46	101.0 nmol/24 St.	—	+	+	+	+	Bronze-Baby	220 IE/1
48	4.7	—	—	—	—	—	Kontrolle	24 IE
50	4.8	—	—	—	—	—	Kontrolle	20 IE
—	2.6	—	—	—	—	—	Kontrolle	10 IE
—	3.4	—	—	—	—	—	Kontrolle	18 IE
—	4.1	—	—	—	—	—	Kontrolle	14 IE

verschiedene im Neugeborenenalter vorkommende Leberläsionen auszubreiten.

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Subdural effusion in the first six months of life

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During the period 1978–1981, among 1280 (0–12 months old) infants suffering from consequences of various pre- and perinatal cerebral lesions, positive neuroradiological changes were found in 148 cases. Of these, 56 proved to be real subdural effusions with elevated ICP and increased protein content. Periodical transfontanellar taps and drainage were effective in 49 cases. A neurosurgical operation was performed in 7 infants because of the poor effect of the above treatment. In these 7 infants the encapsulation process was nearly complete.

Early chronic subdural effusion exerts a devastating influence on the maturing brain partly by the high ICP. Early treatment often cures the process and major surgery is not needed. Early neurorehabilitation and habilitation coordinated with elimination of the effusion is the method of choice. Prudence is recommended in selecting the appropriate cases for instrumental therapy because some small effusions and fluid accumulations may disappear by the end of the first year.

The development of new diagnostic batteries for the early detection of pre- and perinatal brain lesions yielded new information on the occurrence of various morphological and physiological defects in the central nervous system. At present a complete and reliable developmental neurological investigation of the newborn or young infant consists of polygraphic studies of feeding, visual, auditive and motor behaviour, detailed analysis of all elementary motor patterns activated by various stimulus positions, EEG, and in selected cases polymyography. In addition, the investigation should include transillumination. CT, sonography, and, if necessary, transfontanellar tap, subdurography,ventriculography and various laboratory examinations. Such a complex and thorough developmental neurological investigation will reliably reveal early defects in the nervous system, and differentiate between normal variations and defects. Regular transillumination proved to be of considerable help in the early detection of abnormal brain development owing to malformations or trauma (1, 11). Computerised tomography (CT) then revealed an even higher percentage of brain abnormalities during early infancy (2, 5, 8, 10). Subdural effusion was found in a considerable number of young infants (9, 13).

Formerly, a subdural effusion was practically never detected before the second half of the first year, and it was treated by subdural taps, drainage, or neurosurgical intervention. It has been realized only recently that

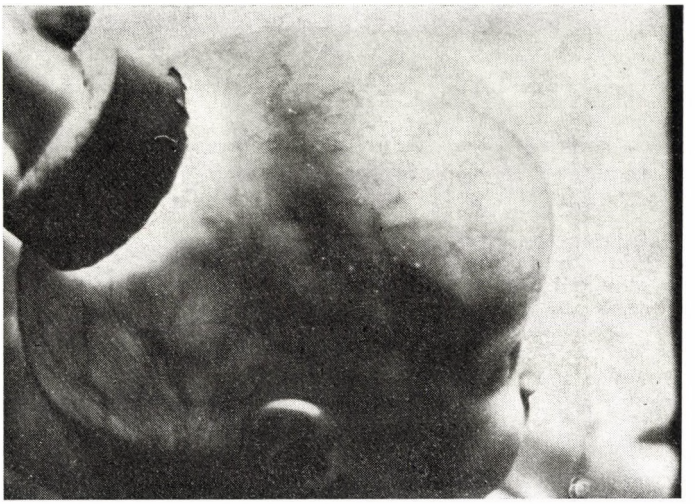


FIG. 1. Transillumination with a floodlight lamp. Large transparency reveals hydrocephalus

chronic subdural effusion may be present much earlier. The present study was focussed on this problem.

MATERIAL AND METHOD

In our department in the period 1978–1981, cerebral lesion and defective neurological function was detected in 1280 infants 0 to 12 months of age. The investigation took one week on the average. During this period a detailed and often repeated study was done. The function of cranial nerves and central control mechanisms were studied by polygraphy, sensory and activated motor functions by a thorough analysis of the stimulus-response relations, and EEG and in selected cases, polymyography were performed. All forms of objective recording of various sensory as well as motor behavioural patterns were used to obtain a sound diagnostic basis.

All infants were transilluminated using a special floodlight lamp (Fig. 1) and followed up by repeated transilluminations. If a suspicious transparency was observed, neuroradiological studies were performed. They consisted of transfontanellar subdurography, vetriculography, CT, and since the last year, transfontanellar sonography. By these means an intracranial morphological defect was revealed in 147 cases (Table I); of these in 56 infants increased intracranial pressure (ICP) and

TABLE I

Frequency of early subdural effusion

	No.	Per cent
Cerebral lesion	1280	100
Positive neuroradiology	147	11.5
Subdural effusion, 0–6 months	56	4.4

an elevated protein level in the CSF were found, with signs of a subdural effusion (Fig. 2). In all these infants, aberrant neurologic symptoms were present. They varied from serious deviations in motor and postural control, defective proportioning of muscle tone and aberrant feeding behaviour, to defective consiousness and vigility. Defective auditive and/or visual function was complicating 72% of the cases (6, 7).

CT proved to be extremely helpful in the detection of early subdural effusions but owing to the fact that laboratory analysis of the CSF was necessary for studying the nature of the subdural fluid. When the subdural space was tapped, air studies were also performed (Figs 3, 4 and 5).

ICP was recorded in selected cases in order to detect a non-manifest elevation of the ICP. An 8 channel Beckman R 411 Dynograph was used to record intracranial pressure and EEG simultaneously. Naturally all interventions were performed under sterile conditions. During the whole study which included not only 147 air studies but several hundred subdural taps, and therapeutic drainages not a single infection occurred.

Early preventive treatment was initiated in cases in which diagnostic batteries revealed subdural effusion. Therapy consisted of regular subdural taps, usually twice weekly on the affected side, or on alternating sides if the effusion was bilateral. In 7 cases a neurosurgical intervention had to be done.

Results

As shown in Table I, early subdural effusion was found in 56 infants aged 0 to 6.5 months, or 11.5% of the 1280 infants who were found to suffer from results of pre and perinatal cerebral lesions. Signs or suspicion of IC haemorrhage at or following birth was found in 26% of these cases; they all had a suspicious or clearly pathological transparency on transillumination. In 6 cases the transillumination was not positive initially but a transparency developed during the next 4 to 6 weeks. It was therefore

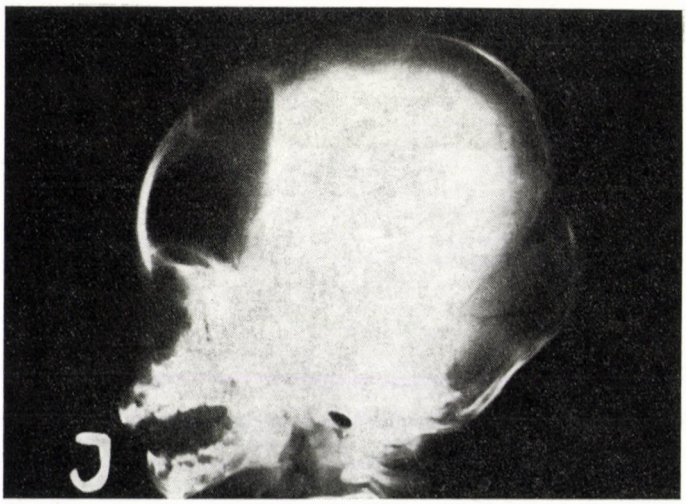


Fig. 2. Subdural effusion in a 4 months old infant. The effusion is mainly frontal

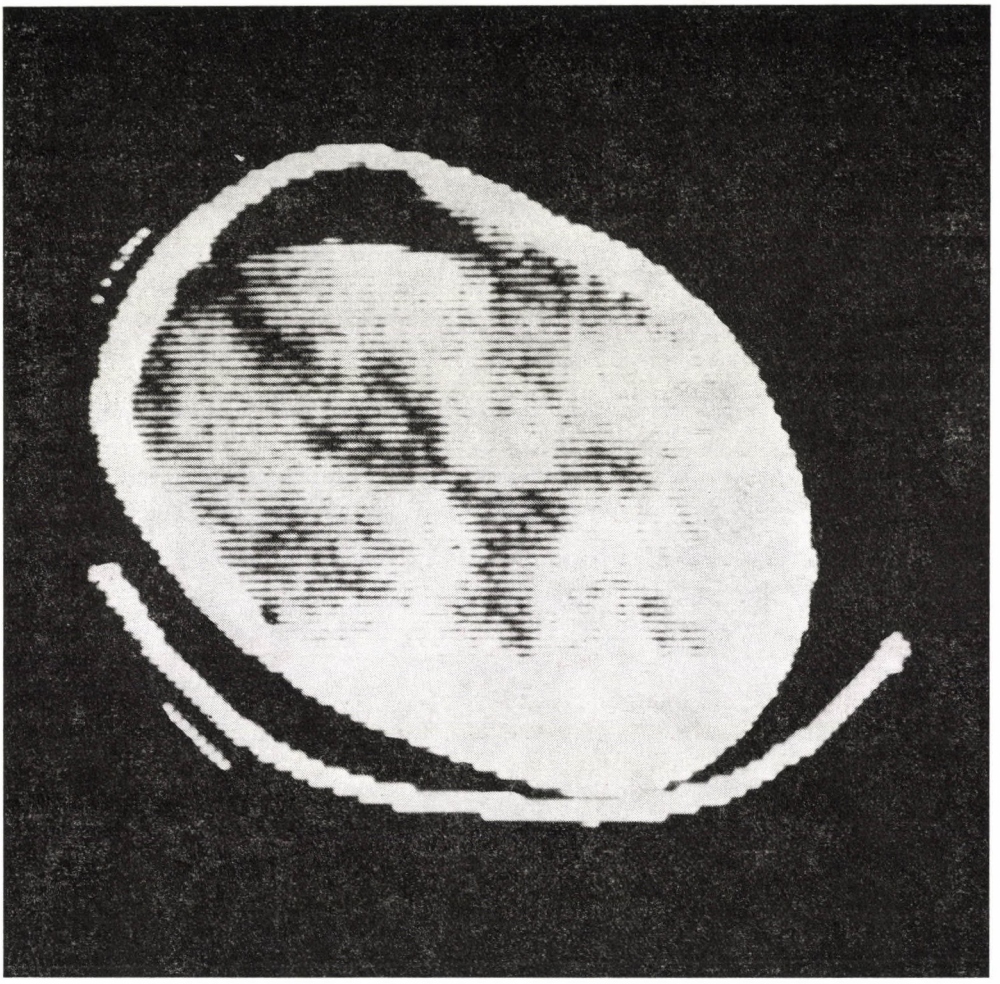


FIG. 3. CT of a subdural effusion over the frontal lobe

considered imperative to repeat regularly transillumination in infants with apparent cerebral lesion or with due suspicion of such a defect.

Repeated tapping or drainage of the subdural space eliminated the effusion in most of our cases. Neurosurgery was necessary in not more than 7 cases. During tapping and drainage the protein content of the effusion dropped from the initial very high level gradually to normal, the effusion diminished and then disappeared.

During therapy, acute and chronic changes in behaviour were recorded. In selected cases drowsiness and loss of contact with the environment were the signs of increased ICP. This was clearly observed in the slow or completely absent stimulus-response correlation, in the absence of any signs

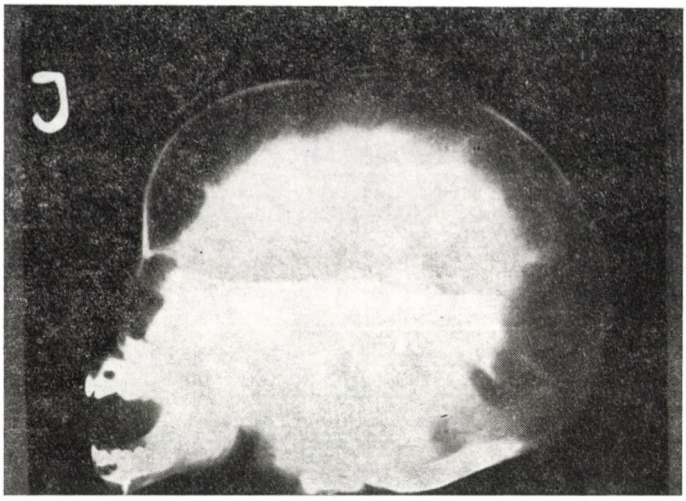


FIG. 4. Subdural effusion over the right hemisphere in a 3 months old infant. Lateral view



FIG. 5. Large subdural effusion over the right hemisphere of a 4 months old infant. AP view

of orientation on polygraphic monitoring or on the EEG. All signs of an active behaviour and environmental contact disappeared. In such cases, aspiration of 20–40 ml fluid from the subdural space produced dramatic changes. Not only the well-known signs of elevated ICP such as an increased fontanellar bulging, precomatose state, bradycardia, and vomiting had disappeared, but even the stimulus-response relations improved when visual or auditive stimuli were applied, and orientative behaviour, habituation and dishabituation returned to the normal level. Eventual changes in ICP during programmed exteroceptive stimulation by sound were studied in selected cases by polygraphy. It was occasionally observed in quiet, resting infants who produced definite behavioural responses to auditive stimulation that the classical orienting reaction was accompanied by a definite elevation in both ventricular and subdural pressure. Continuous monotonous stimulation inhibited the orienting reaction and simultaneously both subdural and intraventricular pressure dropped. When, however, the frequency of continuous monotonous stimulation was changed, the appearance of a second orienting reaction

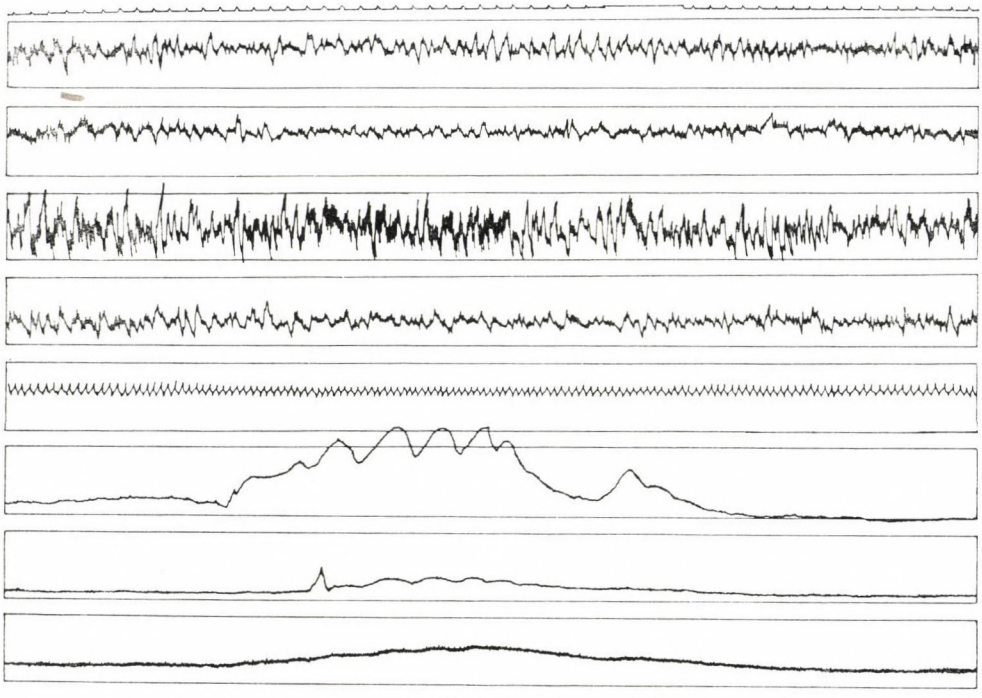


FIG. 6. Simultaneous recordings of spinal (lowest), subdural central and ventricular (upper) pressures, EEG, and ECG. Auditive stimulation was followed by a transitory pressure wave in all pressure recording channels. The 6th channel records right ventricular pressure, the 7th left subdural pressure, and the 8th spinal pressure. Channels 1–5, and paper speed and stimulus parameters as in Fig. 7*a* and *b*

(dishabituation) was again accompanied by a sudden elevation of subdural and intraventricular pressure (Figs 6 and 7*a*, *b*).

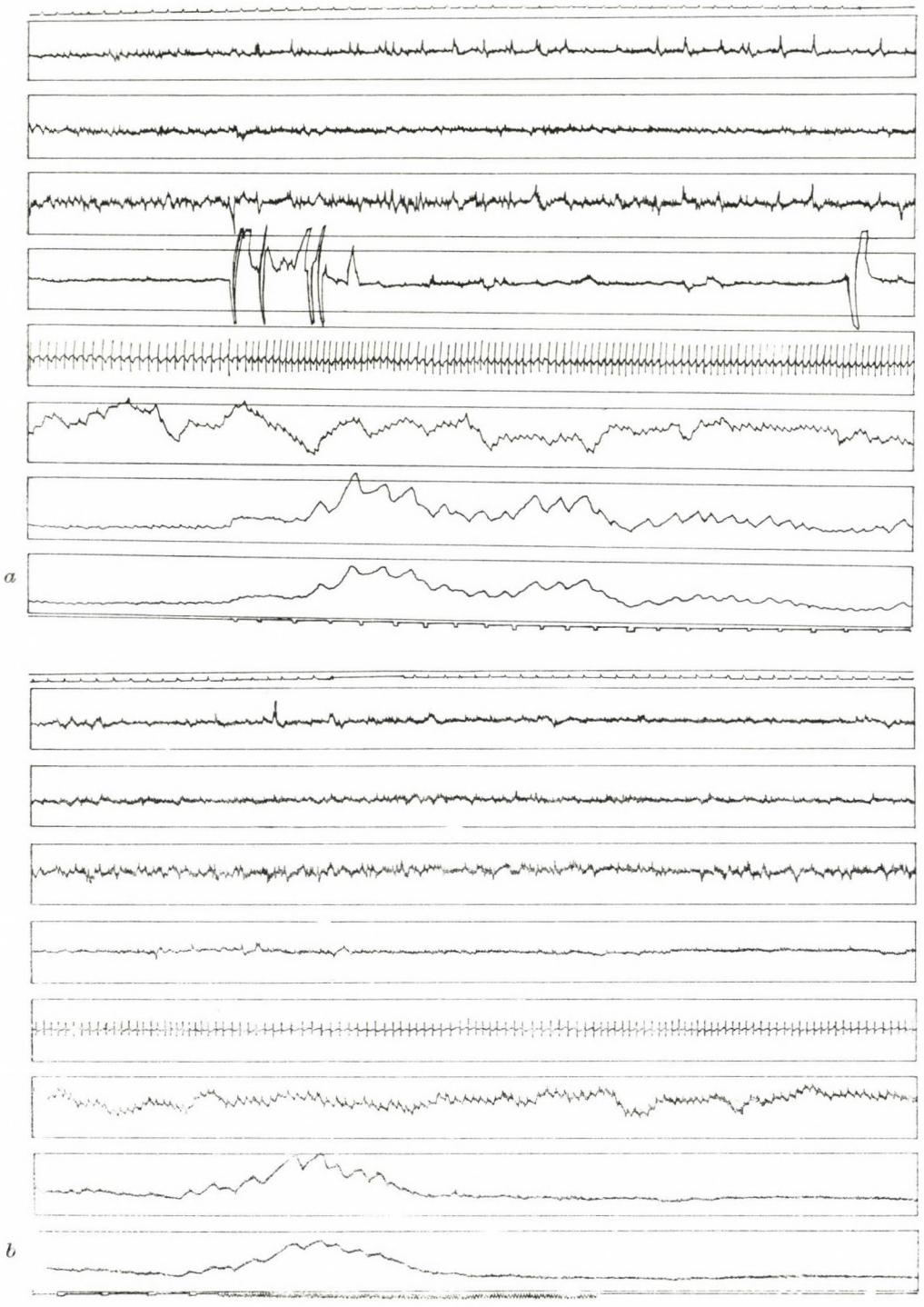
The studies have shown that not only a sudden rise of ICP can depress alertness, vigility and environmental contact of the infant but drowsy states may develop even if the rise is not constant. During prolonged ICP recording an undulating pressure was found.

CT is an important indicator of the fact that fluid has accumulated in the subdural space but not a reliable proof that the fluid represents a real effusion. In our practice the coincidence of positive neuroradiology, increased ICP, elevated protein content up to 600–2000 mg/dl in the subdural fluid seemed to verify the presence of early chronic subdural effusion. A further indicator of its presence was found when 7 of the patients had to be operated upon because of the failure of intermittent transfontanellar taps and drainage: in all these cases the two layers of the fibroblast envelope of the effusion had already begun to form even in 3 infants under the age of 3 months.

In 85% of the cases prolonged treatment by tapping and drainage was successful. The subdural effusion disappeared, the symptoms of increased ICP ceased to exist. Apart from the 7 cases in which this treatment was ineffective, in 6 cases the effusion reappeared, and a new series of tapping became necessary for making the effusion disappear.

Discussion

The occurrence of a subdural effusion with cerebral hypotrophy in the early months of life in such a high percentage means that the effusion may develop earlier than it had been thought; it may be chronic in the first months of life. The mentioned experience during operations revealed the fact that the neomembrane cover enveloping the cerebrum may develop early, though encapsulation is not complete. Thus, early detection of a real effusion may prevent the stabilization of the layers and shorten the period of brain compression. Increased ICP even if it is not constantly elevated exerts a destructive influence on the developing brain. The maturing structural differentiation represented by dendritic arborization, formation of synaptic junctions and glial proliferation are affected by the blood flow being impeded by the increased ICP. According to the classical work of Ingraham and Matson (4) the subdural haematomas discovered in early infancy are usually bilateral. In the 149 cases of Pia (12) 119 were bilateral. In our own material, too, most of the subdural effusions were bilateral and extended to the frontoprecentral region. In some cases they reached the parietal region, but we observed no effusion in the posterior fossa. Development of neomembranes and effusion formation was treated by a number of authors. Friede (3) summarized the recent information. Operative findings and our X ray studies call attention to the


a
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fact that the hypotrophic changes in the brain can be widespread under the layer of the effusion, even in the first months of life. Earlier we have called attention to the fact that neomembrane formation can be a very early process.

According to our experience a large amount of real subdural effusion can accumulate during the first postnatal months with elevated ICP, increased protein level, and definite signs of cerebral hypotropy even in the absence of any suspicion for IC haemorrhage in the perinatal history. Such effusions sometimes accumulate when the newborn had suffered from a verified or suspected IC haemorrhage but this then became completely normal, and no symptoms suggested any danger in the following days or weeks. In these cases transillumination is sometimes negative, and transparency becomes apparent only several weeks later when neurologic symptoms develop. A complete developmental neurological examination detects these symptoms earlier than a quick routine assessment. This is one of the reasons why subdural effusions were detected in our material at an early age and why treatment could be applied with success. Another cause of the high incidence of effusions in our material was that our special department receives a large number of young infants from other neonatal centres where suspicion of a possible pre- or perinatal cerebral lesion had arisen on the basis of certain functional symptoms such as defective feeding behaviour, problems of sucking and swallowing, conspicuous postural and motor signs, disturbed muscle tone, incompetent visual or auditive behaviour, restlessness or prolonged sleeping periods, convulsions, etc. The symptomatology usually represented a mixture of these signs. In 11.5% of these cases neuroradiologic examination revealed hydrocephalus, cystic deformities, porencephaly with fluid accumulation, and real subdural effusions, with a characteristic elevation of ICP and of protein content, a sign which was never detected in other processes associated with IC fluid accumulation as for example in porencephaly.

FIG. 7*a*, *b*. Auditive stimulation was followed by distinct changes on EEG, ECG and waves in the subdural and intraspinal pressures (orientation). Monotonous stimulation produces habituation, and a change in stimulation frequency produces dishabituation with another elevation in subdural and spinal pressures

First line: time marker; paper speed: 10 mm/s

Channels 1–4: EEG (pF_1—T_3 T_3—O_1 pF_2—T_4 T_4—O_2) 30/ · 1 x · 01 10 mV/cm
Channel 5: ECG 30/1 x · 1 10 mV/cm
Channel 6: intraspinal pressure DC/30 x · 01 10 mV/cm, 1 cm = 100 mm H_2O
Channel 7: left side subdural pressure DC/30 x · 01 10 mV/cm, 1 cm = = 100 mm H_2O
Channel 8: right side subdural pressure DC/30 c · 01 10 mV/cm, 1 cm = = 100 mm H_2O
Last line: stimulus marker (stimulus was given by an EMG type PHOTO–PHONO stimulator. Repetition: 0.4 cps in Fig. 7*a* and 4 cps in Fig. 7*b*; duration: 0.2 sec; sound frequency: 1000 Hz; sound intensity: 70 dB

In cases operated upon at the end of the first year or later, the brain showed considerable changes in size, gyrification, electrical activity and function due to the effect of the effusion. There is no way to tell whether an effusion with increased ICP and elevated protein content will be eliminated by spontaneous absorption. It is highly questionable how an accumulation of 150–200 ml can be absorbed if it receives constant supply from the developing vascular layers enveloping the brain and the fact that encapsulation is almost complete at a relatively young age speaks against frequent spontaneous recovery.

Depending on the type of the functional defect rehabilitation consisted in visual and/or auditive training, application of a stereophonic hearing aid, therapeutical training and education of elementary motor patterns, electrotherapy and sensory training. All these served to reinforce the existing functional potentialities and to assist development of the CNS. It would have been paradoxical to begin a medical programme in order to rehabilitate lost functions and to prevent development of other defective functions by habilitation, and to watch meanwhile inactively the continuous compression of the brain. Maximum caution was applied in indicating tapping or drainage. They were done exclusively when clear-cut signs of early chronic subdural effusion were observed. These are a large amount of fluid, elevated ICP, elevated protein level and positive neurological signs. The fact that the first three symptoms were eliminated in 49 cases, speaks for the correctness of diagnosis. Among these 49 cases, 36 infants became completely normal and 13 became subnormal but educable; this result demonstrates the value of the prudent application of coordinated instrumental therapy and neurorehabilitation.

Various strategies have been advised for detecting and treating subdural effusions. The presence of acute and/or chronic neurological symptoms together with positive neuroradiological evidence as well as an increased protein content in the effusion and occasionally the elevated subdural pressure can be indications to adopt an active conduct. The simultaneous presence of all symptoms and evidence of a considerable amount of effusion in the subdural space justifies repeated punctures or subdural drainage, if punctures are not sufficient to diminish the amount of fluid rapidly. We fully share the opinion of those who experienced the disappearance of subdural effusion after persistent taping. As no early chronic subdural haematoma in our patients was due to previous inflammation we considered them to be of traumatic origin if evidence of difficult labour was present, or of unknown origin. Due caution is necessary to decide for a taping regime, but this seems to be a better proposal than to wait until the effusion is absorbed spontaneously. If all the above-mentioned symptoms are present, simultaneously, this waiting attitude may endanger the

developing brain. If tapping or drainage fail to solve the problem, craniotomy, neomembrane stripping, or the insertion of a valve is necessary.

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Trisomy 5q15-q31 due to maternal insertion, ins (6; 5) (q21; q15q31)

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A female child with dystrophy, psychomotor retardation and dysmorphic signs was seen at the age of 20 months. The phenotypic abnormalities were attributed to 5q15–q31 trisomy that was inherited from an insertion, ins (6; 5) (q21; q15q31) of the mother.

Since the original report by Ferguson-Smith et al. (3) of a partial trisomy 5q, 13 further cases have been published (1, 2, 4, 5, 6, 7, 8). In this paper we describe another case of partial trisomy of the long arm of chromosome 5, associated with an abnormal phenotype. Furthermore, we compare the clinical features of all the 15 patients who have been observed till now.

Case Report

E. V. a female patient was born after an uneventful pregnancy at the 38th week of gestation as the second child of her parents. At the time of her birth, her mother was aged 22 years, her father 25 years. The first child of the couple is a healthy, normal boy, who was born two years earlier. The maternal grandmother had 8 pregnancies, one of which ended in stillbirth. No other events worth mentioning had occurred in the family.

Delivery took place without complication and there was no perinatal problem. Birth weight was 2400 g, body length 40 cm, head circumference 37 cm. The child was first seen at the age of 20 months. At that time, the clinical investigations showed a dystrophic patient. Her weight was 7000 g, her length 78 cm, the head circumference 39 cm. All these parameters were under the 3rd percentile value.

The probanda (Fig. 1) had a brachycephalic skull with facial asymmetry. Her flat face and bulbous nose resembled those of the mother. The upper lip was small and moderate macroglossia could be observed. On the left hand, ectrodactyly and on the right feet symbrachydactyly was present (Figs 2–5).

The child was hypotonic and could not stand or sit without help. Psychomotor development was retarded. Heart catheterization revealed a patent ductus arteriosus with pulmonary hypertension.



FIG. 1. The patient at 20 months of age

CYTOGENETIC FINDINGS

Slides from routinely cultivated blood cells were stained for GAG banding. In all investigated metaphases an abnormal chromosome 6 was found (Fig. 6). The father's cells showed a normal karyotype, while in the metaphases of the mother an insertion between the long arms of chromosome 5 and 6 was observed; the segment q15–q31 of the long arm of chromosome 5 was inserted into the long arm of chromosome 6 at the q21 point (Fig. 7).

The karyotype of the mother could be interpreted as 46, XX, ins (6;5) (q21; q15q31). The karyotype of the



FIG. 2. Left hand of the patient



FIG. 3. Feet of the patient

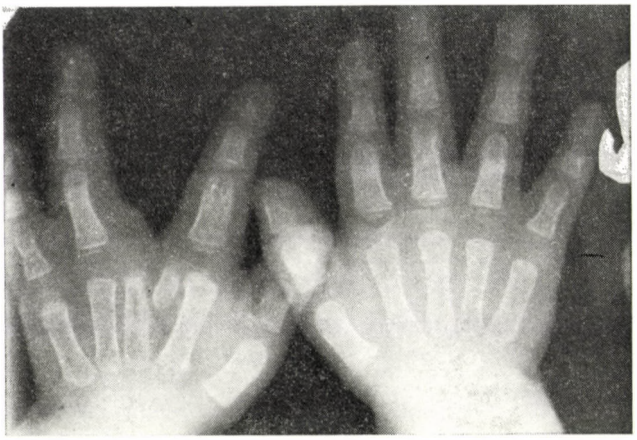

FIG. 4. X-rays of the hands. Right hand: normal osseus development. Left hand: the 3rd finger is missing, only a hypoplastic intrametacarpal mid-phalanx and a small metacarpal bone are present



FIG. 5. X-rays of the feet. Right foot: the distal phalanx of the 2nd toe is missing. A complete cutaneous syndactyly between the 3rd and 4th toes is seen. The distal phalanges of the 3rd, 4th and 5th toes are hypoplastic

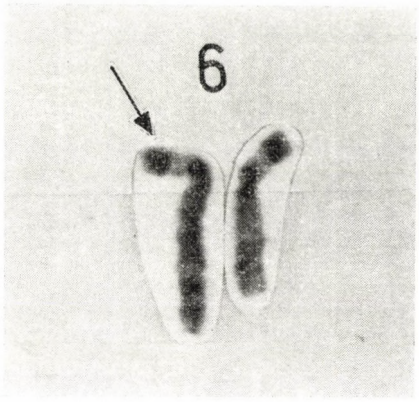

FIG. 6. The abnormal chromosome 6 of the probanda

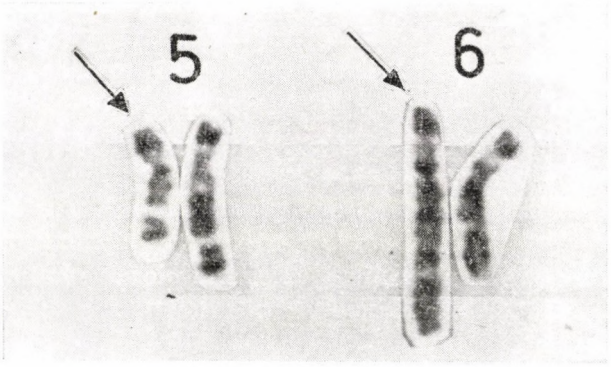

FIG. 7. Chromosomes 5 and 6 of the mother

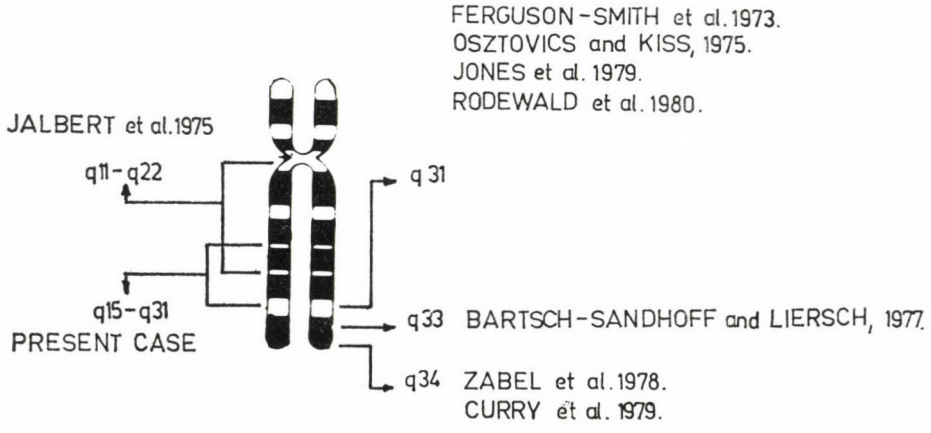

FIG. 8. Distribution of the breakpoints defined in 9 index patients with 5q trisomy

probanda was 46, XX, −6, der (6) (6pter–q21::5q15–q31::6q21–qter) mat. According to the cytogenetic analysis the patient has a 5q15–q31 trisomy.

Discussion

Fifteen cases of partial trisomy of the long arm of chromosome 5 (Table I) were observed on the basis of 9 index patients, as 5q-trisomy occurred four times in close relatives (1, 2, 6, 7). This reduces the number of patients for delineation of the characteristic clinical form of 5q-trisomy. Besides, the trisomic segments of the long arm of chromosome 5 varied (Fig. 8). In one case (4) the proximal part, q11–q22, was involved. Trisomy of the middle part occurred in our present case, where the breakpoints could be localized at q15 and q11. In the remaining 7 cases, a distal type of partial 5q-trisomy occurred, the q31-qter segment was trisomic in 4 cases (3, 5, 6, 7). A q33-qter trisomy was also described (1, 8) as well as a case of q34-qter trisomy (2).

The partial 5q-trisomy proved to be inherited from maternal insertion in 2 out of 9 index patients (4 and in the present case). Partial 5q-trisomy originated from parental translocation in 7 cases, 6 times from the father, once from the mother. In two cases, the second translocation chromosome was chromosome 2 with identical breakpoints (3, 6). In the other 5 families, chromosomes 9, 11, 8, 16 and 22 were defined as second chromosome. Breakage of the second chromosome occurred at the terminal band in each translocation without exception. This circumstance probably allows to disregard the various partial monosomies associated with partial 5q-trisomy when we attempt to compare the clinical picture of the patients.

Independently from the type (proximal, interstitial, terminal), partial 5q-trisomy was associated with low birth weight (under 3000 g), a delay in somatic and psychomotor development, and microcephaly. Congenital heart defect was also frequently found (5/8 index patients and 7/14 investigated patients).

Finger anomalies, too, seemed to be common, although different in severity (8/9 index patients and 9/13 investigated patients); enlarged first toes (4) and clinodactyly by itself (3) or with zygodactyly (8) have also been reported. Preaxial hexadactyly occurred in two patients (6, 7). In one case a hypoplastic thumb connected to the hand by a thin pedicle (5) and in another retroversion of the distal phalanges of the thumbs was found.

One patient showed severe skeletal abnormities (4). This was the only observed case of 5q-trisomy of the proximal type. Therefore, it is not possible to declare that skeletal anomalies are specific for the proximal type of 5q-trisomy. We cannot agree with the recently suggested view (7) that according to the trisomic segments three clinically characteristic phenotypes may be distinguished. We rather believe that many more

TABLE I

Data of patients

	Jalbert et al. (1975)	Present case	Ferguson-Smith et al. (1973)	Osztovics and Kiss (1975)		Jones et al. (1979)
				2*	1	
Age	3y 7/12m	20m	4y	14m	6y	2m
Sex	F	F	M ←	F	F	← F
Growth retardation	–	+	+	+	+	+
Mental deficiency	+	+	+	+	+	+
Birth weight	2880	2400		2700	2650	2190
Microcephaly	M	+	+	+	+	+
Carp-shaped mouth	–	–		–	–	+
Thin lip	–	–		+	+	–
Finger anomaly	+	+	+	+	–	+
Congenital heart defect	VSD	Ductus art. persist.		ASD + VSD	–	ASD + VSD
Origin of 5q-trisomy	ins(1;5) (q32; q11q22) mat	ins(6;5) (q21; q15q31) mat		t(2;5) (p23; q31) pat	t(2;5) (p23; q31) pat	t(5;9) (q31; p24) pat
Trisomic segment	q11–q22	q15–q31		q31–qter	q31–qter	q31–qter

* = index patient

observations are needed to establish the clinical differential diagnosis in the different types of partial 5q-trisomy.

The cranio-facial dysmorphism of patients with distal type 5q-trisomy consists, as far as it has been observed, in microcephaly, hypertelorism, strabism, thin upper lip, carp-shaped mouth and low-set ears.

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with 5q-trisomy

Rodenwald et al. (1980)		Bartsch–Sandhoff and Liersch (1977)		Zabel et al. (1978)	Curry et al. (1979)			
1*		1*	2		1	2	3	4
3y 6/12m	6m	4m	7y	10m	22y	21y	21y	16y
F	F	F	F	M	F	F	M	M
+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	M
1700	2700	2400	3250	2460	2940	2760	2760	2724
+	+	+	+	+	+	+		
+	–	+	+	–	–	–	–	–
–	+	+	+	–	+	+	+	+
+	–	–	–	+	+	+		
ASD	–	VSD	VSD	–	–	–	–	–
t(5;11) (q31; q25)pat		t(5;8) (q33; p23)pat			t(5;22) (q33; p13)pat		t(5;16) (q34; q24)mat	
q31–qter		q33–qter			q33–qter		q34–qter	

M = mild

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11q— syndrome

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A female patient with deletion of the long arm of chromosome 11 is reported. Signs of cranio-facial dysmorphism were a slightly protuberant occiput, dolichocephaly, narrow forehead, antimongoloid slanting of palpebral fissures, carp shaped mouth, mild retrognathia, low hair line, clenched hand with overlapping fingers. The patient died at 3 months of age. Obduction showed a ventricular septal defect and gallstones.

Since the first description of 11q monosomy by Jacobsen et al. (7), 22 further cases have been reported. A delineation of the 11q— syndrome can be based on this material as the reported cases are mostly of de novo type and with few exceptions, the breakage occurred at the same site of the long arm. The present report gives a detailed description of an additional case of 11q deletion and outlines the characteristic features of the syndrome.

Case Report

A. N., a female child, was born from the third pregnancy of her mother. Both parents were healthy and were unrelated. At the birth of the probanda, the mother was 30 and the father 36 years old. The patient had a normal, healthy brother 8 years her senior, born from the first pregnancy. The second pregnancy was aborted in the 10th week of gestation; detailed information concerning this miscarriage was not available. The family history was unremarkable regarding congenital malformations and repeated fetal losses.

The probanda (Fig. 1) was born at the 40th week of gestation. Birth weight was 3350 g, body length, 55 cm, and head circumference, 36 cm. The baby had respiratory difficulties during the first week of life, then she had repeatedly upper respiratory tract infections. She developed normally until the age of 3 months when she suddenly died of pneumonia.

Clinical and cytogenetic examinations were done at 3 weeks of age. Slightly protuberant occiput, dolichocephaly, narrow forehead, clenched hands with overlapping fingers raised a suspicion of 18-trisomy, although prenatal development was not retarded.

Signs of cranio-facial dysmorphism included a slightly protruding forehead with narrow temples, antimongoloid slanting of palpebral fissures, hypertelorism, small upturned nose, down-turned carp shaped mouth, mild retrognathia, high arched palate, low-set ears. The small tongue was

located posteriorly. The posterior hair line was low. The hands were permanently clenched with overlapping of the second and third fingers over the thumb. A slight systolic murmur could be heard.

Radiographs were normal. Red and white blood count, platelet count, serum electrolytes and hepatic enzymes were normal. Autopsy revealed ventricular septal defect of small size and two gallstones.

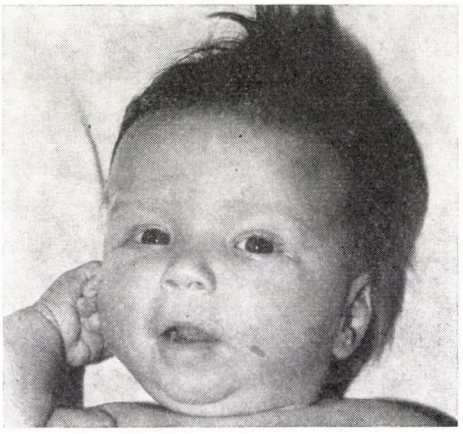

FIG. 1. The patient at 2 months of age

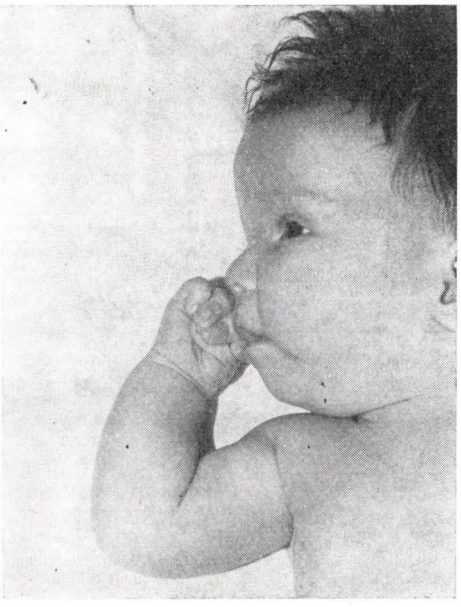

FIG. 2. The clenched hand and overlapping fingers

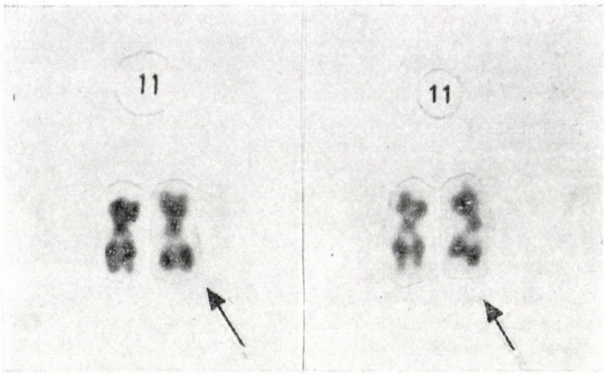

FIG. 3. Chromosome pairs 11 from the patient's karyotype

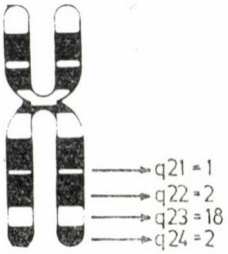

FIG. 4. Distribution of breakpoints in 23 cases of 11q— syndrome

Cytogenetic findings

Slides from routinely cultivated lymphocytes were stained by GAG technique for differential analysis. In all investigated metaphases a deletion of the distal segment of the long arm of chromosome 11, namely q23-qter was observed (Fig. 3).

The karyotype of the patient was thus 46, XX, del(11) (q23). Banded karyotypes of the parents were normal.

DISCUSSION

Up to now, we know of 23 cases of 11q monosomy. 18 originated de novo, 2 were products of a paternal translocation and the type of origin could not be defined in 3 cases (Table I). It is remarkable that the syndrome occurred mostly in females; there were only 3 males among the 23 patients. The average maternal age proved to be 26.7 ± 3.50, the paternal age was 28.9 ± 6.2.

The breakpoints were determined as q23 in most of the cases (18/23). In 4 patients, breakpoints were defined at the adjacent bands q22 and q24 and in one case the deleted chromosome segment was about the half of the long arm (Fig. 4). Fraccaro et al. (5) studied 42 cases with 11q; 22q translocation and breakpoint analyses showed that q23 was a site with high preferential predisposition to breakage.

In the light of the breakpoint analyses it seems probable that a large deficiency, a deletion longer than half of the long arm of chromosome 11 would not be viable. Although nearly half of the patients (11/23) were more than one year old, lethality was considerable in the first year of life. Out of 12 patients under one year of age at the time of the observation, 8 died in infancy (Table I). The postnatal survival rate in cases with small deficiency must also be low, probably not higher than that of patients with other partial monosomy, i.e. 4p–, 5p–, etc.

Severe major malformations are rare in this syndrome. Therefore, the factors leading to early death cannot be general. Severe, multiple major malformations were reported only in a single case (10). It is rather the immaturity (1, 11, 7) and re-

TABLE I

Cytogenetic data, age, sex and parental age in cases with 11q–

		Origin of 11q–	Deleted segment	Age	Sex	Parental age	
						mother	father
Bresson & Noir 1977		de novo	q23–qter	+ 7 d	F	25	21
Cassidy et al 1977		,,	q23–qter	9 m	F	31	35
Engel et al 1976		,,	q23–qter	4.5 y	F	21	20
Faust et al 1974		,,	q21–qter	9 y	F	30	30
Felding & Mittelman 1979		,,	q22–qter	+ 6.5 m		30	30
Frank & Riccardi 1977		,,	q23–qter	7 m	F	33	36
Kaffee et al 1977		,,	q23–qter	+ 22 d	F	23	25
Laurent et al 1979	1		q24–qter	4.5 y	F	26	27
	2	,,	q24–qter	3 m	F	31	31
Lee & Sciorra 1979		,,	q23–qter	+ 23 d	F	24	27
Linarelli et al 1975		,,	q22–qter	12 y	M	32	33
Lippe et al 1980		,,	q23–qter	26 m	F	22	26
Mulcahy & Jenkyn 1977		,,	q23–qter	18 m	F	26	31
Schinzel et al 1977	1	,,	q23–qter	2.5 y	M	24	25
	2	,,	q23–qter	17 m	F	30	31
Turleau et al 1975		,,	q232–qter	14 m	M	24	22
Zabel et al 1977		,,	q23–qter	9 m	F	21	23
Present case		,,	q23–qter	+ 3 m	F	30	36
Larson et al 1976	1	?	q23–qter	23 m	F	34	39
	2	?	q23–qter	15 y	F	15	19
Leonard et al 1979		?	q23–qter	+ 25 d	F	35	43
Jacobsen et al 1973	1	(11 ; 21) pat.	q23–qter	+ 19 m	F	24	25
	2	,,	q23–qter	+ 28 d	F	23	29

TABLE II

Clinical data of patients with 11q—

Investigators	Birth weight	Perinatal problems	Growth retardation	Psychomotor retardation	Recurrent infection	Trigonocephaly	Ptosis of eyelids	Globular nose	Carp mouth	Micro-retrognathia	Clenched hands
Bresson & Noir 1977	2700	+	•	•	•	+	−	+	+	+	−
Cassidy et al 1977	3090	−	−	+	−	+	+	+	+	+	−
Engel et al 1976	2175		+	m	+	+	−	+	−	+	−
Faust et al 1974	2000	−	+	+	+	−	−		−	+	−
Felding & Mittelman 1979	1580	+	+	+	+	+	+	+	+	+	+
Frank & Riccardi 1977	3250	+	−	+	−	+	+		−	+	−
Kaffee et al 1977	3500	+	•	•	+	−	−		−	+	−
Laurent et al 1979 1	2500	−	+	+	−	+	+	+	−	+	−
2	2450	−	−	•	−	−	+	+	−	+	−
Lee & Sciorra 1981	2450	+	•	•	+	+	−	+	+	+	+
Linarelli et al 1975	2350	+	+	+	+	+	+	+	−	+	−
Lippe et al 1980	3400		+	+		−	−	+	+	+	−
Mulcahy & Jenkyn 1977	2600	+	+	+	+	−	−	+	+	+	−
Schinzel et al 1977 1	3090	+	−	+	−	+	+	+	+	+	+
2	3310	−	−	+	+	+	+	+	+	+	−
Turleau et al 1975	2300	−	+	+	−	+	+	+	+	+	−
Zabel et al 1977	2750		+	+	−	+	−	+	−	+	−
Present case	3350	+	+	+	+	−	−	+	+	+	+
Larson et al 1976 1	3480	−	+	+	+	−	+		−	−	−
2	1380	+	+	+	+	−	−		−	−	−
Leonard et al 1979	2350	−	•	•	•	+	−	+	+	+	−
Jacobsen et al 1973 1	2000	+	+	+	−	+	−			+	−
2	1700	+	•	•	•	+	•			+	−

current infections (4, 8 and the present case) which are responsible for death. Perinatal problems, such as respiratory distress, prolonged jaundice, thrombocytopenia, hypoglycaemia were observed in half of the cases with low and normal birth weight alike (1, 4, 6, 7, 8, 9, 10, 12, 14, 15 and the present case). Recurrent infections were mentioned in nearly half of the patients (3, 4, 8, 9, 12, 14, 15 and in the present case).

In the survivors, psychomotor retardation seems to be obligatory although in different degrees, as in some cases mental retardation was slight (3, 17). Growth retardation probably becomes evident only after one year of age (3, 7, 9, 12, 13, 14, 16).

A clinical suspicion of 11q— syndrome can be based on the combination of the following, mainly characteristic, signs of cranio-facial dysmorphism (Table II): the skull is dolichocephalic with keel-shaped protuberant forehead, flattened occiput and narrow temples. This configuration may be very pronounced; out of 23 cases, in 15 cases trigonocephaly was stated radiographically. Nearly constant symptoms are the globular, upturned nose with aplastic ridge, the small, carp shaped mouth, the slightly receding chin and ptosis of the upper eyelids. All these features result in a strikingly characteristic look of the patients, although in several cases it is only the chromosome analysis that reveals the syndrome.

The protuberant occiput, absence of trigonocephaly as well as the clenched hands with overlapping fingers were misleading in the clinical diagnosis of our case. Clenched hands were observed in 3 further patients. Schinzel et al. (15) described in one of their two patients clenched hands with ulnar deviation of the index fingers which overcrossed the thumb and middle fingers. Lee et al. (10) observed clenched hands with bilateral fifth finger camptodactyly accompanied by multiple malformations as an unusual finding in 11q— syndrome. These observes have pointed to the similarity between the clinical picture of 11q- and 18-trisomy. Felding and Mittelman (4) reported clenched hands with supernumerary digits on both sides and the baby was small for dates. This is an association characteristic more of 18-trisomy than of the 11q- syndrome. In view of these observations in 4 patients with 11q— syndrome, the statement of Lee et al. (10) can be supported that 11q monosomy must be considered in the differential diagnostics of 18-trisomy.

Besides the afore-mentioned dysmorphic signs which give the characteristically, funny appearance of patients with 11q— aberration, there are other minor symptoms not consistently observed. The palpebral fissures are variably oriented, epicanthus, hyper- or hypotelorism, long philtrum, coloboma, low set ears have been mentioned in several cases. Out of the major malformations, congenital heart defects occur.

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9p deletion syndrome

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A detailed description of a male patient with deletion of the short arm of chromosome 9 is presented. The most striking finding was that both the phenotype and the behaviour of the patient gave the impression of Down syndrome both after birth and at 3 years of age.

The delineation of the syndrome of 9p monosomy was first given by Alfi et al. (1). Afterwards, several cases were reported in the literature. To our best knowledge, their number now amounts to about 30 (2–8, 10–20). A further case will be reported below. The aim of this report is to point to the similarity between the phenotype of 9p monosomy and that of Down syndrome.

Case Report

F. A., a male patient was born when his mother was 24 years old, the father 25 years old. This was the first pregnancy of the couple. The parents were unrelated and healthy. In the family history there was no event worth mentioning.

After uneventful pregnancy, delivery occurred without complication at the 36th week of gestation. The 1 min Apgar value was 5. The proband did not thrive, his hands and feet were swollen. Birth weight was 3000 g, body length, 51 cm, and the head circumference was 33 cm.

After birth the proband was registered as having Down syndrome. The diagnosis was based on the following clinical features: microcephaly with flattened occiput, ptosis of the upper eyelids, epicanthus, upward and outward slanting palpebral fissures, small nose with anteverted nostrils, small mouth, slight micrognathia, low-set and adherent ears, hypotonicity of the muscles, umbilical hernia and small pelvis (Fig. 1). The only symptom not corresponding to Down syndrome was the size of the hands and feet and the fingers (Fig. 2). In spite of lymphoedema, the hands did not seem broad and the fingers were thin and long. Besides, a protruding frontal bossing, long philtrum, short neck and wide-spaced nipples could be observed. He had also hypospadias.

At 8 months of age when the patient was examined, his weight was 7500 g (25 percentile), length was 70 cm (50 percentile), head circumference was 41 cm (under 3rd percentile). At 20 months of age the psychomotor retardation was expressed. At 3 years, his behaviour resembled that of Down patients, he was amiable and affectionate (Fig. 3).

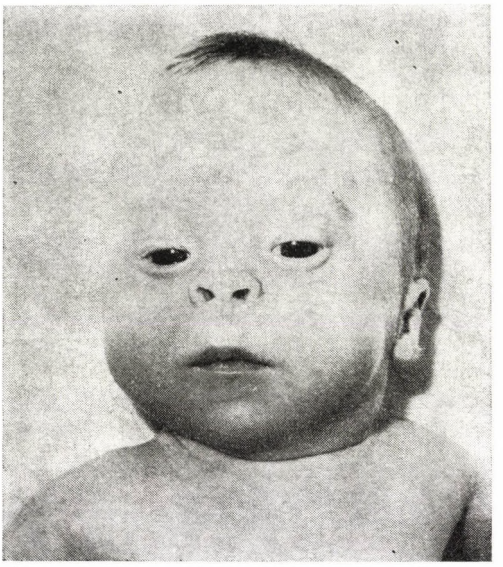

FIG. 1. The patient at 3 months of age



FIG. 2. The hand of the patient

Cytogenetic findings

In routinely cultivated blood cells after GAG staining deletion of the short arm of chromosome 9 was found. The breakpoint was identified as p22. The karyotype of the patient is thus 46, XY, del(9) (p22) (Fig. 4).

Both parents were normal cytogenetically.

DISCUSSION

Partial monosomy of the short arm of chromosome 9 has mostly been observed as a de novo aberration. Parental translocations were found responsible in a few cases (2, 3, 15, 17). An analysis of the breakpoints in 24 reported cases (Fig. 5) showed that the band p22 has mostly been involved; in 18 cases the breakage occurred at this site.

The clinical characteristics of 9p deletion were the subject of several papers (1, 5, 9, 20). The characteristic cranio-facial dysmorphism has been described to consist of trigonocephaly with protruding forehead, mongoloid palpebral fissures, epicanthus, flat and wide bridge of nose, short nose

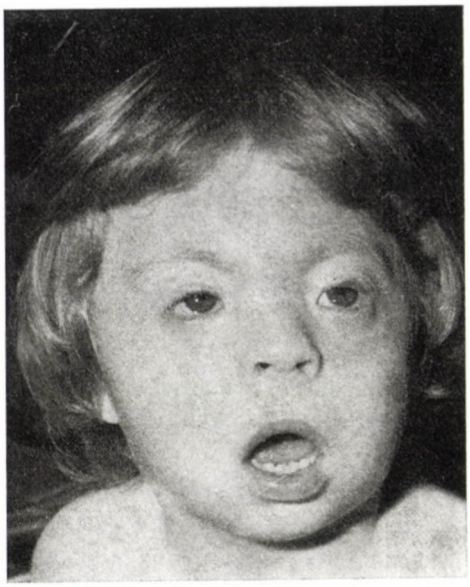

FIG. 3. The patient at 3 years of age

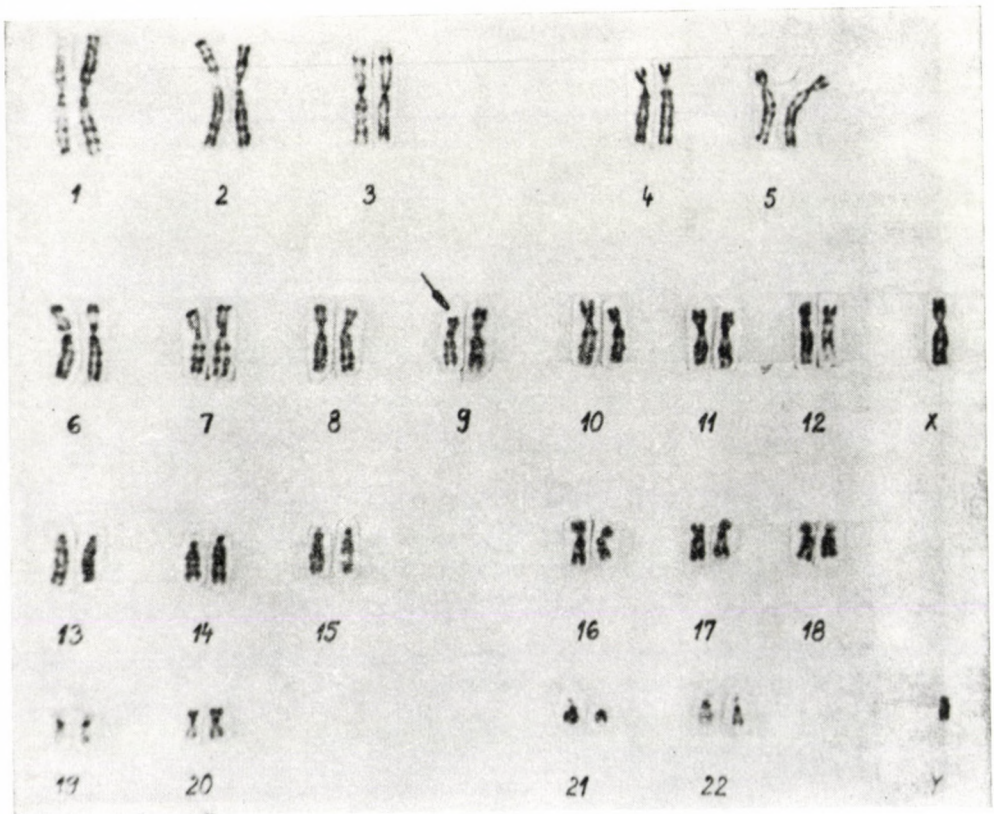

FIG. 4. The karyotype with the deletion of 9p

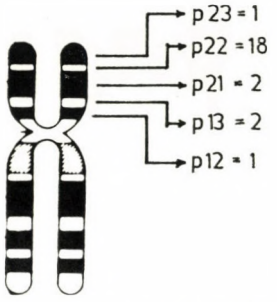

FIG. 5. Distribution of breakpoints in 24 cases of 9p— syndrome

with up-turned nostrils, small mouth, slight micrognathia, low-set and adherent ears. The neck is short, sometimes with more or less pronounced pterygium colli. The fingers and toes are long. The I. Q. is 30–60. Life expectancy does not seem to be diminished significantly.

Listing the main specific symptoms, the likeness between 9p— syndrome and Down syndrome is striking (Table I). There is no other chromosome

TABLE I

Comparison of the clinical symptoms in patients with 9p— and 21-trisomy syndromes

Clinical symptoms	9p—	21-trisomy
Microcephaly	+	+
Trigonomicrocephaly	+	−
Flat occiput	+	+
Short neck	+	+
Protruding forehead	+	+
Upward and outward slanting palpebral fissures	+	+
Epicanthus	+	+
Flat nasal bridge	+	+
Short nose with anteverted nostrils	+	+
Small mouth	+	+
Protruding tongue	−	+
Small, adherent ears	+	+
Broad hands with short fingers	−	+
Long fingers	+	−
Hernia	+	+
Other malformations	−	+
Hypotonicity	+	+
Mental retardation	+	+

aberration where the phenotype would remind of the appearance of patients with 21-trisomy to such a degree. Even our patient's behaviour resembled that of Down patients. In clinical differential diagnostics this has no practical importance, since 9p deletion is a much less frequent chromosome aberration than 21-trisomy. Studies of the molecular basis of the likeness of these two chromosomal syndromes would be more interesting.

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Professor L. Kulin: 80 years



László Kulin was born in the Transylvanian town Zilah in 1901. He studied medicine first in Budapest, then in Debrecen where he joined the staff of the University Paediatric Department in 1925; he remained a member of this institution in all his professional life. In 1933 he spent a year in Vienna working in the Department of Paediatrics. In 1949 he was appointed to the chair of paediatrics in Debrecen where he remained active until his retirement in 1972.

He worked much also outside the walls of his department: From 1946 he participated in the National Committee for Child Welfare. In 1956–57 he was dean of the University Medical School of Debrecen and its rector over the subsequent three years. In 1962–65 he was president of the Hungarian Paediatric Association.

Professor Kulin published nearly hundred papers on a wide range of paediatric topics. His main interest was directed to infantile marasmus.

He observed that high ambient temperature and humidity promoted the weight gain of marasmic infants who would not survive at room temperature; his ideas found their way into textbooks of paediatrics.

Under his leadership a great number of paediatricians grew up, including four professors and twenty-nine department heads.

The secret of Professor Kulin's success lies in his deep humanity, his community minded mentality, his extended knowledge of the full scale of paediatrics and his excellent organisatory capacity. He was devoted to his university and proved to be an excellent leader. He cared for the interests of all his subordinates with understanding.

On the occasion of his eightieth birthday his pupils wish him health, vigour and unchanged hilarity for each day of his subsequent years.

L. KARMAZSIN

Acta Paediatrica Academiae Scientiarum Hungaricae, Vol. 23 (2), pp. 255–260 (1982)

Book reviews

W. H. GADDES: *Learning Disabilities and Brain Function. A Neuropsychological Approach.* Springer Verlag. New York–Heidelberg–Berlin 1980. 403 pages with 45 figures. Price DM 49.50

The book has been written principally for the use of clinical psychologists and teachers of mentally disabled children, but it may be useful for physicians, especially for child neurologists or psychiatrists, for teachers and parents.

The introduction into anatomy and physiology is followed by eleven chapters dealing with nervous system changes leading to learning disabilities. The author cites the most recent results of research in this field. A better understanding is promoted by descriptions of illustrative case at the end of each chapter and there is a short summary for each chapter.

A 10 page summary at the end of the book describes (A) the tests for neuropsychological examination, (B) the syndromes of neuropathological nature, (C) the cerebral nerves and (D) offers literature for more extensive study.

The next 10 pages offer the exact definition of the Latin words and expressions used in the text. There is an extensive literature index and an author and subject index.

In Chapter 1 the author tries to defend his physiological views against anticipated criticism. He is obviously aware of the fact that his approach deals with only one side of the problem, therefore he recommends studies from other sources on psychosocial factors. These factors have to and can be explored in each individual case. The various degrees of cerebral damage are described.

Chapter 2 deals with the incidence and aetiology of learning disabilities.

Structure and function of the nervous system are the topic of Chapter 3, and the next chapter offers a neurophysiological explanation of the learning disorders. A detailed analysis of disabled perception can be found in Chapter 5; abnormalities of perception, of attention and the various forms of agnosia are dealt with here.

The symptoms caused by damaged function of the sensory and motor tracts and the commensurate examination methods are considered in Chapter 6. A classical description of apraxia and agnosia is followed by a presentation of the mild forms often overlooked in childhood, and their impact on special functional disabilities.

Chapter 7 treats cerebral dominance and handedness. Here the combinations of inherited patterns with abnormalities caused by cerebral damage are listed.

Skills needed for writing, syllabication and calculation are dealt with in Chapter 9 together with the disabilities caused by the corresponding structures.

The last two chapters sum up the preceding ones. The therapy of the disabilities are described here; the author emphasizes that improvement can be achieved only when the diagnosis is correct. He stresses the necessity of teamwork in treatment and education.

The book will be of considerable value for the expert interested in learning disabilities; for a deeper knowledge of some special questions he will need to consult more detailed works.

A. MÁTTYUS

Problems in Pediatric Endocrinology. LA CAUZA, C., ROOT, A. W. (eds). Proceedings of the Serono Symposia, Vol. 32. 486 pages. Academic Press, London, New York, Toronto, Sydney, San Francisco 1980. Price: £ 25.20

The Proceedings of the Serono Symposia, published since 1973, mainly cover endocrinological topics. Three years after the 12th volume of the series (Chiumello and Laron: Recent Progress in Pediatric Endocrinology, 1977) a new book has been made available containing the papers presented at the First International Symposium on Problems in Pediatric Endocrinology held in Florence in 1979.

The titles of the first three sections (The Physiopathology of Growth Hormone, pp. 3–151; Perinatal Endocrinology, pp. 155–318; and Evaluation of Skeletal Age, pp. 321–387) show the main topics of the Symposium. The majority of the speakers came from Italy and the USA.

In the introductory report of the first section (Regulation of Secretion of HGH) Root emphasizes the heterogeneity of the HGH molecule (monomeric, dimeric, and probably polymeric forms) and the clinical importance of the new radioreceptor assays of GH (e.g. short children with normal immunoreactive but radioreceptor inactive GH respond with growth to exogenous HGH). From the studies on the control of GH secretion and somatomedin it is again concluded that the regulation of sleep-related secretion of HGH and of stimulus-provoked GH release seem to differ. — The diagnostic value of the short-sleep test with a continuous EEG monitoring system in the study of short stature was confirmed by Bottene. Kaplan and Grumbach introduce their current classification of GH deficiency and disorders of GH metabolism, distinguishing 18 forms. Fig. 1 presenting the frequency of hormonal disorders in 1843 children referred for short stature shows that constitutional short stature was the most common form (50%), and 1° hypothyroidism and gonadal dysgenesis the next most common (11.9% and 11.2% respectively). Also shown was the incidence of congenital hypothalamic hypopituitarism (8%) and of hypothalamic hypopituitary tumours (6.3%). In the chapter on neonatal hypothalamic hypopituitarism there is a description of 13 early diagnosed cases with the conclusion that HGH treatment can be delayed till between the 6th month and the 2nd year of life because in this period, despite evidence of deficient GH responsiveness to stimuli, the growth rate is in the normal range.

An excellent report by Gardner discusses deprivation dwarfism. La Cauza et al. write about the limiting factors of somatomedin activity, and Cacciari et al. report on HGH therapy and 5α-reductase activity. Some interesting case reports are also found in this section.

The second section contains a very good review about congenital Graves's disease written by Hollingworth et al. Infants coming from families in whom several generations of Graves's disease have been diagnosed have often severe and prolonged forms of the disorder. The findings in twins indicate that two infants exposed to the same maternal intrauterine environment may respond in different ways. It is emphasized that the thyroid stimulating immuno-globulins (TSI) are immunological markers for Graves's disease rather than its definitive cause. The chapter of Reynolds about fetal and neonatal steroid metabolism deals with many important questions. The rest of the papers discusses congenital hypothyroidism (specially the Italian experience), diabetes mellitus of mothers and infants, and some

hormones in perinatal age i.e. LH, prolactin and plasma renin activity.

In the introductory report of the 3rd section (Principles in the Evaluating of Skeletal Age) Tanner emphasizes that the so-called Tanner–Whitehouse bone-ages are logically and mathematically different from the bone-ages derived by any of the Atlas, direct to bone-age techniques, and their system makes it possible to distinguish skeletal maturity versus skeletal age. The seven following papers discuss some aspects of this topic. — After 14 further short communications there is a subject index to help the reader.

The book is a great asset to paediatric endocrinologists and a further indication of the high scientific level of the Serono Symposia.

F. PÉTER

J. M. BEASLEY, S. E. F. JONES: *A Guide to Paediatric Anaesthesia.* Blackwell Scientific Publications, Oxford 1980. 167 pages. Price £ 5.80

This thin paperback by two Birmingham anaesthesists is intended, as it is said in the Preface, to be a practical guide for those in training and for those who only occasionally anaesthetize children. As it explains all possible details and many of the knacks and tricks of the know-how of anaesthesia and even intensive care, the book will have success, especially in the U.K. as practically all of the apparatus and most of the devices recommended are British made. The chapters deal with preoperative assessment, premedication, induction of anaesthesia and intubation with a list of the commonly used drugs, the maintenance and termination of anaesthesia, with neonatal anaesthesia including fluid replacement and blood transfusion, anaesthesia for special operations, monitoring, anaesthesia for radiological investigations (where computer tomography is not mentioned), postoperative and intensive care, the conditions affecting anaesthesia and its intraoperative complications including scoline apnoea and malignant hyperpyrexia, among others

K. JELLINEK

K. JÄHRIG, D, D. JÄHRIG, P. MEISEL: *Phototherapie.* Georg Thieme, Leipzig 1981. 138 Seiten mit 23 Abbildungen, 7 Tabellen und 2 Beilagen. Preis M 26,–

Das in der Reihe »Moderne Pädiatrie« erschienene Büchlein bietet eine ausgezeichnete, ausführliche Darlegung der Probleme hinsichtlich der beim Neugeborenenikterus angewandten Phototherapie.

Einleitend wird die Geschichte besprochen, deren Grundlagen bis zum 18. Jahrhundert zurückgehen. Dann werden die photochemischen und physiologischen Grundlagen: der Mechanismus des physiologischen Ikterus des Neugeborenen, die physiologischen und biochemischen Grundlagen des Bilirubinstoffwechsels und der Photokatabolismus des Bilirubins erörtert. Ein Kapitel schildert die Technik der Lichtbehandlung; hier werden die verschiedenen Strahlungsquellen und deren physikalischen Eigenheiten und biologische Effektivität angeführt. Ein anderes Kapitel befaßt sich mit der physiologischen Wirkung des Lichtes. Der überwiegende Teil der Monographie ist jedoch dem Klinikum gewidmet. Es werden erst die praktische Ausführung der Bestrahlung, die Indikationen und Kontraindikationen, Nebenwirkungen die Wirkung beeinflussenden klinischen Faktoren, die ergänzenden und zusätzlichen therapeutischen Maßnahmen behandelt. Sodann werden die Erfahrungen bei der Nachuntersuchung der Neugeborenen, ferner die Problematik der Phototherapie im Zusammenhang mit den angewandten Behandlungsmethoden bei den verschiedenen Formen des Neugeborenenikterus besprochen. Abschließend wird über jene Fragen berichtet, die weitere Klärung erfordern, wie z.B. die

photopharmakologische Wirkung, die Interaktion von Medikamenten und Licht oder das Verhältnis zwischen Licht und zellulärer Immunantwort und der komplexe photobiologische Effekt. In dem Inhaltsverzeichnis finden wir von der heute schon kaum übersichtbaren Füllen von Publikationen die etwa 135 wichtigsten Übersichtsarbeiten.

Die Monographie vermittelt dem praktisch tätigen Neonatologen ein gutes und auch kritisches Bild hinsichtlich des gegenwärtigen Standes der Kenntnisse über die Phototherapie.

E. VÁRADY

K. AAS: *Das allergische Kind*. Deutsche Übersetzung von W. Werner. 2., überarbeitete Auflage. G. Thieme Verlag, Stuttgart–New York 1981. X + 264 Seiten mit 8 Abbildungen. Preis DM 19,80

Der große Erfolg dieses vielseitig verwertbaren Buches rechtfertigte die vorliegende zweite deutsche Auflage. Die Verwendung des Werkes kann auf drei Ebenen erfolgen: 1. allergische Kranke und Eltern allergischer Kinder können fachmäßige Information und praktische Ratschläge in leicht faßbarer Weise erhalten; 2. allgemeine pädiatrische Praxis ausübende Ärzte können gut zusammengefaßte und auf den neusten Stand gebrachte Kenntnisse über die kindlichen allergischen Erkrankungen erwerben; 3. die sich mit allergischen Krankheiten intensiver befassenden Spezialisten können für den entsprechenden Kontakt mit den Eltern und auch zum Unterricht einige gute Ideen verschaffen.

Das erste Viertel der Arbeit befaßt sich mit allgemeinen Kenntnissen in bezug auf das Wesen des allergischen Zustandes, mit dem Pathomechanismus der Allergie und den wichtigsten Allergenen, den Möglichkeiten der Allergieprophylaxe. Die weiteren Kapitel sind den zur Zeit als allergisch betrachteten Krankheiltsbildern gewidmet, unter denen man auch solche findet, die in anderen Fachwerken nicht zu finden sind, wie z.B. die Allergie der Augen, Kopfschmerzen, usw. In zwei kurzen Kapiteln wird auf die Bedeutung der psychosomatischen, meteorologischen- und Umweltfaktoren hingewiesen. Hiernach behandelt das Buch die Allergiediagnostik, die verschiedenen Testmethoden; die Anweisungen für die Eliminations- und Provokationsdiäten sind besonders nützlich. Hiernach werden die heute zur Verfügung stehenden Behandlungsmaßnahmen geschildert. Abschließend werden Physiotherapie und Heilgymnastik kurz — vielleicht zu kurz — erörtert und die verschiedenen Impfstoffe, ferner einige praktische Probleme besprochen.

Mehr Abbildungen hätten den informativen Wert des Buches noch steigern können.

E. CSERHÁTI

Amniotic Fluid and Its Clinical Significance. M. SANDLER (ed.) Marcel Dekker, New York 1981. 328 pages. Price SFr 98.—

Amniotic fluid has become one of the important materials for prenatal diagnosis. Details of its different aspects are presented in this excellent book. The first chapter, discussing the biochemistry of amniotic fluid, offers a good survey of the origin of its chemical constituents and their concentrations in normal pregnancies. The second chapter deals with amniocentesis, its ethical questions, and its possible complications in the mother and fetus. The next part discusses the cytology and the chromosomal analysis of cultured cells. Gosden et al. describe in detail the characteristics of the rapidly adhering cells and their use in prenatal diagnostics of developmental anomalies. It must be mentioned that most of these cells can well be examined without culturing and so rapid orientation will almost always be possible even in smears (Clinical Genetics 16: 282–290, 1979).

A chapter is devoted to the techniques of sonography and its use for estimation of the volume of amniotic fluid. Then Sutcliffe gives an excellent review of the amniotic fluid proteins and their clinical significance. The next subject is the prenatal screening of neural tube defects and the use of amniotic fluid cholinesterase examination. The biochemical methods of estimation of the maturity of the fetal lung in late pregnancy are described in two chapters; they will be useful for the obstetrician rather than the paediatrician. Finally, we find a very good review on the prenatal diagnosis of inherited metabolic disorders; it goes into many details but the subject would have deserved a deeper discussion.

All in all, this is a very useful book; it will offer many useful informations to the clinical geneticist, obstetrician, paediatrician and to all those interested in this recently explored field of medicine.

Z. PAPP

Kinderärztliche Notfälle. Begründet von B. DE RUDDER. Neubearbeitet von A. WINDORFER, H. TRUCKENBRODT. 10., neubearbeitete und erweiterte Auflage. Georg Thieme Verlag, Stuttgart–New York 1981. XII + 386 Seiten mit 48 Abbildungen und 24 Tabellen. Preis DM 36,—

Das Taschenbuch ist die 10. deutsche Auflage des von de Rudder unter dem Titel »Kinderärztliche Notfallfibel« 1949 herausgegebenen Werkes. Es ist ein ausgesprochen für praktische Zwecke konzipiertes Buch, das bei Notfällen als Wegweiser zur raschen Erfassung und zum raschen ärztlichen Handeln dienen soll.

Der sich gut bewährte Aufbau wurde beibehalten, die einzelnen Kapitel jedoch überarbeitet und ergänzt, wie z.B. die technischen Hinweise zur Notfalltherapie. Ganz neu verfaßt wurde die medikamentöse Notfalltherapie, und als neue Beiträge wurden unter anderen das mukokutane Lymphknotensyndrom, die maligne Hyperthermie, akute Kälteschäden, Drogennotfälle, die hypertonische Krise, Puderaspiration usw. aufgenommen.

Dem obigen Zweck entsprechend werden die Krankheitsbilder, ohne weitläufigen Ausführungen, von Leitsymptomen und -syndromen ausgehend differenziert und sodann die dringlichen therapeutischen Maßnahmen angeführt. Ausgezeichnete Abbildungen, Tabellen und ein ausführliches Sachverzeichnis unterstützen die schnelle Orientierung.

Das Buch enthält wohl alles, was dem Arzt in schweren dringlichen Situationen helfen kann. Den Wert der Arbeit beweist nichts besser, als die Tatsache, daß sie in den verstrichenen 32 Jahren außer den 10 deutschen wiederholte italienische und spanische, ferner französische, japanische und polnische, insgesamt 19 Auflagen erlebt hat.

K. SCHMIDT

Pädiatrische Diagnose und Differentialdiagnose. Herausgegeben von A. DITTMER unter Mitarbeit von 16 Autoren. Georg Thieme, Leipzig 1981. 926 Seiten mit 186 Abbildungen und 205 Tabellen. Preis M 78.—

Die Verfassung eines derartigen Werkes hat die 17 Autoren vor eine beträchtliche Aufgabe gestellt. Das Ziel der Unternehmung war, auf die durch die Lehrbücher vermittelten Grundkenntnisse bauend, doch diese überschreitend, ein Gesamtbild der pädiatrischen Diagnostik und Differentialdiagnostik zu bieten. Dies dürfte vorbildlich gelungen sein.

Das Buch besteht aus 2 Bänden. Der erste enthält 7 Kapitel, die den Allgemeinen Teil darstellen. Hier werden die Grundlagen der Diagnosestellung, die medizinisch-genetische Diagnostik, das Immunsystem und die Diagnostik der Immunpathologie, Serumeiweiß und seine Bedeutung für die Diagnostik, ausgewählte mikrobiologische bzw. klinisch-chemische Untersuchungen besprochen. Das letzte Kapitel dieses Bandes gehört

bereits zum Speziellen Teil und befaßt sich mit dem Neugeborenen.

In dem zweiten Band werden im Rahmen von 12 Kapiteln die somatischen Entwicklungsstörungen, die systematischen Skeletterkrankungen, Störungen der endokrinen Drüsenfunktionen, die Malabsorptionssyndrome, Stoffwechselstörungen und Avitaminosen, die Erkrankungen des Verdauungstraktes und jene mit zellulärer Blutveränderungen oder verstärkter Blutungsneigung, sodann Infektionen, rheumatische Krankheiten, Erkrankungen der Harnwege, die des Herzens, der Atemwege und Lunge und schließlich die neurologischen Erkrankungen und Verhaltensstörungen behandelt. Außer einem Gesamtschrifttum findet man abschließend ein klinisch-syndromal und dann ein diagnostisch-methodisch zusammengestelltes Sachwortverzeichnis.

Der Grundkonzeption entsprechend wurde getrachtet, die richtigen Diagnosen von zwei Richtungen aus zu erreichen, einerseits aufgrund des in den Lehrbüchern dargestellten Diagnose-Differentialdiagnosesystems und anderseits symptomatologisch, ausgehend von den dominierenden Leitsymptomen und den spezifischen diagnostischen Verfahren. Zur entsprechenden Diagnosestellung brauchen wir jedoch stets mehr physiologische, pathophysiologische und pathogenetische Kenntnisse und auch stets bessere Untersuchungsmethoden. Den einzelnen Autoren wurde es überlassen, diese Gesichtspunkte in ihren Kapiteln nach eigenem Ermessen zur Geltung zu bringen.

Die demonstrativen Abbildungen, geistreichen Tabellen und Piktogramme und die gute Drucktechnik sollen hervorgehoben werden.

Das Ziel jeder Diagnostik ist die rasche und beste Therapie, und auf diese praktische Forderung ist das Werk angelegt; dem praktisch tätigen Kinderarzt wird also ein Buch in die Hand gelegt, das ihm eine bedeutende Hilfe leisten wird.

K. SCHMIDT

S. ESSBACH und AUTORENKOLLEKTIV: *Ein Kind kann keine Schule besuchen — hat es überhaupt eine Entwicklungschance?* Verlag Volk und Gesundheit, Berlin 1981. 190 Seiten mit 21 Abbildungen und 3 Tabellen. Preis M 18.—

In den letzten 12 Jahren ist in der DDR eine große Anzahl von rehabilitationspädagogischen Fördereinrichtungen entstanden, die etwa 70% der schulbildungsunfähigen Kinder und Jugendlichen aufnehmen, um sie auf das Leben in der Gesellschaft zu vorbereiten. Große Anstrengungen werden unternommen, um in den nächsten Jahren für die restlichen gleichartigen Kinder und Jugendlichen ebensolche Möglichkeiten zu schaffen. Die Bestrebung des aus neun Mitgliedern bestehenden multidisziplinären Autorenkollektivs — der Forschungsgruppe der Sektion Rehabilitationspädagogik der Humboldt-Universität zu Berlin, unter der Leitung von Doz. Eßbach — war die Bearbeitung des Problemenkreises der wissenschaftlichen Erkentnisse als Voraussetzung für die erfolgreiche Gestaltung der rehabilitativen Bildung und Erziehung. In der Publikation werden die Erkenntnisse und die daraus abgeleiteten Schlußfolgerungen für den Förderungsprozeß vorgelegt. Die theoretisch begründeten Aussagen werden auch in enger Zusammenwirkung mit vielen bewährten Praktikern auf ihre Anwendbarkeit untersucht.

Die Publikation, als Informationsschrift zur Bildung und Erziehung schulisch nicht mehr bildbarer, rehabilitationspädagogisch jedoch noch förderungsfähiger hirngeschädigter Kinder, wendet sich nicht nur an Fachkräfte, sondern schließt als Leser Eltern und die gesellschaftliche Öffentlichkeit ein, weil die Autoren — ohne die Bedeutung des Förderungsprozesses in den verschiedenen Einrichtungen mindern zu wollen, — überzeugt sind, daß dieser vor allem von Eltern und auch weiteren gesellschaftlichen Kräften mit unterstützt werden muß.

J. FALK

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CONTENTS

Intravenous glucose tolerance test in childhood obesity. *D Molnár, M Kardos, G Soltész, S Baranyai* 127

Effect of glucagon infusion on some plasma metabolites and hormones in obese children. *G Soltész, D Molnár, M Kardos, Zs Baranyai* 137

Haemodialysis treatment of acute renal failure in hypertonic dehydration. *I Marosvári, S Köves* 145

Dynamics of adrenal steroidogenesis in childhood: steroid excretion in prepubertal and pubertal girls. *L Kecskés, Zs Juricskay, Zs Tatai, M Szécsényi* 151

Skin haemangioma: treatment by cryosurgery. *K Pólus* 167

Lymphocyte markers in childhood ALL: the lack of correlation with prognosis. *E Cristo Morón, T Révész, D Schuler* 171

The cerebrospinal fluid of newborn infants with perinatal hypoxia. *M Fekete, Gy Soltész, M Horváth* 179

Thyrotropin and prolactin response to ambient temperature in newborn infants. *F Ruppert, E Sulyok, F Varga, I F Csaba, A Petz* 189

Effect of intravenous alanine on blood glucose, glucagon and growth hormone levels in diabetic children. *G Soltész, D Molnár, J Mestyán* 195

Current issues in the practice of genetic counselling. *A Czeizel* 201

Über die Pathogenese des Bronze-Baby-Syndroms: Koproporphyrinentleerung hyperbilirubinämischer Neugeborenen. *F V Lukács, M Breckner, E Lázár, K Sziráki, C Sulyok* 211

Subdural effusion in the first six months of life. *E Katona, M Halász, M Berényi, P Szabados, K Izsák* 219

Trisomy 5q15—q31 due to maternal insertion, ins (6; 5) (q21; q15q31). *M Osztovics, P Kiss* 231

11q—syndrome. *M Osztovics, G Jójárt* 239

9p deletion syndrome. *O Wilhelm, M Osztovics* 247

Professor L Kulin: 80 years 253

Book reviews 255

Index: 26 021

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Down syndrome: correspondence of clinical diagnosis and karyotype

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Of 286 children diagnosed clinically as Down syndrome, in 7% the cytogenetic result did not support the original diagnosis. Errors were more frequent in the age group below one year (8%) than beyond that age (3.5%). In half of the misdiagnosed cases the karyotype revealed some rare chromosomal abnormality. The main cause of the errors is that the diagnosis of Down syndrome in infancy rests on the craniofacial and other dysmorphic signs while the equally important psychomotor disturbances cannot be judged reliably in early age.

Studies of the correlation between phenotype and karyotype have significantly been promoted by the application of new banding techniques. The so-called clinical mapping of chromosomes, the analysis of the pattern of symptoms common in individuals with the same chromosome aberration permitted to identify the clinical manifestation not only of the different chromosomes, but also of the different chromosome segments. During the last decade, data have been collected on the responsibility of e.g. the 4p16 and 5p15 and 21q22q segments for the Wolf-Hirschhorn, cri du chat and Down syndrome, respectively. Furthermore, as Shapiro has postulated [29] a chromosomal imbalance increases the instability of the embryonic development of the traits which show the greatest variation in the average population, thus the traits of the head and face. The craniofacial dysmorphia is much more characteristic of chromosome aberrations than are gross malformations [18]. Its detailed description has made it possible to define "chromosome syndromes" in increasing number.

The purpose of this paper is to present data on the correspondence of the clinical and cytogenetic findings in Down syndrome, the most common chromosome aberration.

Material and Methods

The study was carried out on a material consisting of children born with clinically stated Down syndrome in Budapest between 1970 and 1979. The material has been divided into two groups (Table I).

Group 1 includes cases which had been registered in the Hungarian Congenital Malformations Registry (HCMR) during the above mentioned period. According to the basic principle of the HCMR [9], the clinical diagnosis was registered within the first year of life. Out of 245 registered cases, 201 have been karyotyped; the rate of cytogenetic verification amounted to 82%.

Group 2 consists of cases sent to our laboratory by district paediatricians in Budapest who have been asked to inform us

bi-yearly on children with Down syndrome previously not notified to the HCMR. These patients were born at the same period and at the time of notification they were older than one year. 85 of 95 cases were cytogenetically investigated, the rate of cytogenetic verification was 89.5%.

Chromosome analysis was carried out, with few exceptions, in peripheral blood cultures. In certain cases skin biopsy was performed. In all cases the GAG technique [33] was used, in some cases the CB technique [34] was also applied.*

Results

Out of 286 cytogenetically investigated subjects suspected for having Down syndrome, in 267 trisomy 21 was revealed. The rate of misdiagnosis was higher in the group of patients under one year of age (8%) than in older children (3.5%).

Table II shows the cases where no trisomy 21 was found. Some important data of these patients are as follows.

Patient No. 1. After birth, the dysmorphic phenotype characterized by a round face with flat profile, epicanthus, flat nasal bridge and slight muscle hypotonicity indicated Down syndrome; therefore, the newborn male infant was reported to the HCMR. At 3 months of age he was admitted with an aspecific hyperthermia; clinical and electronmicroscopic examinations revealed a hypo-

Table I

Yearly distribution of number of HCMR registered and referred patients with Down syndrome, rate and results of cytogenetic verification

Year of birth	Group 1			Group 2		
	Registered in HCMR No.	No. of cytogenetically investigated subjects	Trisomy 21 not verified No.	Reported by paediatricians No.	No. of cytogenetically investigated subjects	Trisomy 21 not verified No.
1970	23	22	3	9	9	0
1971	19	16	2	12	10	1
1972	15	12	3	11	10	0
1973	23	16	2	9	7	0
1974	28	22	2	8	8	1
1975	30	25	1	11	8	0
1976	28	23	0	6	5	0
1977	25	23	1	11	11	0
1978	31	25	1	7	7	0
1979	23	17	1	11	10	1
Total	245	201	16	95	85	3
Per cent		82%	8%		90%	3%

* In 46 cases, chromosome investigation was done at the Second Department of Paediatrics, Semmelweis University Medical School, Budapest. For submitting the results we are indebted to Drs J. Szollár and M. Dobos.

TABLE II

Data of patients diagnosed as mongoloids whose karyotype did not show trisomy 21

No.	Age at clinical diagnosis	Age at chromosome investigation	Phenotype at chromosome investigation	Karyotype
1.	6 d	3 m	dysmorphic, hypohidrotic ectodermal dysplasia	46, XY
2.	5 d	5 m	dysmorphic, not suspected DS	47, XXX
3.	6 d	14 m	dysmorphic, suspected DS	49, XXXXY
4.	4 w	10 m	dysmorphic, suspected DS	47, XX, +mar
5.	8 w	18 m	dysmorphic, not suspected DS	46, XX/47, XX, +22
6.	3 w	8 m	dysmorphic, suspected DS	46, XY, del(9) (p22)
7.	8 d	8 d	dysmorphic, suspected DS	46, XY, der(9), t(3; 9) (q27; q34)mat.
8.	14 d	10 m	dysmorphic, not suspected DS	46, XX, der(2), t(2; 5) (p23; q31)pat.
9.	8 d	3 m	dysmorphic, not suspected DS	46, XX, der(15), t(6; 15) (q27; q24)mat.
10.	9 w	12 m	dysmorphic, not suspected DS	46, XX, ? i(16p)
11.	6 d	9 m	dysmorphic, not suspected DS	46, XX
12.	7 d	4 m	dysmorphic, not suspected DS	46, XY
13.	3 d	3 m	dysmorphic, not suspected DS	46, XY
14.	6 d	6 m	eumorphic, normal	46, XX
15.	4 w	2 m	eumorphic, normal	46, XY
16.	3 w	3 w	eumorphic, normal	46, XY
17.	2.5 y	2.5 y	eumorphic, normal	46, XX
18.	4 y	4 y	eumorphic, normal	46, XX
19.	1.5 y	2 y	eumorphic, normal	46, XX

hidrotic ectodermal dysplasia.* The karyotype proved to be normal. In the following years, the phenotype of the patient corresponded to that usually observed in hypohidrotic ectodermal dysplasia (Fig. 1).

* We are indebted to Dr. E. Török, Chief of the Department of Dermatology, Heim Pál Children's Hospital, Budapest, for submitting the data.

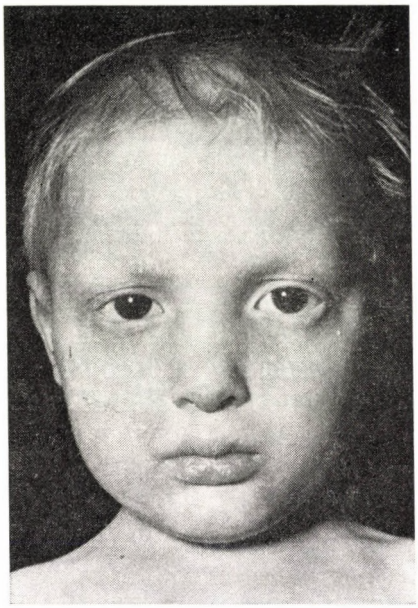


FIG. 1. Patient No. 1 at age of 2 years

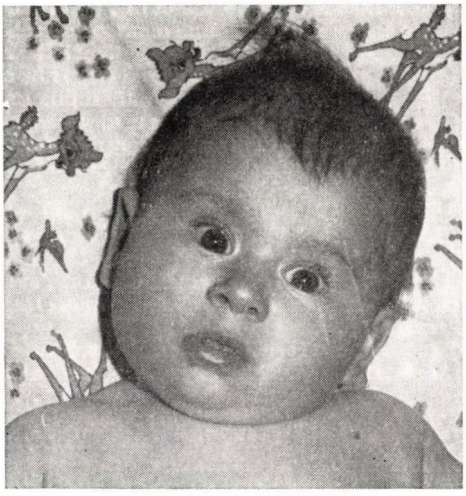


FIG. 2. Patient No. 2 at age of 5 months

Patient No. 2. The female infant was diagnosed as having Down syndrome at birth. Flat occiput, upward and outward slanting palpebral fissures, small nose, small mouth, hypotonicity, bilateral transverse palmar creases, clinodactyly were observed. At 5 months of age (Fig. 2) her psychomotor development was normal and except for a mongoloid ocular

region, her appearance and behaviour did not remind of Down syndrome; her karyotype was 47, XXX.

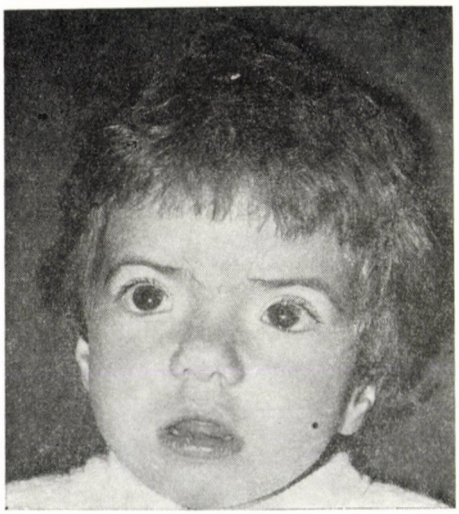
Patient No. 3. This dysmature male newborn was delivered at the 36th week of gestation, with 1880 g weight and 36 cm length. He was cyanotic and had no spontaneous respiration. A small, round head 30 cm in circumference, hypertelorism, slightly slanting palpebral fissures with epicanthal folds, small nose, short neck, brachymesophalangy, clinodactyly, unilateral transverse palmar crease were the significant signs evaluated as features of Down syndrome.

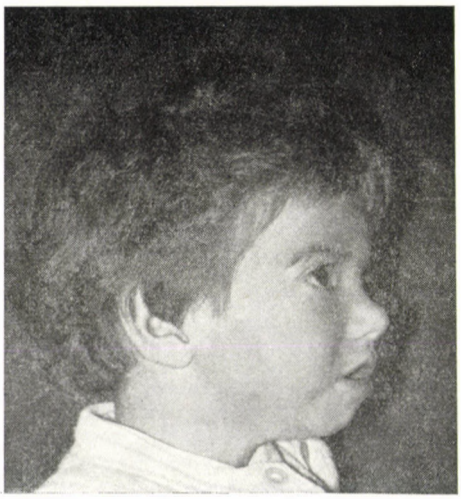
When the patient was 14 months of age a chromosome investigation was carried out. As the clinical diagnosis could not unequivocally be rejected, although the head configuration, the upturned nose and the severe malformation of the external genitalia (hypoplastic scrotum, bilateral kryptorchism) did not fit into the picture of Down syndrome (Figs 3, 4). The karyotype was 49, XXXXY.

Patient No. 4. This female infant at the 4th week of life was registered as having Down syndrome. She had a small head with flat occiput, round face with flat profile, epicanthal folds, slightly upward and outward slanting palpebral fissures, small nose and mouth, protruding tongue, brachymesophalangy and clinodactyly. She was referred for chromosome analysis when she was 10-month-old. The mental retardation was obvious, the ocular movements and her friendly behaviour gave the impression of a Down patient (Fig. 5).

Chromosome analysis revealed a supernumerary small, metacentric, chromosome, shorter than chromosomes 19—20 (Fig. 6). The karyotype was 47, XX, +mar.

Patient No. 5. Registration with Down syndrome occurred when the girl was 2-month-old. Chromosome





FIGS 3—4. Patient No. 3 at age of 23 months

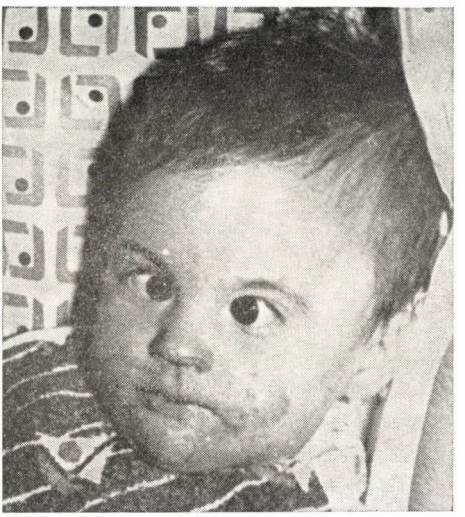


FIG. 5. Patient No. 4 at age of 20 months

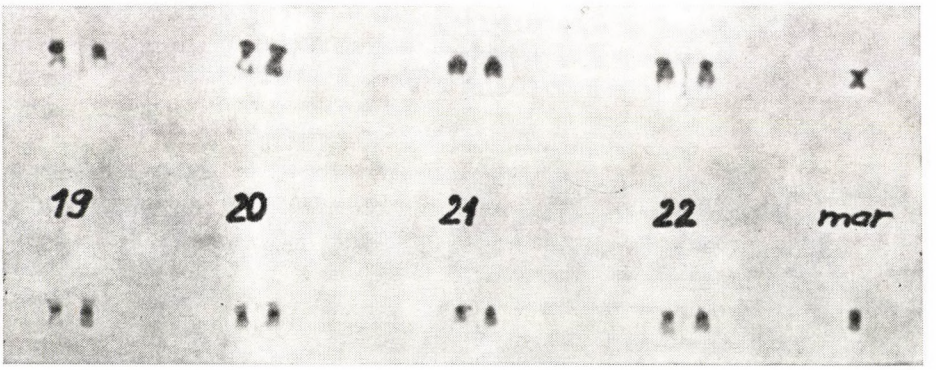
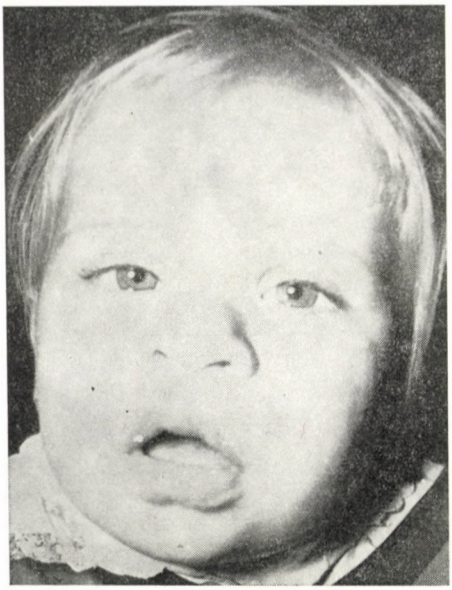


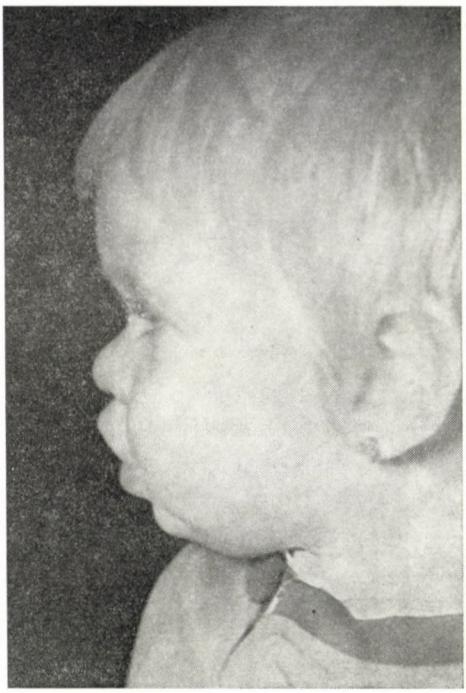
FIG. 6. Chromosomes 19—20 and the marker chromosome from karyotypes of patient No. 4 (GAG and CB staining)

investigation was performed at 18 months of age, when her appearance did not indicate the preceding clinical diagnosis (Figs 7, 8). Her karyotype was stated as 46, XX/47, XX, +22. A detailed description was given previously [21].

Patient No. 6. This male infant was recorded by the HCMR with Down syndrome at the age of 3 weeks. At the time of cytogenetic investigation, at 8 months of age, his external appearance and psychomotor status reminded of mongoloid patients but his long fingers and toes were inconsistent with Down syndrome (Fig. 9). His karyotype was 46, XY, del(9) (p22). The case has been reported recently [38].

Patient No. 7. This dysmature baby was born at the 40th week of gestation, with 2500 g body weight and





FIGS 7—8. Patient No. 5 at age of 18 months



FIG. 9. Patient No. 6 at age of 3 months

50 cm length and 31 cm head circumference. After birth he had melaena and respiratory difficulties. He died on the 8th day of life. Down syndrome was diagnosed on the basis of a small, round head, short neck with an excess of skin, outward and upward slanting palpebral fissures, epicanthal folds, hypoplastic nasal bridge, low set ears, brachymesophalangy, clinodactyly, bilateral transverse palmar creases, bilateral deep longitudinal furrows between the first and second toes, diastasis of the rectus abdominis muscle, umbilical hernia, congenital heart failure and malformations of the external genitalia such as a hypoplastic scrotum, scrotal hypospadias, kryptorchism on both sides. In addition to the observed features, obduction showed multiple visceral malformations: ventral septal defect, coarctation of the aorta, patent ductus arteriosus, atypical lobation of the lung, absence of the gall bladder and renal agenesis on the right side.*

Karyotype analysis was carried out in skin fibroblasts obtained postmortally, they revealed a structurally abnormal chromosome 9. After investigation of the parents, partial trisomy 3q, trisomy of 3q27-qter



FIG. 10. Structurally abnormal chromosome 9 of patient No. 7 and translocated chromosomes 3 and 9 of his mother

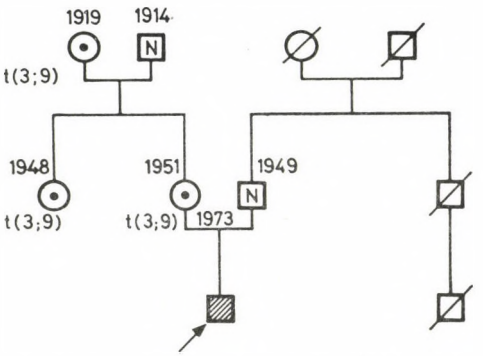


FIG. 11. Pedigree of patient No. 7

* We are indebted to Dr. E. Barb, Heim Pál Children's Hospital, Budapest, for submitting data of the patient.



FIG. 12. Patient No. 8 at age of 4 weeks

segments inherited from maternal translocation t (3; 9) (q27; q34) was stated. The abnormal chromosomes of the child and his mother are shown in Fig. 10. The balanced chromosome aberration, the translocation proved to be familial (Fig. 11).

Patient No. 8. This infant was reported to the HCMR with Down syndrome at 2 weeks of age (Fig. 12). The clinical diagnosis was based on two symptoms, *viz.* a flat, round face and epicanthus. At the time of chromosome investigation, the 10-month-old child did not remind of mongoloids. Her chromosome aberration, partial trisomy 5q, trisomy of 5q31-qter segments was inherited from a



FIGS 13—14. Patient No. 9 at age of 2 years

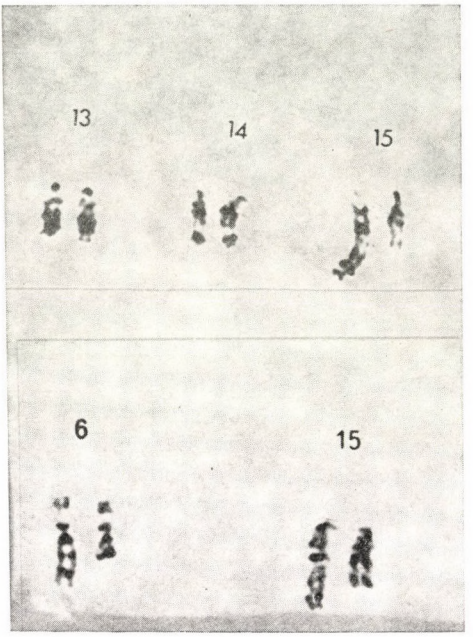


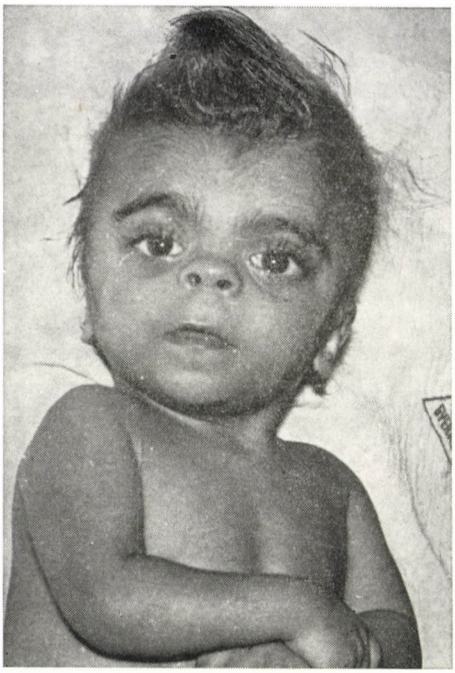
FIG. 15. Structurally abnormal chromosome 15 of patient No. 9 and the translocated chromosomes 6 and 15 of her mother

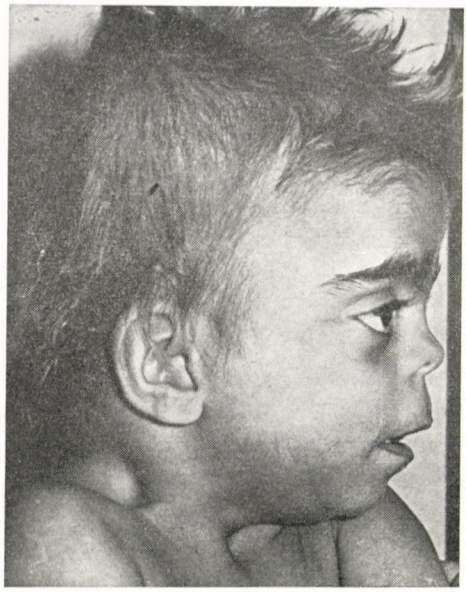
paternal translocation, t (2; 5) (p23; q31). The case was published elsewhere [20].

Patient No. 9. This small-for-date newborn infant was born in the 40th week of gestation with 2400 g weight and 46 cm length. Hypertelorism, flat nasal bridge, short neck, bilateral transverse palmar creases, clinodactyly, muscle hypotonicity, umbilical hernia were interpreted as features of Down syndrome. At the time of chromosome investigation, when the baby was 3 months of age, the clinical diagnosis could be rejected. The most striking features of the statomotor retarded child, in addition to the above mentioned signs, were a quadrate head, slight exophthalmos, synophrys, long eyelashes, stubby nose, broad depressed nasal bridge, gently protruding jaw. She was apathic with poor movements and could not be contacted (Figs 13, 14).

Her cytogenetic abnormality was partial trisomy 6q, trisomy of 6q21-qter segments inherited from a maternal translocation t (6; 15) (q21; q24). The abnormal chromosome of the patient and those of the mother are shown in Fig. 15. The same unbalanced translocation was found in her first cousin who displayed a striking likeness to our patient (Figs 16, 17).* The

* This patient was seen at the Second Department of Paediatrics, Semmelweis University Medical School, Budapest. We are indebted to Dr. Dobos for the photographs and data.





FIGS 16—17. The first cousin of patient No. 9 at age of 13 months

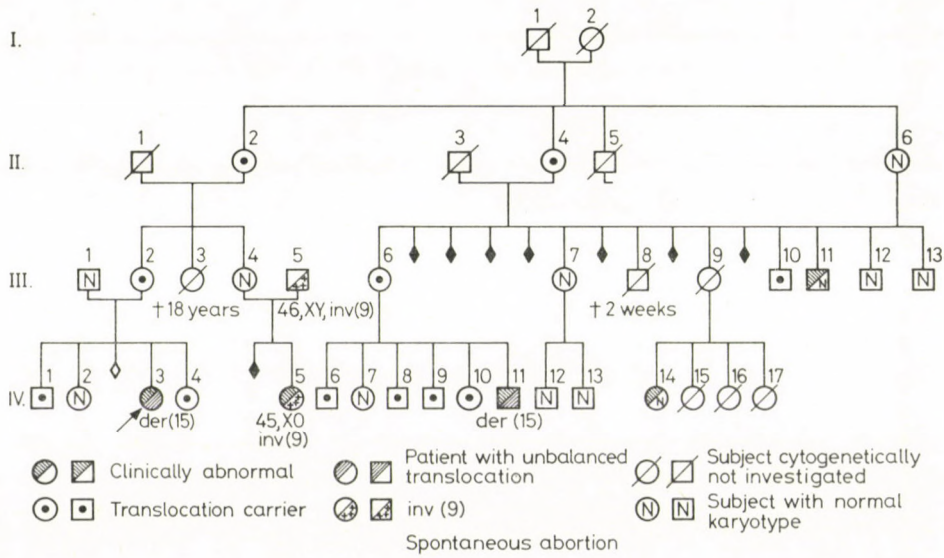


FIG. 18. Pedigree of patient No. 9

translocation could be identified in three generations in this family (Fig. 18).

Patient No. 10. At 9 weeks of age, this female infant was notified as having Down syndrome. The misleading clinical symptoms were epicanthus, outward and upward slanting palpebral fissures, small nose, small mouth, clinodactyly, hypotonicity of the muscles and hyperflexibility. Besides, she had to be operated upon for progressive hydrocephalus with increasing intracranial pressure. At 12 months of age, she was investigated cytogenetically. At that time the resemblance to Down syndrome was not convincing (Fig. 19). In addition to the above symptoms she had small dysplastic ears, short fingers and toes and severe psychomotor retardation with visual and hearing disability.

As a new mutation, a structurally abnormal chromosome 16 was found. Using G and C banding techniques, the abnormality has been supposed to be an isochromosome of the short arm (Fig. 20), thus a trisomy of the short arm with monosomy of the long arm of chromosome 16.

Patient No. 11. The female newborn on the 6th day of life was diagnosed as having Down syndrome. Flat occiput, mild hypertelorism, epicanthus, transverse palmar creases on both hands, clinodactyly, hypotonicity were the clinical findings. Cytogenetic investigation took place at 9 months of age, when no specific features could be observed (Fig. 21) Her karyotype was normal.

Patient No. 12. The baby was born at the 35th week of gestation with 1560 g and 36 cm. After delivery, he had respiratory difficulties. Due to the slightly outward and upward slanting palpebral fissures with epicanthus and hypotonicity of the muscles Down syndrome was diag

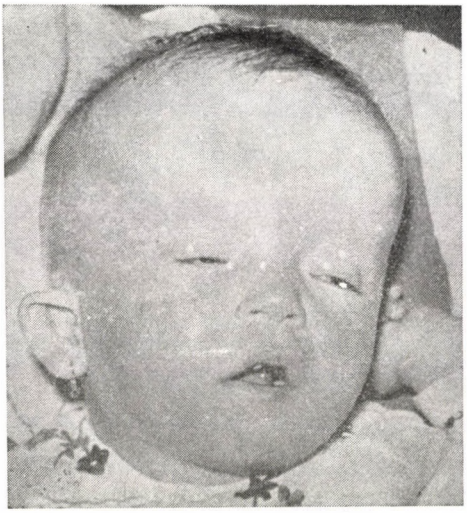


FIG. 19. Patient No. 10 at age of 12 months

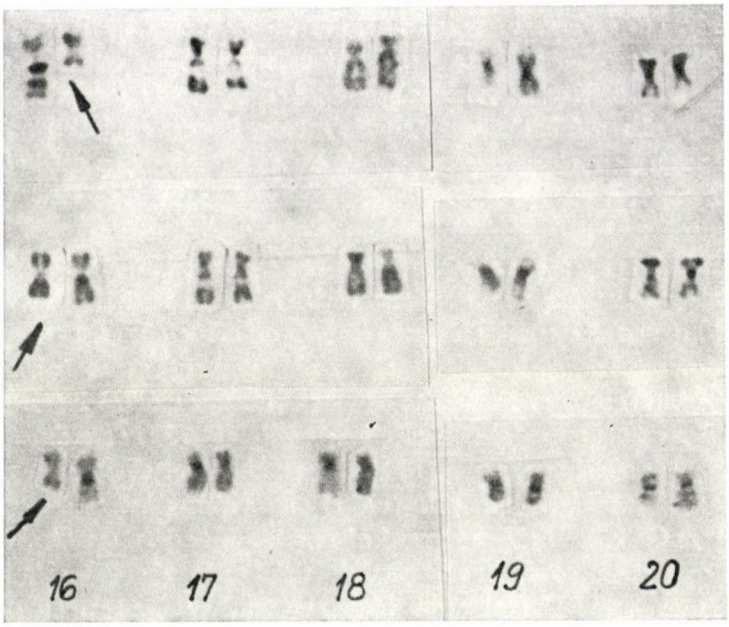


FIG. 20. Chromosomes 16—20 from the karyotypes of patient No. 10 (GAG and CB staining)

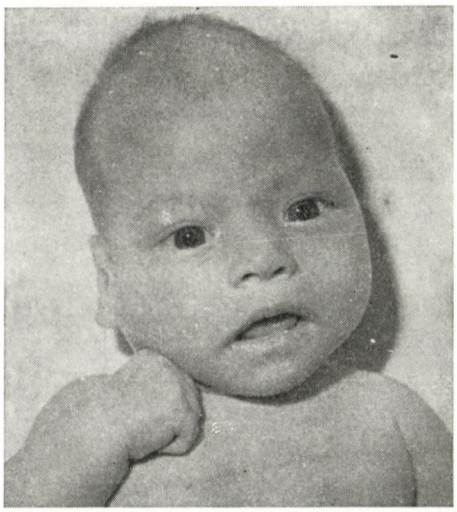


FIG. 21. Patient No. 11 at age of 9 months

nosed. When he was 4 months old, the karyotype was found normal and his development seemed to be completely normal (Fig. 22).

Patient No. 13. This male infant was reported to the HCMR with Down syndrome at the age of 3 days. The clinical diagnosis was based on

mongoloid palpebral fissures and unilateral transverse palmar crease. Otherwise, no abnormality was observed. At 3 months of age, psychomotor development was normal. The karyotype did not show any aberration.

Patients 14, 15 and 16. In each of these cases, the clinical diagnosis of

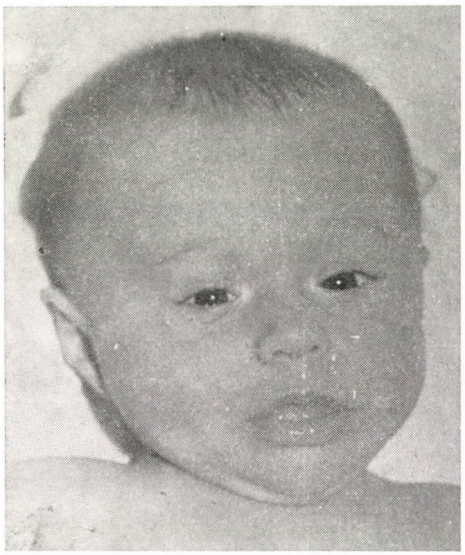


FIG. 22. Patient No. 12 at age of 8 months

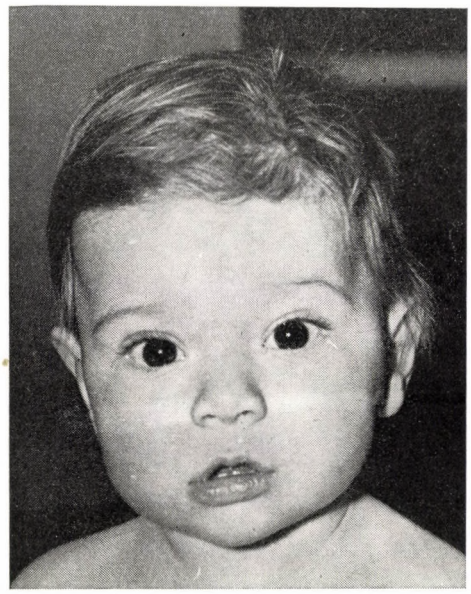


FIG. 23. Patient No. 17 at age of 2.5 years

Down syndrome was based on a single symptom. In one case epicanthus, in the two other cases a unilateral transverse palmar crease were observed in the otherwise normal, healthy infants. Karyotyping was performed between 3 weeks and 6 months of age and all the three infants proved to be normal.

Patient No. 17. This girl was born in the 34th gestational week with 1300 g. Her somatic development was normal but the mental development was supposed to be delayed, since she did not speak at 2.6 years of age. Taking into account the epicanthus (Fig. 23) she was referred to our laboratory as having Down syndrome. The karyotype was normal and now, at 6 years she is completely normal.

Patient No. 18. This 4-year-old, mentally retarded girl was referred to the laboratory as a Down patient since she had seizures since birth but is eumorphic. The karyotype proved to be normal.

Patient No. 19. This girl patient was born in the 38th week of gestation with 1900 g and 36 cm. In the first year, her development was retarded. Apart from the bilateral transverse palmar creases, no other dysmorphic signs could be observed. At 2 years of age, she looked normal and her karyotype was normal.

Discussion

Out of 286 patients only 6 were eumorphic, incorrectly stated as having Down syndrome. In these cases the clinical diagnosis was completely erroneous (2%). In 13 further misdiagnosed cases (5%) dysmorphia was present and in 9 out of these, the underlying defects were rare chromosome aberrations of different types but not trisomy 21 (3%).

XXXXY aberration is a rare finding, although the phenotype was defined more than 10 years ago on the basis of 50 observations [15]. The number of reported patients with del(9p) amounts to about 30 [15, 38]. Not more than 15 cases with trisomy 3q [1, 3, 4, 6, 13, 19, 25, 27, 28, 30, 32], 14 cases of trisomy 5q [2, 8, 12, 16, 17, 20, 22, 24, 39] and 10 cases with trisomy 6q [5, 7, 10, 11, 14, 23, 26, 31, 35, 37] have been published. Trisomy 22 mosaicism is very exceptional [15] and, to our best knowledge, a structural aberration of chromosome 16, like in our patient No. 10, has not been described. Supernumerary marker chromosomes on the whole occur with a frequency of about 3/10,000 newborns, but most of them remain unidentified, so that the frequency of a single type must be much lower.

The characteristics of the external appearance of patients with these rare chromosome aberrations are as follows. A phenotype that could be mistaken for Down syndrome has never been observed in the few reported cases of trisomy 5q and 6q, and our two patients with trisomy 5q and 6q had also been misdiagnosed. On the other hand, in 3 further cases (patients Nos 3, 6 and 7) the clinical diagnostic mistake was understand-

able, since children with XXXXY aberration have often been reported to resemble mongoloids due to the craniofacial dysmorphia, especially the mongoloid palpebral fissures [15]. In many cases with del(9p), it is not only the craniofacial dysmorphia but also the patient's behaviour that

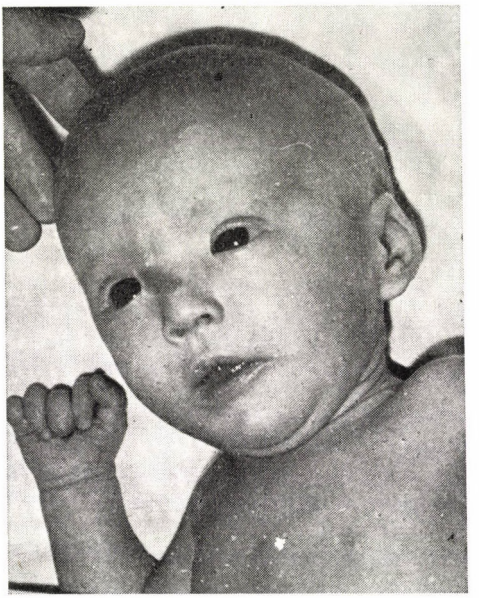


FIG. 24

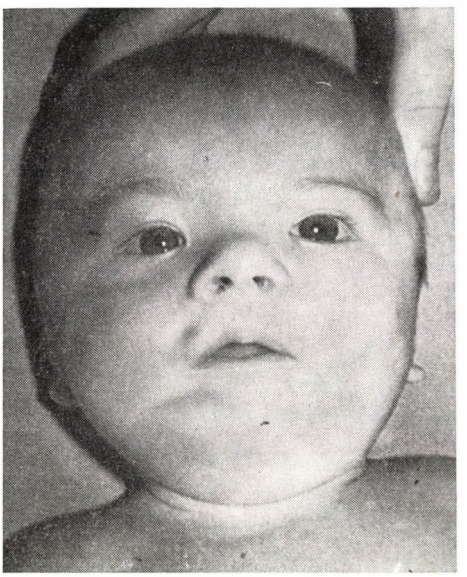


FIG. 25

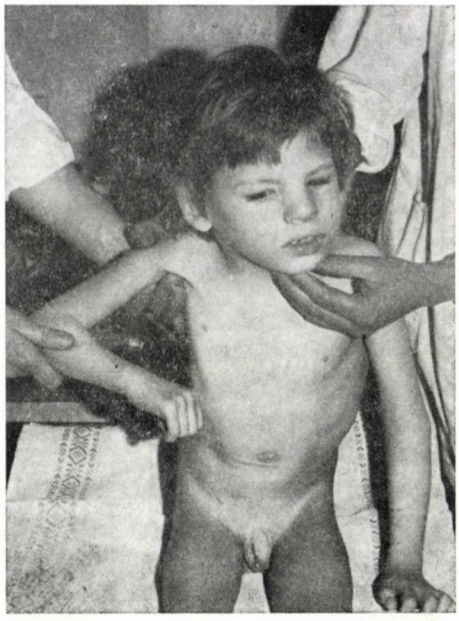


FIG. 26

FIGS 24—25—26. One-week-old infant with del(5) (p13), another infant with del(5) (p11) at age of 6 weeks and a boy with del(5) (p13) at age of 6 years

makes the child resemble one with Down syndrome [15, 38]. From the description of children with trisomy 3q, it seems that mongoloid slanting of palpebral fissures, epicanthus, flat occiput, small nose with anteverted nostrils and flat bridge, hypotonicity and clinodactyly may be present in different combinations [4, 19, 25, 27].

Since a dysmorphic sign by itself is not more than a variation without absolute specificity, the question sometimes emerges whether there is a certain number beyond which the dysmorphic symptoms indicate an abnormal phenotype. In our opinion the question is absurd. Any description of Down patients is inadequate if the characteristic behaviour is not taken into account. The primary cause of diagnostic errors lies rather in the fact that movements, motions, imitation ability, amiability, etc., contribute as much as does the craniofacial dysmorphia to the characteristic clinical picture of Down syndrome, but they cannot be judged correctly in young infants. One might enumerate many symptoms that are useful for differentiation. To mention some of them, the severe malformations of the external genitalia and the severe mental retardation in patients with XXXXY; the long fingers and toes found in del(9p), and the grave multiple visceral malformations in cases of partial trisomy 3q, etc., but the difficulty in evaluating the symptoms plays a smaller role than the age of the patients.

The age-dependent variations of the mongoloid phenotype have recently been demonstrated by Tolksdorf and Wiedemann [36]. Still, differences in the characteristic features between children and adults with Down syndrome do not mean at the same time significant diagnostic difficul-

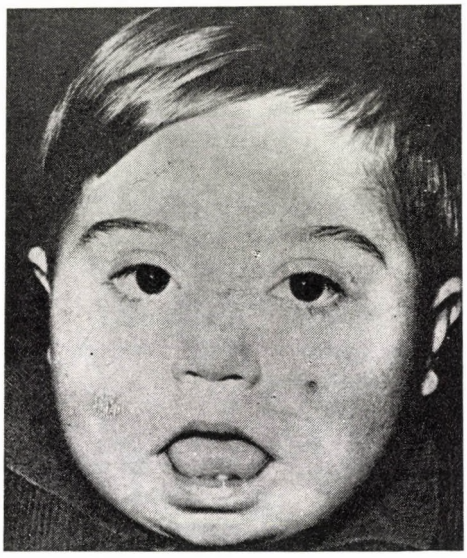


FIG. 27



FIG. 28

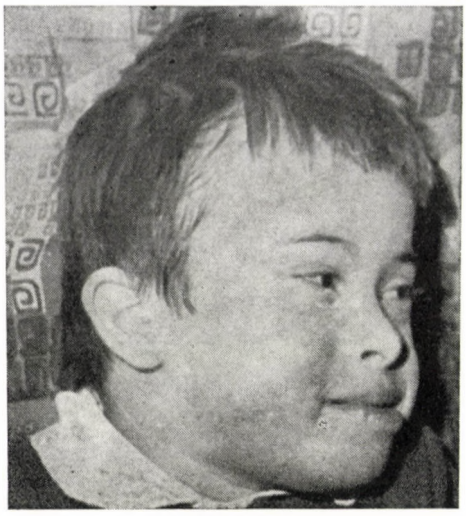


FIG. 29

FIGS 27—29. 3 gypsy patients with trisomy 21

ties. Clinical diagnostic problems arise mostly in the newborn period; in our material, the majority of erroneous diagnoses occurred during the first 3 weeks of life. Since certain combinations of the frequent dysmorphic signs, e.g. those of the ocular region, transverse palmar crease, clinodactyly and hypotonicity occur also in chromosome aberrations other than trisomy 21, it is only natural that the paediatrician observing a dysmorphic young infant and being unable to evaluate his psychomotor status, will diagnose the case as Down syndrome, the aberration best known to him.

Age-dependent variations of the dysmorphic pattern of Down syndrome during the first ten years are generally not so significant than those observed in other chromosome aberrations. The best example is the cri du chat syndrome. This syndrome has two clinical forms, the "type in infants" and "the type in older children and adults". The two forms can easily be differentiated, as the round face, small nose and short neck belong to infants with del(5p) and disappear in older children (Figs 24, 25, 26).

The resemblance of patients with Down syndrome is scarcely influenced by familial or ethnic traits. In our material there were 7 gypsy children and in our experience gypsy mongoloids are more similar to non-gypsy mongoloids than to their parents or other family members (Figs 27, 28, 29). Just the opposite was the case with the patients with partial trisomy 6q. The probanda and her first cousin with the same aberration were strikingly similar, but the resemblance resulted from familial-ethnic traits. The configuration of the head, the great and deeply sited eyeballs,



FIG. 30. Gypsy patient No. 9 and her mother

the synophrys, long eyelashes, stubby nose, gently protruding jaw were observed in the healthy members of the gypsy kindred, too (Fig. 30). These features predominate the dysmorphia described by Tipton et al. [35] as characteristic of trisomy 6q: microcephaly, flat facial profile, prominent forehead, flat nasal bridge and carp mouth.

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Unusual chromosome aberrations in 3 children with Down syndrome

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In 3 children with Down syndrome extremely rare chromosome aberrations were found. In the first patient, the karyotype showed 46 chromosomes with a de novo duplication of the q22-qter segment. This finding supports that the 21q22-qter band was responsible for the characteristic mongoloid features. In the second case, trisomy 21 was present and out of 78 investigated cells, 60 contained a small, supernumerary marker chromosome in addition to trisomy 21. The parents were cytogenetically and clinically normal. In the third case trisomy 21 with inv(10) (p13q22) occurred. The inversion was inherited from the mother with diminished fertility.

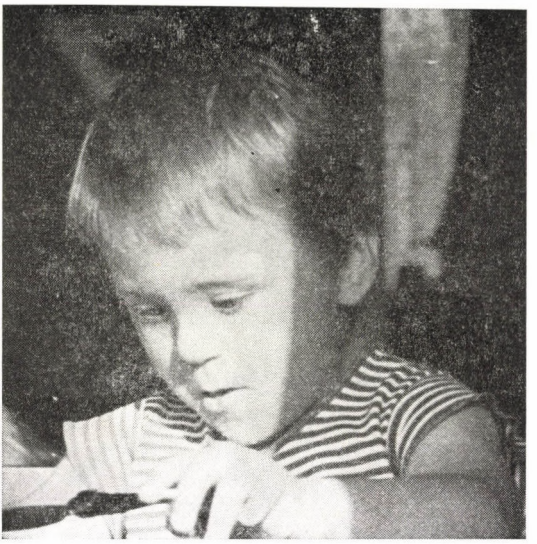
Chromosome aberrations other than regular and Robertsonian translocation trisomy 21 are rare in patients with Down phenotype. The number of observations on trisomy 21 resulting from the fusion of the telomeric regions of the long arms of the two chromosomes 21 amount to about 10 [4, 6, 13, 14, 18, 26, 27, 32, 33]. Trisomy 21 as a result of a parental translocation of chromosome 21 with a non-acrocentric chromosome has been reported by several investigators [1, 2, 5, 11, 12, 15, 16, 17, 19, 20, 21, 24, 29, 35, 36]. Trisomy 21 with other chromosomal imbalance also occurred in some cases; these have been summarized by Schwanitz and Hagner [28].

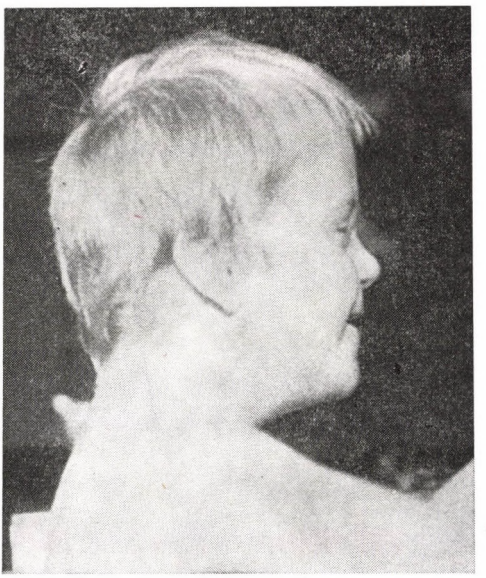
We describe here 3 patients with clinical features of Down syndrome whose karyotypes showed extremely rare aberrations.

Case No. 1

V. H., a female patient (Figs 1 and 2) was referred to chromosome investigation when her mother had become pregnant for the second time.

At birth, her mother was 26 and her father 29 years old. Both are healthy. The patient was born after a normal pregnancy in the 39th week of gestation. Birth weight was 2850 g, length was 54 cm. There was no complication after delivery. Down syndrome was detected clinically in infancy and the baby was sent for chromosome analysis to two different laboratories; both found a normal karyotype. At 3 years of age, the child could not speak, she showed many signs of craniofacial dysmorphia characteristic of Down syndrome including oblique palpebral fissures, epicanthus, flat occiput, flat facial





FIGS 1—2. Patient No. 1 at 3 years of age

profile, small nose and small mouth, short neck, small ears. Her hands were broad, with short fingers, clinodactyly and unilateral transverse palmar crease. An umbilical hernia was also present.

Karyotype analysis of a peripheral blood culture showed 46 chromosomes in the cells and one of the chromosomes 21 was structurally abnormal. The long arms of this chromosome were elongated, as a result of the

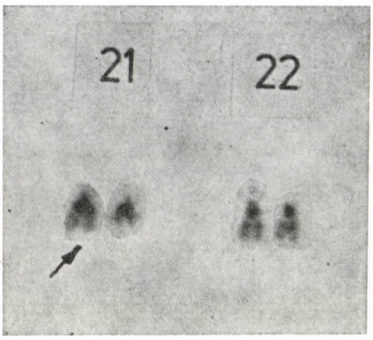


FIG. 3. Patient No. 1. Chromosomes 21—22 from the karyotype

elongation of segment q22-ter (Fig. 3). Duplication of the chromosome segment 21q22-qter has been supposed. The karyotype of the parents was normal.

Case No. 2

The boy Z. M. was 2 years old at the time of chromosome investigation. He was born when the mother was 36 and the father 35 years old. This was the 8th pregnancy of the couple. From the first pregnancy, 16 years before the proband's birth, a normal, healthy boy was born. In the subsequent years, 7 pregnancies were interrupted artificially. The parents are healthy. The mother has a mentally retarded brother, 15 years her junior, who was born when his mother was 39 years of age. The family did not agree to his examination.

Delivery took place after normal pregnancy at term. Birth weight was 3000 g, length was 57 cm. There was no perinatal complication. The patient seemed to be mongoloid, having brachycephaly, flat occiput, flat face,

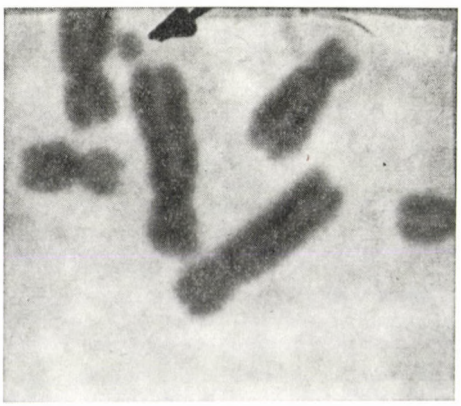


FIG. 4. Patient No. 2. The small marker chromosome in a mitosis

mongoloid palpebral fissures, epicanthus, small nose, small mouth with protruding tongue and a short neck. Hypotonicity and hyperflexibility were also observed, with severe mental retardation.

Cytogenetic investigation of the peripheral blood culture revealed in 60 investigated cells trisomy 21 and, in addition, a minute supernumerary chromosome (Fig. 4). In further 18 cells the marker chromosome was not present. The patient's karyotype was defined as 47, XY, +21/48, XY, +21, +mar; the karyotype of the parents was normal.

Case No. 3

A. B., a gypsy girl, was seen at the age of 3 years. She was born from the third marriage of her mother who was pregnant only twice during a period of 20 years. One pregnancy ended in spontaneous abortion at about the 10th week of gestation. After 17 years, the proband was born from the second pregnancy when the mother was 39 and the father 36 years of age. Both are healthy.

The external appearance of the patient was typically mongoloid. She had no congenital visceral malformation.

Cytogenetic investigation showed a supernumerary chromosome 21 and besides a structurally abnormal chromosome 10 that was stated as inv(10) (p13q22) (Fig. 5). In the maternal karyotype the number of chromosomes was 46; the inv(10) aberration was also present. The father had a normal karyotype.

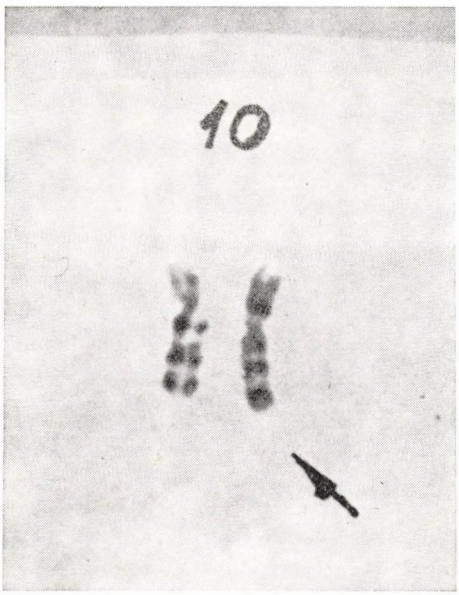


Fig. 5. Patient No. 3. Normal chromosome 10 and its homologous one with pericentric inversion

Discussion

De novo duplication within the same chromosome in abnormal individuals, as in our patient No. 1, has been observed by some authors [8, 10, 31, 32, 34]. Analysing these cases, Vogel et al. [34] stated that the most likely explanation for this aberration type is a translocation between the homologous chromosomes during meiosis. Studying the different cases of partial trisomy 21, several investigators indicated that the trisomy of band q22-qter on chromosome 21 was responsible for the clinical characteristics of Down phenotype [13, 20, 25, 35, 36]. Our observation supports this statement. The rarity of this type of partial trisomy 21 explains why at two previous investigations the karyotype with a chromosome number 46 was declared to be normal although the patient's appearance left no doubt that he had Down syndrome.

Trisomy 21 with a supernumerary minute chromosome was found previously by Calabro et al. [3]. In their case, the small marker chromosome had segregated from the father. In two other cases [22, 23] recurrence of Down syndrome was observed in the investigated families; the subjects with Down syndrome had only trisomy 21, the microchromosome was carried by healthy family members. Ramos et al. [23] assumed the possibility of a connection between the meiotic non-disjunction and the familial occurrence of microchromosomes. In our case, the supernumerary minute chromosome without any modifying effect on the Down phenotype was considered a de novo aberration appearing in mosaic form. Still, we could not exclude an unidentified low rate mosaicism in one of the parents and the absence of the small marker chromosome may have been due to its loss during preparation.

As far as we know, trisomy 21 with inv(10) aberration has not yet been observed in the same subject. This aberration, inherited from the mother, did not alter the mongoloid appearance of patient No. 3. It may have played a role in the diminished fertility of the structurally heterozygote mother. While De la Chapelle et al. [7] concluded that the pericentric inversion of chromosome 10 segregated without consequences, Dutrillaux et al. [9] described a recombinant chromosome 10 in a malformed boy whose mother had inv(10). Recently, inv(10) (p13q22) has been found in our laboratory in a man whose wife had repeated spontaneous abortions. All these data call for further studies definitely to clarify the importance of inv(10).

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Familial occurrence of partial trisomy 4q and probable monosomy 5p due to 4q/5p translocation

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Translocation between chromosomes 4q and 5p has been observed in 12 members of a family. Multiple deaths occurred in early childhood in the family but spontaneous stillbirth did not show an increased frequency. In two children, identical cytogenetic findings were found consisting of translocation between 4q and 5p, resulting in trisomy 4(q31 → qter) and, based on clinical symptomatology, of a probable monosomy 5(p15 → pter). The clinical picture of both children included the main features of the cri du chat syndrome: a low birthweight, catlike cry, severe psychomotor retardation, hypotonicity, antimongoloid slant of the eyelids, microcephaly together with other symptoms determined by trisomy 4q.

The characteristic cri du chat is known to occur in patients with deletion of the 5p distal portion [13, 14], with ring chromosome 5 [20] as well as in translocation to 5p of various chromosome materials [8, 9, 16, 18, 22].

We shall report on two patients with manifestations of the cri du chat syndrome and with other phenotypic symptoms due to an unbalanced chromosome translocation 4q/5p.

Family history

The family pedigree is shown in Fig. 3. Thirty-one members of the family could be investigated cytogenetically. Balanced translocation could be detected in 12 cases including the parents of both described cases. Transmission of the translocation could be followed up over 4 generations. There were several other children with congenital developmental defects in the family; their symptomatology might have suggested a chromosome anomaly. All these children, however, died before our investigations had started, but no spontaneous stillbirths could be recorded within the entire large kinship.

Case reports

Case No. 1. The propositus was born at 42 weeks gestation by normal delivery with 2850 g weight and 49 cm length from the second pregnancy of the 21-year-old mother. The child

was brought to our laboratory at the age of 4 months, with marked hypotrophy, hypotonicity and unphysiological cry. She had a microcephalic asymmetrical head with plain occipital area, antimongoloid slant of the eyelids, broadened nose root, lower set and dorsally rotated ears, sunken mouth corners, micrognathia, short neck, laterally sited mamillae, and congenital heart defect. There were flexion creases on both palms, thin digits, a double toe on the right foot in full syndactyly with a common nail, high set toe on the left foot, digits on both feet in tibial deviation. The skull length was normal, the width parameters were subnormal, thus, the head was dolichocephalic. The cephalic index was 80, mesocephalic. The head circumference was by 3 sigmata smaller than normal.

The child had suffered from repeated respiratory tract infections. At the age of 12 months she underwent surgery for persistent arterial duct. Neurological examination at the age of 17 months revealed psychomotor retardation and a smaller neurocranium with slightly asymmetric sutures. She did not sit or stand spontaneously. PEG: asymmetric skull. The lateral chambers filled poorly, the subarachnoid spaces were not enlarged, they filled symmetrically. Transillumination showed normal limits.

At the age of 31 months her body weight was 10 kg, and her height, 82.5 cm. The child still walked on a broad basis in an uncertain way, did not speak and could not vocalize. She was not able to retain urine or stools. Psychomotor development was at the level of a 1-year old child. Control craniometric examination: within the limits of variability, breadth parameters were significantly lowered. The distance between the inner eye corners was increased (intercanthal index 40.25 — euryopia). The head circumference was small (−2SD), the intermammal index was 24.1 (23.4 ± ± 1.9).

Dermatoglyphic examination: pattern intensity on palms was low. Carpal triradius was in position t. Both palms showed transversal flexion creases.

Case No. 2. This 3rd grade cousin of the proband was born in the 42nd week of pregnancy (occipital position), birth weight was 2700 g, body length was 46 cm.

Physical findings at the age of 24 months: unphysiological cry, only weak murmur. She begins to walk. Tells only "mama", cannot retain urine and stools. Plagiocephaly, broad nose root, narrow eyelids, small neurocranium. Antimongoloid slant of the eyes, hypotonicity of the extremities, normal reflexes, deformities on plants, tibial deviation of digits, congenital dislocation of the hip.

Craniometric examination: head circumference 43.2 cm (−2.5 SD). The basis of the skull is smaller than average at the limit of significance. The distance between both the inner and outer eye corners is significantly longer than average. Cephalic index was 95.

Dermatoglyphic examination: the main line A has a tendency to course

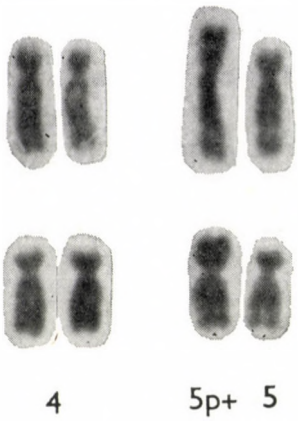

FIG. 1. Patient 1, chromosomes 4 and 5 from G banded karyotype

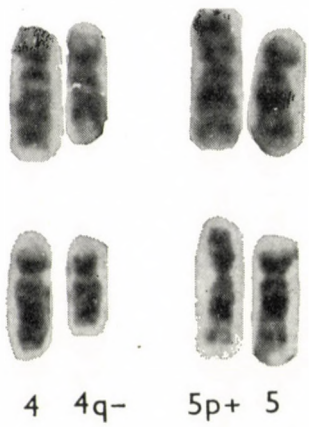

FIG. 2. Mother of patient 1, chromosomes 4 and 5 from G banded karyotype

transversally. The other palm lines are normal. The carpal triradius is in position t. Transversal flexion creases are found on both palms.

*

Both affected girls had additional chromosome material on the short arms of chromosome 5: this material could be identified as the distal portion of the long arm of chromosome 4, (q31→qter) (Fig. 1).

A balanced translocation 4; 5 (q31; qter) (Fig. 2) was found in the mother of Case 1, in the father of Case 2, and in 10 other relatives.

DISCUSSION

Up to now, 33 cases of partial trisomy of the long arms of chromosome 4, and in translocation to other chromosomes or in association with

monosomy of other portions of the karyotype, have been described [21, 2, 6, 10, 5, 24, 1, 3, 7, 11, 19].

As to the extent of the trisomy, 2 cases of 4 (q21 → qter), 5 cases of trisomy 4 (q25 → qter), 2 cases of trisomy 4 (q26 → qter), 6 cases of trisomy 4 (q27 → qter), 2 cases of trisomy 4 (q28 — qter), 6 cases of trisomy 4 (q31 → qter), and 2 cases of trisomy 4 (q32 → qter) have been reported. The other cases were published before a more exact determination of the trisomic portion had become avilable [21, 23]. A review of the symptomatology grouped according to the trisomic portion together with the frequencies of occurrence is given in Table I. Psychomotor retardation, hypotrophy, abnormally shaped, large, lower set ears, microcephaly, hypertelorism, epicanthic folds, short neck, narrow palpebral fissures and/or ptosis, cryptorchidism, congenital heart defect, renal malformations and abnormities of the feet are the common signs in trisomy 4q [24]. Some authors even speak of a distinct syndrome [4]. As shown in Table I, a typical clinical picture corresponding to partial trisomy 4q can hardly be established. Symptoms occurring in more than 50% of the cases are not typical, and may appear in several chromosome anomalies. The cases reported allow a classification of trisomies 4q according to the size of the trisomic portion. Still, even such an arrangement does not allow to distinguish a typical clinical picture of individual partial trisomies except for some cases detailed below, also with respect to the fact that trisomy 4q is not a distinct reduplication of the chromosome material. It is accompanied by a further chromosome reconstruction, frequently by monosomy, which itself may markedly influence the symptomatology, as in the case of translocation to 5p. The criteria defined in this way as well as a comparison to the clinical picture of a similar chromosome reconstruction involving the same chromosomes, give a more homogeneous picture (Table II). The increased frequency of cryptorchidism in all trisomy 4q types is noteworthy; this sign may therefore be put in relation with the loss of the distal portion of 4q. In contrast, in trisomies of the proximal portion of band q28 (or q31), symptoms such as a prominent nasal bridge, slanting forehead and short philtrum are only seen.

Although cytogenetic methods failed in revealing a loss of chromosome material in the terminal portion of the short arms of chromosome 5, such a loss might be suggested on the basis of the typical cry and of other symptoms in the patient. Other authors reported similar observations in association with translocations of various chromosome portions to 5p [13, 16]. As can be seen in Table II, it may be a phenotypic picture of partial trisomy 4 (q31 → qter) in translocation to 5p. Such a chromosome reconstruction is dominated by signs typical of the cri du chat syndrome, since autosomal monosomy, though not involving a large portion, is a more serious defect than partial

trisomy in the genetic material of an individual. This is also confirmed by the clinical symptomatology observed in all the reported cases, *viz.* a low birth weight, catlike cry, marked psychomotor retardation, a lag in postnatal development, hypotonicity and hypertelorism [17, 15]. Other symptoms present in more than 50% of the cases include an antimongoloid slant, large, malformed and low set ears, microcephaly, micrognathia and/or retrognathia, and congenital heart defect.

Of the 6 cases with trisomy 4q with simultaneous translocation to 5p,

TABLE I

Symptomatology observed in 33 cases of partial trisomy 4q grouped according to the size of the trisomic portion

Features	Partial trisomy for 4q							
	21→ter	25→ter	27→ter	31→ter	31→ter t4/5	32→ter	Other	Total
Psychomotor retardation and/or hypotrophy	2/2	6/8	8/8	4/5	6/6	2/2	2/2	30/33
Large and law set ears	2/2	8/8	7/8	4/5	4/6	2/2	2/2	29/33
Cryptorchidism	1/1	2/3	4/5	2/2	2/2		2/2	13/15
Short neck	1/2	6/8	6/8	3/5	3/6	1/2	2/2	22/33
Hypertelorism	1/2	3/8	5/8	3/5	6/6		2/2	20/33
Microcephaly	2/2	4/8	3/8	3/5	4/6	2/2	2/2	20/33
Hypotonicity	2/2	2/8	6/8	1/5	6/6		1/2	18/33
Congenital heart defect		5/8	3/8	3/5	4/6	1/2	2/2	18/33
Narrow palperbal fissures and/or ptosis	2/2	4/8	4/8	4/5	2/6		1/2	17/33
Feet abnormalities	2/2	3/8	3/8	4/5	2/6		2/2	16/33
Antimongoloid slant		3/8	6/8	1/5	5/6		1/2	16/33
Epicanthic folds	1/2	1/8	5/8	3/5	3/6	1/2		14/33
Micrognathia	1/2	4/8	2/8	1/5	4/6	1/2		13/33
Carp mouth	2/2	3/8	4/8	1/5	1/6	1/2		12/33
Renal abnormalities	1/2	3/8	3/8		2/6		2/2	11/33
Depressed nasal root	1/2		3/8	3/5	2/6	2/2		11/33
Slanting forehead	1/2	3/8	5/8	1/5				10/33
Sclerodermia and/or feet oedema	2/2	2/8	2/8	2/5	1/6		1/2	10/33
Short philtrum	2/2	4/8	3/8			1/2		10/33
Protruding forehead	1/2	3/8		3/5		2/2		9/33
Prominent nasal bridge	1/2	4/8	2/8	1/5				8/33
Widely spaced nipples		2/8	2/8	1/5	2/6		1/2	8/33
Hip anomalies		2/8	1/8	2/5	2/6			7/33
High pitched cry					6/6			6/33

2 patients were members of one family [17] and they might be suggested to carry identical chromosome reconstruction. Despite this, a comparison of the clinical pictures of these pairs reveals several differences,

TABLE II

Features observed in 6 cases of trisomy 4 (q31 → qter) in translocation to 5p (with suggested monosomy 5 (p15 → pter))

	Schwingshackl	Gann	McDermott et al. 1977	Oka et al. 1978	This report		[Present/ described
	Case 1	Case 2			Case 1	Case 2	
Sex	F	M	F	M	F	F	
Age	2 M	2 M	3 M	76 d	31 M	24 M	
Weight at birth	2280 g	2870 g	2778 g	2380 g	2850 g	2700 g	
Length at birth	46 cm	48 cm		46 cm	49 cm	46 cm	
Head circumference	30.5 cm	33 cm	35 cm	33.5 cm			
Psychomotor retardation and/or hypotrophy	+	+	+	+	+	+	6/6
Hypotonicity	+	+	+	+	+	+	6/6
High pitched cry	+	+	+	+	+	+	6/6
Hypertelorism	+	+	+	+	+	+	6/6
Cryptorchidism		+		+			2/2
Antimongoloid slant	+	+	+		+	+	5/6
Large and low-set ears	+		+	+	+		4/6
Microcephaly	+	+			+	+	4/6
Micrognathia	+	+	+		+		4/6
Congenital heart defect	+	+		+	+		4/6
Short neck	+	+			+		3/6
Epicanthic folds	+	+	+				3/6
Depressed nasal root		+				+	2/6
Narrow palpebral fissures	+					+	2/6
Feet abnormalities					+	+	2/6
Renal abnormalities		+		+			2/6
Hip abnormalities	+					+	2/6
Widely spaced nipples				+	+		2/6
Long and tapering fingers	+			+			2/6

obviously suggesting participation of other exogenous factors operative in forming the phenotype of an individual.

Twelve members of the family at issue, all translocation carriers, who could be examined cytogenetically and clinically, showed no signs of defective somatic development. This clinically negative finding suggests that there is no loss of the terminal portion of 5p due to translocation to 5p of chromosome material and that these carriers bear a reciprocal and balanced translocation of 4q and 5p.

Based on an analysis of literary data, Yunis et al. [24] concluded that 34%—40% of the descendants of individuals with balanced translocation are carriers. An analysis of our pedigree gave 32.5%, a similar value. No repeated spontaneous stillbirths could be detected in the kinship despite a good cooperation of the family. Fourteen persons with supposingly normal karyotype (10 cytogenetically verified), 14 carriers and 15 early deaths in childhood with congenital developmental anomalies (2 cases of cytogenetically confirmed trisomy) with similar clinical findings were recorded among descendants of balanced carriers of the chromosome anomaly (Fig. 3). Conception of some further 15 individuals with unbalanced karyotypes, likely monosomic for a portion of chromosome 4, is to be supposed; such individuals are lost during early embryonal development without the parents becoming aware of the conception. This could explain the higher proportion of carriers of balanced chromosome anomalies observed in the family, and the literary data that record higher incidences as compared with theoretical assumptions based on segregation relations.

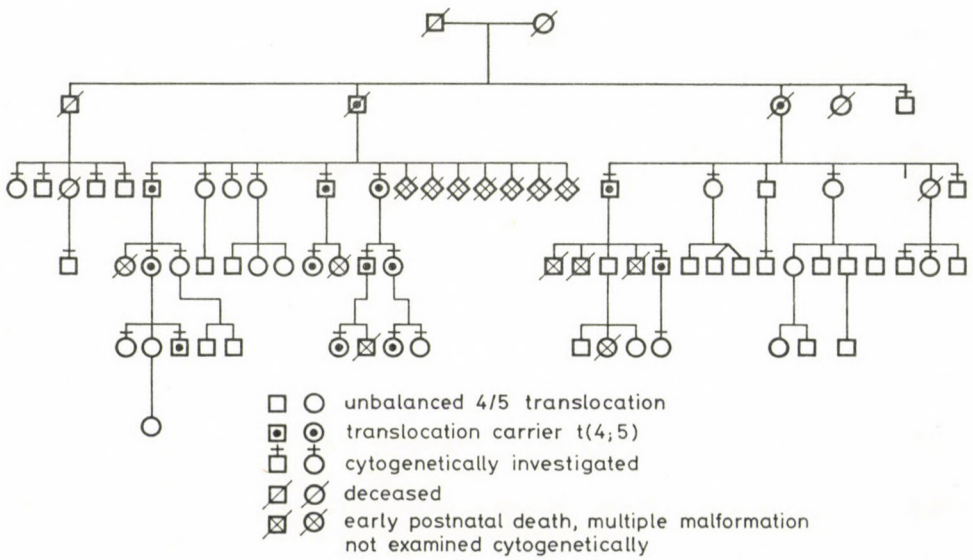

FIG. 3. The family pedigree

Acknowledgements

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Developmental changes in lymphocyte surface markers

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The aim of the study was to establish normal values for T_G and T_M cells in neonates and small babies who are more susceptible to infections than children and adults. For the same reason, the T- and B-lymphocyte ratios were also determined. The percentage of B- and T-cells in neonates was significantly lower, while their absolute number higher, than the adult value. The percentage of T_G cells was significanlty higher in neonates than in the other age groups. There was a significant decrease in the ratio of T_M cells as compared to adult values. Spontaneous thymidine uptake showed an inverse correlation with age, but the difference was not significant. There was a nearly identical response to PHA in the observed age groups, at least to the single dose of PHA applied.

The cell surface markers of lymphocyte populations and the relevance of their functional changes were extensively studied during the past decade. Recently, T-cell subsets became the prime targets of these investigations, revealing further heterogeneity of surface membrane components and functional characteristics [8, 35, 36, 39]. One of the most accessible approaches for the counting of T-lymphocyte subpopulations employs their capacity to form EA-rosettes. Moretta et al. [24, 26] described the T_G and T_M cells that possess specific receptors for the Fc-fragment of either the IgG (T_G cells) or the IgM molecules (T_M cells). These two lymphocyte populations differ markedly in their morphological, cytochemical and functional characteristics [16, 28]. The IgM-Fc "positive" subpopulation provides help for B-lymphocytes in their PWM-induced proliferative response and differentiation process. The T_G (IgG-Fc positive) cells, on the other hand, cannot provide help. Furthermore, upon contact with IgG, T_G cells inhibit the PWM-induced maturation of B cells into plasma cells [28, 29]. Although some authors ascribe this inhibitory effect in cord blood to cells other than T_G lymphocytes [18, 30], these two cell types seem likely candidates for the suppressor and inducer subpopulations described originally for mouse lymphocytes.

The ratio of T_G and T_M cells was found to be significantly altered in a number of primary and secondary immunodeficiencies [11, 17, 27, 40]. A close study of this question seems particularly relevant in neonates since most of the primary immunodeficiencies appear in the first few weeks of life. Further, newborn babies and especially prematures are "naturally"

more susceptible to viral, bacterial and fungal infections [10, 43]. In the present study we describe our findings in normal babies and children. Apart from T- and B-lymphocyte counts, the number and ratio of T_G and T_M cells was determined along with the PHA-induced proliferative response of their cells.

Materials and Methods

Subjects. Group 1 consisted of 0 to 6-day old mature, healthy newborns, Group 2 of 1 to 20-month old babies admitted to the hospital for minor surgical interventions. Two to 18-year old children hospitalized for similar reasons formed Group 3, while Group 4 consisted of 20 to 40-year old healthy blood donors. The number of subjects for each test is indicated in the legends to the figures.

Sera. Antibodies used in the EA-rosettes were obtained by repeated immunization of rabbits with ox red blood cells (haemolytic titre in the IgM-rich serum was 1 : 4000, in the IgG-rich serum 1 : : 16 000). IgM was obtained by precipitation with 33% ammonium-sulfate followed by Sephadex G-200 gel filtration. An IgG fraction was prepared by DEAE-cellulose (REANAL) chromatography following the salt-fractionation procedure [11, 33].

Purity of the IgG- and IgM-fractions was established by immunoelectrophoresis.

All sera were inactivated at 56°C for 1 hour.

Preparation of erythrocytes for EA-rosettes. Less than a week old ox RBCs were washed three times inPBS. A 2%RBC suspension was incubated at 37°C for 60 min with a subagglutinating concentration of anti ox-RBC IgG or anti ox-RBC IgM in buffer. Sensitized cells were washed in PBS [11, 24, 33].

Lymphocyte separation. Lymphocytes were separated from heparinized venous blood by Ficoll-Uromiro gradient centrifugation [4]. Monocytes were removed from the aliquots used for surface marker analysis by adherence to a glass surface followed by carbonyl iron treatment. Cell viability was determined using the trypan blue dye-exclusion method. T-cells used in Fc-receptor analysis were separated by the E-rosette technique [15].

B-lymphocytes were counted by direct immunofluorescent technique using FITC-labelled anti-human pig IgG (SEVAC) and also by a heterologous anti-B-cell serum used in an indirect fluorescence system.

T-lymphocyte numbers were assayed by the E-rosette technique [20] and by indirect immunofluorescence using a heterologous anti-T-cell serum.

EA-rosettes. 5×10^6/ml lymphocytes or separated T-cells were mixed with an equal volume of 2% sensitized ox-RBCs and centrifuged at 100 *g* for 5 min. Rosettes were counted after 1 hour incubation at 4°C. Prior to assaying the EA–IgM rosettes, the lymphocytes were incubated overnight in serum-free medium [11, 22, 24, 33].

Lymphocyte cultures. 1) 72 hour cultures were set-up from separated lymphocytes in TC-199 medium containing Hepes buffer and 20% inactivated normal human AB-positive serum. Cell concentration was 2×10^6/ml; 200 μl aliquots were cultured in Microtest II tissue culture plates with or without 5 μg/ml PHA (Wellcome) at 37°C for 56 hours. A further 16 hour incubation period followed the addition of 1 μCi ^{3}H-TdR (spec. activity: 150 mCi/mM, Amersham). Thymidine uptake was measured by liquid-scintillation counting of the TCA-precipitable material in an Intertechnique spectrometer. 2) In some instances whole-blood cultures were also set up. 0.2 ml of heparinized peripheral blood was diluted to 4 ml with the above medium and cells were cultured in the same way as in the case of separated lymphocytes.

Statistical analysis. Kruskal-Wallis H-test was used [46]. A p value of less than 0.01 was considered significant.

Results

Results are summarized in Figs 1 and 2. Since the absolute number of lymphocytes is high in neonates and small babies, the absolute number of lymphocyte subpopulations is also higher than later in life. The percentage of B-lymphocytes in neonates is significantly lower than in the other 3 age groups. After a few months the ratio of B-cells to total lymphocytes reaches adult values and it is at this time that the highest B-cell numbers are detected. T-cell ratios show an increase parallel to development, with low values in the first few weeks and a subsequent rise to adult values. T_M lymphocyte percentages show a similar tendency. Neonatal T_M percentage values are significantly lower than those of adults.

There was a revers tendency in the percentage of T_G-cells: it was highest

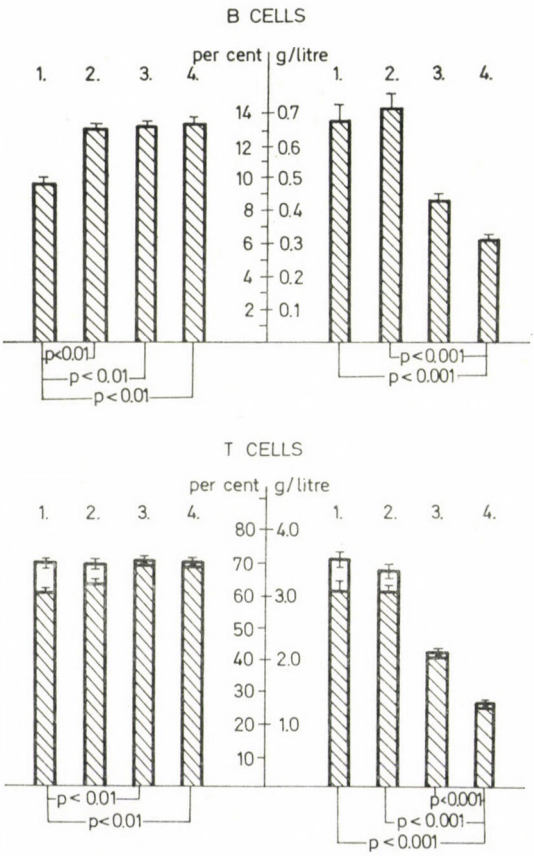

Fig. 1 Distribution of B and T lymphocytes according to age. Columns represent the mean percentage values or the mean absolute numbers with the standard error of the mean. Open columns represent the T-cell ratios as determined by a hetero-antiserum to T-cells. Number of subjects tested: 40

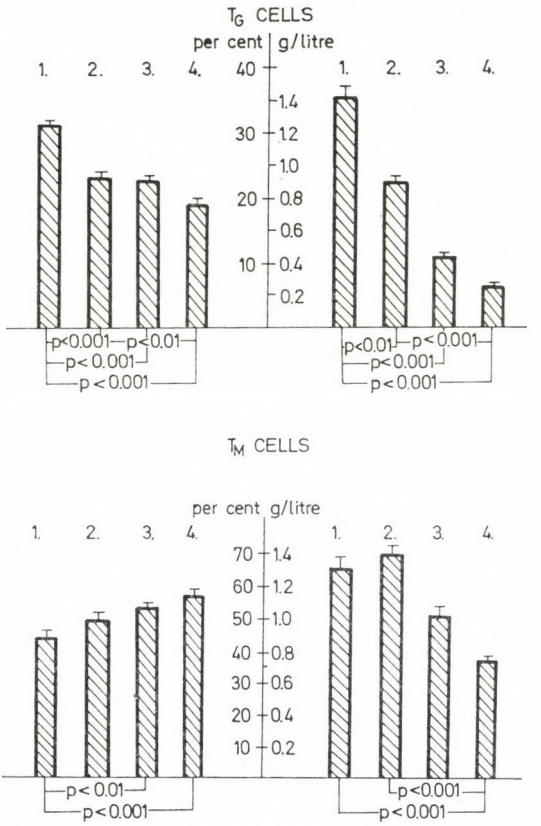

Fig. 2 Distribution of EA-IgG and EA-IgM rosette-forming cells in isolated T-lymphocytes Symbols as in Fig. 1. Number of subjects tested: 30

in the group of neonates and showed a gradual decline to adult values in small babies.

When we analysed the ratio of IgG–Fc and IgM–Fc receptor positive cells from unfractionated lymphocytes, we found results similar to those obtained from isolated T-cells. Significant alterations were found only in the youngest age-group.

Comparing the values from fractionated and unfractionated cells, it became apparent that while IgM–EA rosettes were only formed by T-cells, there were other cell types (B- and L-lymphocytes) involved in the formation of IgG–EA rosettes.

PHA-stimulated lymphocyte transformation showed no significant differences in the investigated age groups (Table I). Spontaneous metabolic activity as measured in unstimulated cultures, was also the same in the neonates as in older children or adults. Their PHA reactivity was nearly the same as that of the others. Whole blood cultures gave similar results. Comparing the stimulation values in

TABLE I

PHA*-induced lymphocyte transformation

	1.			2.		
	U	S	SI	U	S	SI
Separated lymphocytes 5×10^5 cells/well	1850 (±1090) n = 12			1710 (±1260) n = 20		
		40 040 (±28200) n = 12			50 300 (±31 700) n = 20	
			24 (±14) n = 12			42 (±39) n = 20
Whole blood microcultures 5×10^4 cells/well	640 (±370) n = 23			490 (±290) n = 14		
		45 830 (-32 450) n = 23			42 870 (±20 370) n = 14	
			96 (±90) n = 23			112 (±83) n = 14

	3.			4.		
	U	S	SI	U	S	SI
Separated lymphocytes 5×10^5 cells/well	1680 (±990) n = 26			1230 (±680) n = 21		
		71 920 (±58 060) n = 26			58 950 (±29 480) n = 21	
			57 (±53) n = 26			62 (±37) n = 21
Whole blood microcultures 5×10^4 cells/well	440 (±230) n = 6			320 (±170) n = 6		
		45 850 (±33 680) n = 6			36 090 (±25 670) n = 6	
			115 (±99) n = 6			118 (±54) n = 6

* PHA dose, 5 μg/ml
U: unstimulated, cpm
S: stimulated, cpm
SI: stimulation index

separated and whole blood cultures it appeared that stimulation indices rose considerably in the presence of autologous erythrocytes.

Discussion

Primary and secondary immunodeficiencies are characterized by varying degrees of T-cell dysfunction. These functional changes, however, are often not reflected in the number of peripheral T-cells or in the usual ***in vitro*** functional tests [41]. At the same time a shift in the ratio of T-cell subsets is demonstrable in the majority of cases [5, 11, 25, 27, 42]. One of the more widely used and accepted methods for the identification and isolation of T-cell subsets is the EA-rosette technique, outlined by Moretta et al. [16, 26, 28, 29].

The aim of this study was to establish the normal values of T_G and T_M cells in neonates and small babies who are more susceptible to infections than children and adults. For the same reason the T- and B-lymphocyte ratios were also determined. In contrast to the findings of other authors [2, 3, 6, 13, 14] we found that the percentage of B-cells in neonates is significantly lower than in adults. At the same time, however, the absolute number of B-cells is higher than the adult value. This is explained by the high initial WBC count and relative lymphocytosis in neonates. The percentage of B-cells reaches the adult values at a few months of age, while the absolute number attains the peak in this age group.

As far as T-cells are concerned the results in the literature are somewhat contradictory. Some authors found in neonates normal "adult" values [2, 10], while others reported reduced T-cell ratios [1, 3, 13, 14, 37, 43]. According to our findings the percentage of T-cells in neonates and young babies as determined by E-rosetting is significantly lower than in adults. It is around the age of 2 years when this value reaches that of the adults. When the number of T-cells was estimated with a heterologous anti-T-cell serum we found nearly identical percentages in all the examined groups. It seems therefore that noenates have a larger proportion of immature, E-rosette negative, T-cell pool, that may be demonstrated by other means, such as anti-T sera [1, 2, 38].

As regards the T_G—T_M cells of newborn babies, in most of the studies cord blood lymphocytes were tested [14, 18, 19, 23, 30, 31]. T_G cells were found to be increased in cord blood, showing a rapid decline to adult values by about 3 months. A reverse tendency was shown for T_M cells. We also found the percentage of T_G cells significantly higher in neonates than in the other 3 groups. Thus, the absolute number of T_G cells was highest in newborn babies and lowest in adults. These tests were carried out on peripheral blood lymphocytes.

There was a significant reduction in the ratio of T_M cells of neonates as compared to adult values. In young babies both the percentage and absolute number of T_M cells showed an

increase. In older children the percentage of these cells rose further, while their absolute number gradually declined. Similar results were obtained when the tests were carried out on unfractionated lymphocytes rather than separated T-cells. Naturally, these suspensions also contained some B-cells and other non-T lymphocytes as well as a few monocytes. In comparison to adult values both the relative and absolute number of EA-IgG positive cells was significantly higher in neonates in this test-system too. The ratio of IgM-Fc receptor positive cells, on the other hand, showed no significant difference from the adult value.

Looking at the results obtained with unseparated lymphocytes and isolated T-cells it appears that cells other than T-lymphocytes also take part in the formation of IgG-EA rosettes. In contrast, the IgM-EA rosettes in this test system are formed entirely from T-cells.

According to some of the earlier papers, B-cells and other non-T lymphocytes take no, or very little part in the formation of IgG- and IgM-EA-rosettes [44, 45]. Others, however, claim that under optimal conditions these receptors can be found on a significant proportion of the above-mentioned cells [12, 33]. Because of all this uncertainty, we feel that the enumeration of T_G and T_M cells should be carried out from isolated T-cells.

There are also somewhat conflicting reports concerning the lymphocyte responses to PHA in cord blood. Most authors found a higher spontaneous ^{3}H-thymidine uptake by neonatal lymphocytes [9, 24, 32, 34]. Maximum PHA responses were higher than those of the adult controls and were found at lower PHA concentrations (1—10 μg/ml). With higher PHA doses the responses were comparable in the two age groups [3, 6, 7]. In our experiments spontaneous thymidine uptake showed an inverse correlation with age, but the differences were not significant.

There was a nearly identical response to PHA in the observed age groups, at least to the single dose of PHA used in the experiments. Separated lymphocytes and whole blood cultures gave essentially identical results. Since this latter test requires only minute amounts of blood, ongoing studies are designed to establish the responses to various doses of mitogens as well as the kinetics of these responses.

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Hormonal control of sodium content in human milk

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The possible role of prolactin (PRL) and aldosterone (Aldo) in regulation of Na content of human milk was investigated.

Simultaneous determinations of Na and K in milk, PRL and Aldo in maternal plasma and milk were performed on postpartum days 2, 4 and 6, in samples from 15 mothers giving birth at term and from 8 mothers giving preterm birth. Na concentration and Na/K ratio in milk, Aldo concentration in milk and maternal plasma decreased significantly with advancing lactation. In preterm plasma and milk PRL concentration remained unchanged, whereas in term plasma PRL decreased, milk PRL increased significantly by the 6th day post partum. After preterm delivery milk Na and K levels were significantly higher, maternal plasma and milk Aldo slightly lower than after delivery at term. Daily Aldo and PRL excretion into the milk markedly increased during lactation irrespective of the length of gestation and showed a significant negative correlation with milk Na level and Na/K ratio.

It is suggested that the increasing rate of Aldo and PRL excretion into the milk may be of importance in the regulation of Na concentration in human milk.

In recent years, there appeared several reports on the postpartum fall of sodium concentration in human milk [1, 2, 12, 23]. The concomitant decline of the Na/K ratio suggested that the fall may be a result of the increasing rate of active reabsorption of sodium in exchange for potassium by the mammary glands [2]. In view of the increased urinary sodium loss, the frequently occurring hyponatraemia and the high daily sodium requirement of very low birth weight premature infants [7, 8, 15, 26, 30], particular attention has been paid to define the milk sodium content of mothers delivering premature infants.

In recent studies convincing evidence has been provided that sodium concentration of milk produced by mothers giving birth prematurely is significantly higher than that produced by mothers delivering at term during the first month post partum [11]. This observation raises the possibility that after preterm delivery the mother's own milk meets better the comparatively high sodium need of premature infants than would any natural pooled mature breast milk. On the contrary, Sann et al [27] and Schanler and Oh [28] did not find significant differences in the milk sodium content at any stage of lactation between mothers delivering at or before term. No reports have been published, however, as to the factors controlling the sodium content of human milk. Consequently, the reason for the rapid post partum decline and

for the difference, if any, between mature and premature milk sodium content remained to be clarified.

With respect to the fact that sodium retaining hormones like prolactin (PRL) [14] and aldosterone (Aldo) [31] have been demonstrated in human milk, it seemed relevant to assume that these hormones might play a role in the control of milk sodium concentration.

In the present study an attempt has been made to obtain information about the role of PRL and Aldo excretion into milk in the changes of milk sodium concentration in the first week after preterm and term delivery.

Materials and Methods

Two groups of healthy lactating mothers volunteered for the study. They were on a normal diet of 100—120 mmol/day sodium content without diuretic therapy. None had a history of renal disease or toxaemia of pregnancy. All had an uncomplicated vaginal delivery. No hormonal therapy was given to suppress lactation. Group I consisted of 15 mothers 22.1 (15—30) years of age, giving birth between 37—41 (mean, 38.7) weeks of gestation. The birth weight of their infants ranged from 2600 to 3840 g (mean 3017 g).

Group II included 8 mothers 22.5 (17—28) years of age delivering preterm babies with a mean gestational age of 31.8 (28—33) weeks and a mean birth weight of 1580 (1200—2050) g.

Maternal plasma and milk samples were obtained on days 2, 4 and 6 post partum. Blood was collected into chilled tubes containing heparin. Milk samples of about 1 ml were expressed by hand at the beginning and the end of feedings, at least three times each day. After separating the plasma, the samples were frozen and stored at —20°C until analysed. The total amount of milk was estimated by weighing the baby before and after each feed or by measuring the expressed volume of milk. Sodium and potassium concentrations were determined by flame photometry. Aldo was measured in plasma and milk by radioimmunoassay using SORIN-CEA-IRE ALDOK kits (32). The detection limit of Aldo was 4 and 15 pg/ml in plasma and milk, respectively. PRL was measured by radioimmunoassay as reported by Healy et al [14] using SERONO-BIODATA kits. Statistical analysis was performed by calculating the coefficient of correlation, the equation of regression and Student's t test.

Results

The sequential changes of sodium concentration and sodium to potassium ratio in milk obtained from mothers of term and preterm infants during the first week after delivery are shown in Figure 1. It can be seen that sodium concentration was the highest on the 2nd day and averaged 44.7 ± 3.9 mmol/l in term and $52.2 \pm \pm 2.7$ mmol/l in preterm milk. With progressing lactation sodium concentration decreased significantly at a similar rate in both groups and reached by the 6th day a level of 17.8 ± 1.7 mmol/l and 23.6 ± 2.5 mmol/l in term and preterm milks ($p < 0.01$), respectively. On days 4 and 6 post partum the mean concentration of sodium proved to be significantly higher in the milk of mothers of preterm infants than in that of mothers delivering at term ($p < 0.05$). Potassium concentration

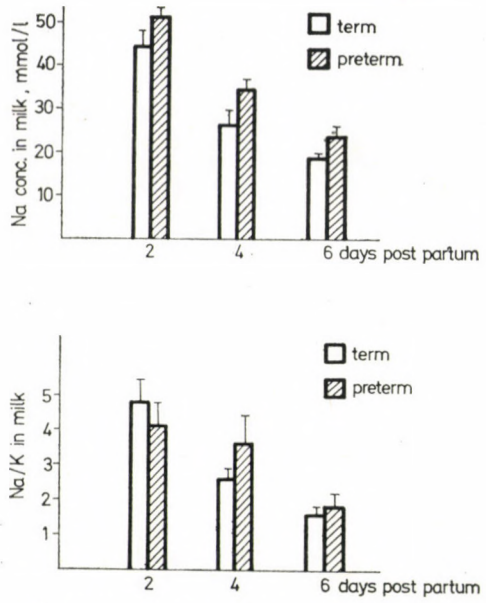

FIG. 1 Postnatal changes in sodium concentration Na/K ratio of human milk after term and preterm delivery

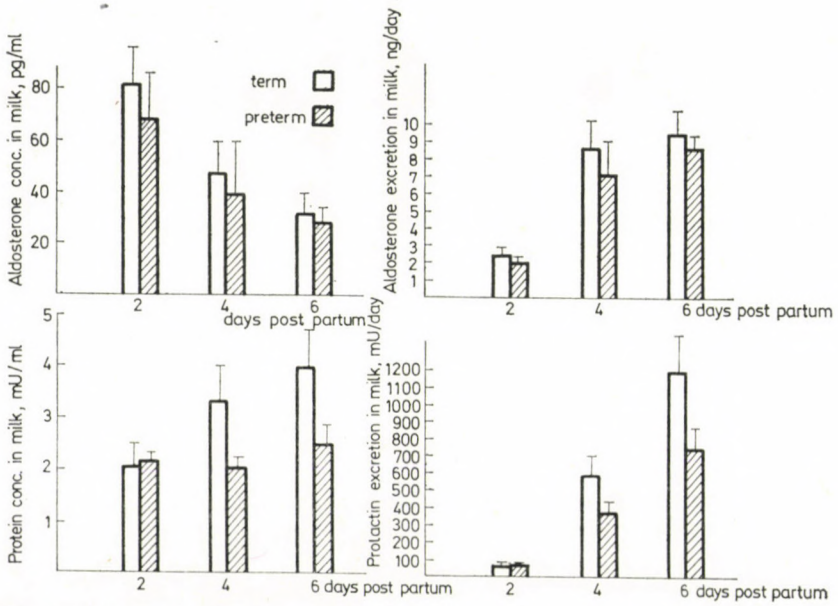

FIG. 2 Postnatal changes in the concentration and daily excretion of milk Aldo and PRL after term and preterm delivery

showed no consistent change over the course of the study and remained at the mean level of about 11 mmol/l after delivery at term and 13—14 mmol/l after preterm delivery. The difference between the two groups was significant during the whole period of study ($p < 0.05$).

As a result, the Na/K ratio was significantly higher in both groups on the 2nd than on the 4th ($p < 0.01$) and 6th day ($p < 0.05$), and so significant difference could be observed between the two groups as the lactation progressed (Fig. 1).

The postnatal changes in milk Aldo and PRL levels are set out in Fig. 2. Aldo could be detected in the milk of 5 out of 8 mothers of preterm and in the milk of 10 out of 14 mothers of term infants. The highest Aldo concentration of 80.6 ± 15.0 pg/ml and 66.7 ± 9.6 pg/ml in term and preterm milk, respectively, was found on the 2nd day. This was followed by a progressive decline in both groups to about 30 pg/ml by the 6th day ($p < 0.01$).

PRL was present in a measurable amount in all milk samples irrespective of the length of gestation and the time elapsed after delivery. After delivery at term, milk PRL concentration rose significantly from 2.1 ± 0.4 mU/ml on the 2nd day to 3.9 ± 0.7 mU/ml ($p < 0.01$) on the 6th day. Preterm delivery, however, was found to be associated with an unchanged milk PRL level of about 2 mU/ml resulting in a significantly higher PRL concentration in term milk on days 4—6 ($p < 0.05$). Due to the rapid increase in milk volume, the estimated rate of daily Aldo excretion rose considerably with the progress of lactation from 2.4 ± 0.5 ng/day

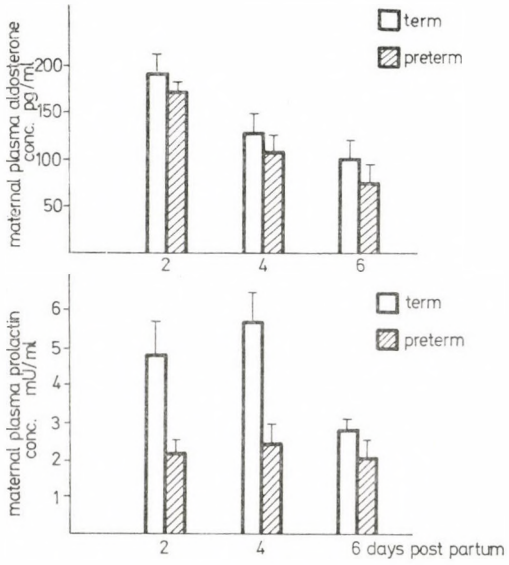

FIG. 3 Postnatal changes in maternal plasma Aldo and PRL concentration after term and preterm delivery

on the 2nd, to 9.4 ± 1.4 ng/day on the 6th day ($p < 0.05$) in the term group, and from 2.0 ± 0.3 ng/day on the 2nd, to 8.6 ± 0.6 ng/day on the 6th day ($p < 0.01$) in the preterm group.

The estimated daily PRL excretion was also found to increase significantly in both groups of mothers ($p < 0.01$), but on days 4 and 6 post partum it remained significantly lower ($p < 0.05$) after preterm delivery than after delivery at term (Fig. 2). Simultaneous measurements of maternal plasma and milk hormone levels revealed that the fall of milk Aldo concentration was fairly consistent with the fall of plasma Aldo concentration from 193 ± 22 pg/ml to 120 ± ± 20 pg/ml in the term group ($p < < 0.01$) and from 171 ± 12 pg/ml to 75 ± 21 pg/ml ($p < 0.01$) in the preterm group on day 2 to 6 post partum (Fig. 3).

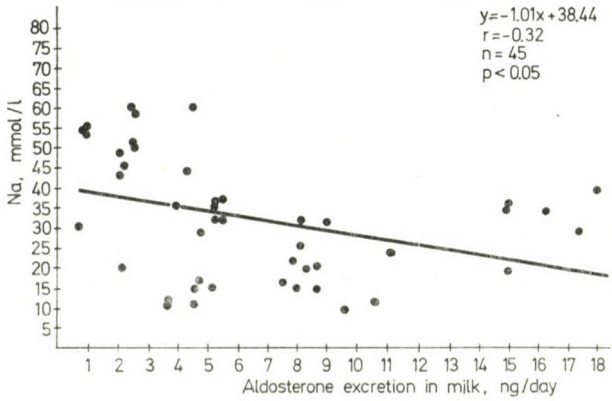

FIG. 4 Relationship between milk sodium concentration and daily Aldo excretion during first week post partum

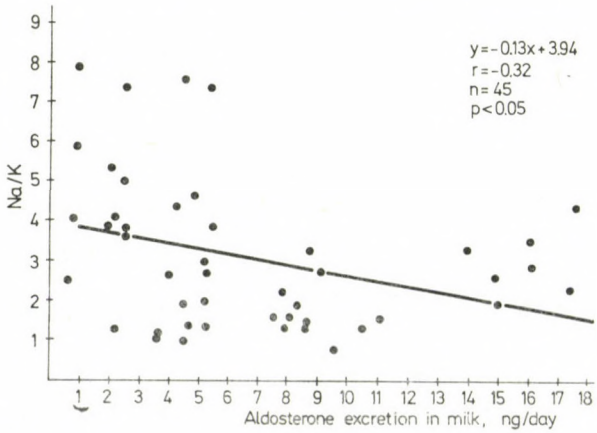

FIG. 5 Relationship between milk Na/K ratio and daily Aldo excretion during first week post partum

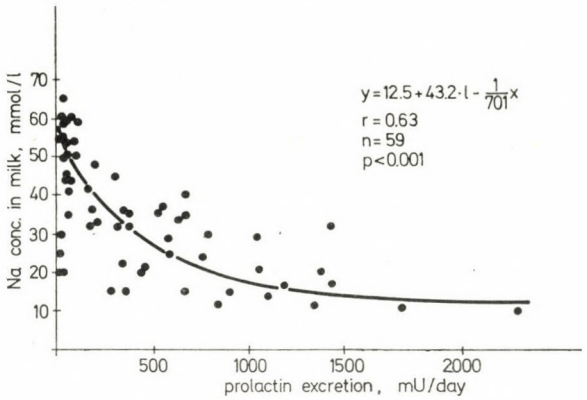

FIG. 6 Relationship between milk sodium concentration and daily PRL excretion during first week post partum

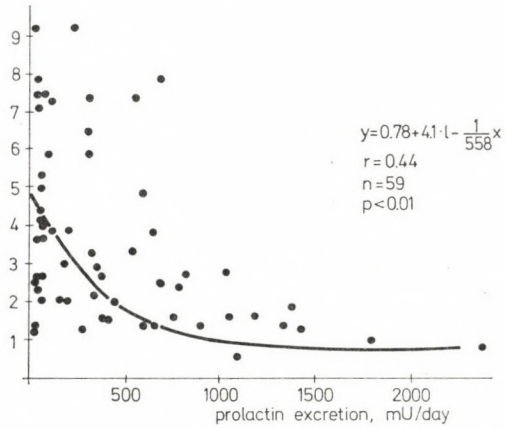

FIG. 7 Relationship between milk Na/K ratio and daily PRL excretion during first week post partum

The mean maternal plasma and milk Aldo concentrations tended to be lower in mothers delivering before term than in those delivering at term. The differences between the two groups did not, however, reach the level of statistical significance (Fig. 3).

On the basis of these measurements, the Aldo transfer ratio into milk was approximately about 3 : 1. Alterations in plasma PRL concentration had no influence on the milk PRL level. Mean plasma PRL was found to increase slightly, from 4.84 $\pm$ 0.88 mU/ml to 5.45 $\pm$ 0.75 mU/ml between days 2 and 4, followed by a significant decrease to 2.81 $\pm$ 0.49 mU/ml by the 6th day ($p < 0.01$) in the term group.

On the other hand, in the preterm group the plasma PRL concentration did not change over the course of the study and remained at the mean level of 2.11 to 2.46 mU/ml, being significantly lower on days 2 and 6 than in

the mothers delivering at term ($p < < 0.01$) (Fig. 3).

A significant negative correlation was found between milk sodium concentration, sodium to potassium ratio and daily Aldo and PRL excretion in the milk (Figs 4, 5, 6, 7), indicating the possible role of these hormones in the control of the sodium content of human milk.

Discussion

Recent studies have indicated that the milk of mothers of young preterm infants contains substantially more nitrogen, protein, sodium, chloride and calcium than the milk of mothers of full-term infants [3, 11]. Based on these observations, it has been recommended that preterm infants should be fed with their own mother's milk to meet the higher nitrogen and mineral requirements of these infants.

Moreover, it has been shown that milk sodium decreased from early to established lactation, whereas milk potassium showed no consistent change over the same period [11, 16, 27, 28]. No reasons have, however, been given to account for either the rapid postnatal fall in sodium concentration, or the striking differences in the mineral composition of milk following preterm and term deliveries.

The present findings are in good agreement with the observation showing that the sodium content of the milk of mothers delivering before term is in the first week post partum about 6 mmol/l higher than in the milk of those delivering at term [11]. It was also found that a decline occurs in sodium concentration in both groups of mothers, irrespective of the length of their gestation.

As to the decrease of the Na/K ratio it was justified to assume that the postnatal fall of the milk sodium level did not result from dilution; it was due rather to the increasing rate of active reabsorption of sodium in exchange for potassium by the mammary glands. Furthermore, the difference in the efficiency of active sodium transport might be expected to account for the higher sodium content after preterm delivery.

Abundant evidence has been provided to suggest that the activity of (Na-K) activated ATP-ase which is believed to represent the enzymatic equivalent of active sodium transport [29] is regulated by various hormones. In search of hormonal factors presumably involved in the control of milk sodium concentration we measured maternal plasma and milk levels and approximated the daily excretion rates of PRL and Aldo.

Like others [10, 14] we could demonstrate a considerable amount of PRL in human milk. Milk PRL concentration and daily PRL excretion was found to be significantly lower after preterm delivery than after delivery at term. Moreover, daily PRL excretion correlated inversely with the milk sodium and Na/K ratio indicating that the rising PRL excretion may play a role in activating the sodium transport mechanism in the mammary gland and so in the decline of the milk sodium level.

In support of this possibility are the following findings.

1. PRL has been regarded as a regulator of fluid and electrolyte metabolism [19]. It was found to enhance renal reabsorption of sodium and water [16, 22] through either its direct tubular effect or indirectly through increasing the sodium retaining action of Aldo and the water retaining action of antidiuretic hormone as observed in sodium-loaded or glucocorticoid-treated sheep [6, 17, 18].

2. Cell membrane and cytoplasmatic PRL receptors have been identified in the lactating mammary gland [4, 25] and also in milk [33]. In the rabbit, the PRL receptor content in milk, which was found to reflect the receptor content in mammary gland, was low after parturition but increased thereafter to reach its maximum by day 21 post partum. This high level was maintained over the next days of lactation [33].

3. In vitro and in vivo studies of the lactating rabbit mammary gland revealed that PRL caused a decrease in intracellular sodium and an increase in potassium concentration. The effect could be reversed by ouabain, a specific inhibitor of (Na-K) ATP-ase [9].

4. Sodium and potassium composition of normal milk reflects the intracellular ionic composition [9]. The declining mik sodium may therefore reflect the PRL-induced increase in the rate of active sodium transport. In fact, PRL treatment during late lactation resulted in an increase of milk potassium and in a decrease of milk sodium and chloride concentration [21]. Also, during the early post partum period, milk sodium concentration was slightly higher in bromocriptine treated women than in those taking placebo [2].

5. PRL level in maternal plasma increases significantly as gestation progresses [5]. Consequently, the plasma PRL level is lower at preterm than at term delivery. In addition, the lack of lactation-induced rise in PRL secretion in the case of a preterm infant may result in a low plasma PRL level after delivery. Experiments in rats revealed that larger litter size was associated with higher rates of PRL secretion [24].

6. Abrupt termination of breast feeding caused a rapid increase of milk sodium and chloride, and a concomitant fall in potassium concentrations [13]. These changes too may be attributed to the declining PRL level.

These observations could be interpreted to mean that the higher sodium concentration in milk from mothers delivering before term may be due to the reduced maternal plasma PRL, provided that the milk PRL level and the PRL-induced increase in active sodium transport are proportional to plasma PRL. Evidences have been provided to indicate, however, that no clear relationship exists between the serum PRL level and the receptor content of tissues or milk [33].

Beside PRL, Aldo excretion into the milk could also be demonstrated.

On the basis of the significant inverse relationship between daily Aldo excretion and milk sodium concentration or milk Na/K ratio it seems reasonable to expect that Aldo may have a regulatory role in the electrolyte composition of milk. Still, in several milk samples no measurable amount of Aldo could be detected. Moreover, to date no specific Aldo receptors have been identified in the lactating mammary gland and there is no evidence that Aldo is specifically taken up and concentrated by the breast gland epithelium. For this reason, the relationship between Aldo excretion and milk sodium content may only be incidental and should not be interpreted as a direct evidence of the role of Aldo in the control of milk sodium content.

Further studies are to be conducted to explore the hormonal control of active sodium transport in the mammary gland. The apparent difference in the electrolyte composition of milk after preterm and term delivery offers an opportunity to obtain valuable informations.

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Damaging effect of free radicals liberated during the reduction of oxygen: its influencing by drugs

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An experimental model has been elaborated for measuring the effect of various factors on oxygen toxicity in newborns. The preventive effect of the compounds with antioxidant properties, D-penicillamine, 6,6'-methylene-bis (2,2,4-trimethyl-1,2-dihydroxyquinoline), and vitamin E, on damage caused by hyperoxygenation was judged by measuring superoxide dismutase activity and the tissue content of malonyldialdehyde. Both compounds were found to possess antioxidative effects by either method; their use for therapeutic purposes is thus recommended.

Oxygen is the third most common element in nature. Five per cent of the human organism as a total and 67.8% of its aqueous compartment consist of oxygen. Molecular oxygen gas, playing a central role in vital functions, is a homogeneous, ubiquitous compound with excellent diffusibility. Most physiological reactions of molecular oxygen are mediated by enzymes. Reduction of molecular oxygen in aqueous solution under the effect of various catalysts leads to liberation of certain free radicals like superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl (OH.); their biological role has become a central topic of medical research during the last decade.

Some of the radicals arising from molecular oxygen play an important role in cellular respiration, metabolism and multiplication. Electron paramagnetic resonance spectroscopic studies revealed that these bioradicals are indispensable in subcellular reactions of mitochondrial oxidation. The free radical concentration shows a gradual increase during mammal organogenesis; after completion of this early embryogenic phase their concentration decreases. Figure 1 illustrates the production of free radicals as a result of electron transport, drugs, enzymes, air pollutants and ionizing radiation; in addition, the effects of enzymes on the free radicals are also shown. Free radicals induce autooxidation which is a typical chain reaction of free radicals. These are highly reactive atomic groups without electric charge, possessing an electron of paramagnetic property without a mate. They can attach to

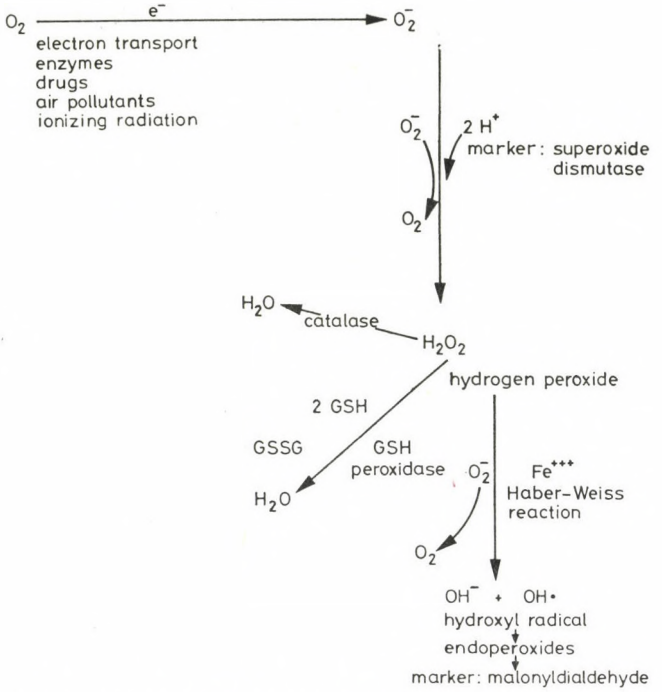

Fig. 1 Development of free radicals as a result of electron transport. The effect of certain enzymes on these radicals

carbon, nitrogen, halogen or other atoms.

Auto-oxidation proceeds through several phases.

a) Slow induction period: a radical arises by detachment of a hydrogen from a methyl group activated by an adjacent unsaturated bond.

b) The radical reacts with oxygen resulting in a peroxide radical.

c) The peroxide radical captures a hydrogen atom from the activated methyl group of another molecule, and hydroperoxide develops.

d) This latter radical, reacting with oxygen, is transformed to peroxide; this in turn reacts in the same way as described above, thus leading to a chain reaction.

Auto-oxidation in the living organism is a frequent event on membranes rich in lipids. Reactions resulting in liberation of free radicals may be enzymic or non-enzymic. No notable auto-oxidation occurs in the healthy human organism; in fact, the lipids resist oxidation for several hours after death. This points to the existence of extremely effective bio-auto-oxidants within the human organism [1, 6]. One of the most important among them, vitamin E, has been found in decreased quantity in prematures; in several diseases characteristic of premature age, a deficiency of bioantioxidants has indirectly been shown by administration of synthetic antioxidant compounds [12, 14].

Among the biological reactions mediated by oxygen radicals, liberation of superoxide anion during cellular aerobic glycolysis plays an important role in the bactericidal effect during phagocytosis [2, 7, 13, 20, 21].

In paediatrics, a metabolic disorder induced by the superoxide anion radical has gained special importance: elevated oxygen concentration in bradytrophic tissues may lead to cellular and vascular proliferation resulting e.g. in blindness caused by obstruction [14, 26].

One of the factors governing tissue oxidation is the activity of superoxide dismutase (SOD) and registration of its concentration by chemical or biophysical methods may cast light on the intensity of tissue auto-oxidation. More precise knowledge in this field may facilitate the elaboration of effective therapeutic measures against the cellular damage caused by radical reactions.

SOD is a metalloprotein enzyme, its physiological role is to govern the reaction

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

in other words, inactivation of the superoxide anion [8, 9, 19].

D-penicillamine (DPA) is an important drug in the therapy of rheumatic arthritis. Later it was shown to decrease the elevated bilirubin level of jaundiced newborn babies [4, 15]. The complex of DPA and copper exerts an effect similar to that of SOD: it inactivates the toxic oxigen radicals [17, 27].

Proteolysis in pulmonary tissue is enhanced by the free radicals arising during the reduction of molecular oxygen [10, 22]. To counteract this damage, some defensive mechanisms are activated, viz. a mobilization of oxidable substrates [24], and an increase in SOD activity for dismutation of the toxic radicals [5].

In an earlier study we have shown that changes in SOD activity are a good marker of tissue auto-oxidation; in the present work we performed measurements of the malonyldialdehyde concentration for the same purpose and, in addition to DPA, the effects of a synthetic and of a natural antioxidant, 6,6'-methylene-bis/ /2,2,4-trimethyl-1,2-dihydroquinoline (MTDQ) and vitamin E [3, 14].

Materials and Methods

The studies were performed in 3—4 days old Wistar rats. The newborn animals were allotted to 16 groups.

Group 1: 16 animals kept in atmospheric air.

Group 2: 12 animals, in 95% oxygen environment. Figure 2 shows the time of administration of oxygen and the drugs.

Group 3: 12 animals, receiving daily 400 mg/kg MTDQ for three days.

Group 4: 12 animals, receiving MTDQ and kept in oxygen rich environment for three days.

Group 5: 8 animals received a dose of 1 g/kg of intraperitoneal DPA following a time-table similar to that used in animals treated with MTDQ.

Group 6: 8 animals given DPA treatment in oxygen rich environment.

Group 7: 9 animals were treated with intraperitoneal doses of 45 mg/kg vitamin E daily over three consecutive days.

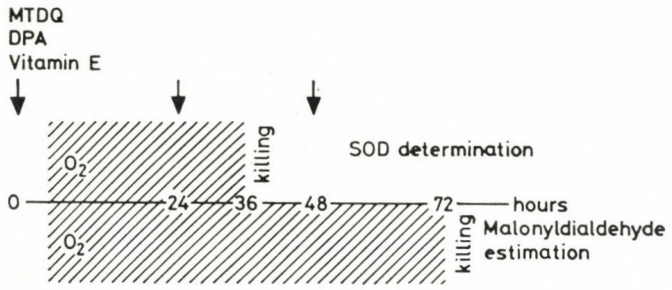

FIG. 2 Time-table of treatment of the experimental animals

Group 8: 7 animals received vitamin E, and were kept in 95% oxygen.

Groups 9 to 16 were corresponding controls.

The animals were killed by exsanguination. Malonyldialdehyde concentration and SOD activity were measured in liver and lung homogenates. The results were calculated for 1 g tissue. The method of SOD activity measurement has been described earlier (8, 14, 18, 19).

The concentration of malonylaldehyde was measured as follows. The tissue particles were homogenized in eightfold quantity of buffer, incubated at 37°C for 1.5 hours, then 100 μl trichloroacetic acid was added to 200 μl suspension, this was followed by centrifugation at 2000 g for 10 minutes. To 150 μl supernatant 100 μl thiobarbituric acid was added then kept in a boiling water bath for 10 minutes after cooling, and photometry at 532 nm was carried out. The results were calculated by aid of a calibration curve and expressed in mol/g wet tissue.

The results were analysed by Student's two-tailed t test.

RESULTS

Figure 3 shows the malonylaldehyde content of the lung tissue of newborn rats. The mean value for the control group kept in atmospheric air was 2.96×10^{-8} mol/g. Prolonged oxygenation markedly increased this value, by about 60% ($p < 0.001$). MTDQ caused a decrease of the tissue malonyldialdehyde level in animals

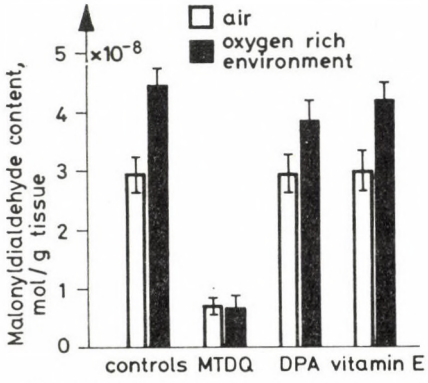

FIG. 3 Pulmonary malonyldialdehyde content of newborn animals kept in air and high oxygen environment, and treated with MTDQ, and vitamin E

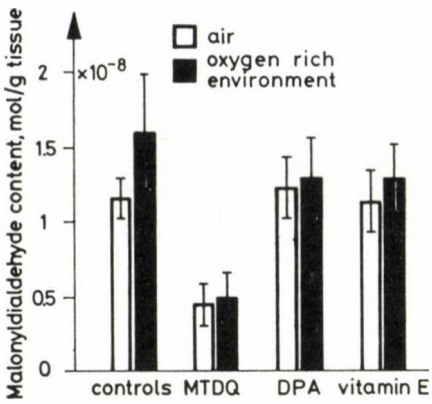

FIG. 4 Liver malonyldialdehyde content of newborn rats kept in air and high oxygen environment, and treated with MTDQ, DPA and vitamin E

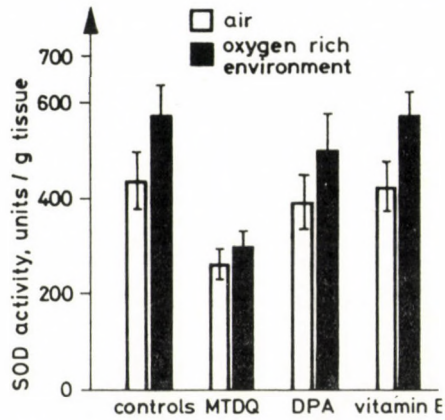

FIG. 5 Lung SOD actvity of newborn rats kept in air or oxygen rich environment, and treated with MTDQ, DPA and vitamin E

kept in both air and oxygen-rich environment, but the mean of these groups did not significantly differ from each other: 7.35 and 7.05×10^{-8} mol/g, respectively. This was a reduction by 75% compared to the value of the control group and by 85% if compared with the controls kept in oxygen rich environment. These differences were strongly significant ($p < 0.001$). DPA caused a less pronounced change; an insignificant decrease could only be shown in animals kept at high oxygen concentration ($p < 0.001$). Vitamin E had no demonstrable effect under either environmental condition. The most pronounced changes were caused by MTDQ, which significantly superior to the other antioxidants ($p < 0.001$).

Hepatic malonyldialdehyde concentrations reflected similar changes (Fig. 4). MTDQ elicited a significant decrease in either type of ambient

oxygen concentration, e.g. in animals kept in high oxygen the mean value dropped from 1.72×10^{-8} mol/g to 4.98×10^{-9} mol/g, corresponding to a reduction by 83% ($p < 0.001$). The two other antioxidants had an insignificant effect.

Figure 5 illustrates the SOD activity values in the lung. Oxygen alone increased the basal value of 436 U/g to 571 U/g ($p < 0.001$). MTDQ treatment decreased the mean value for the normal control group to 264 U/g, and that by 53% for the animals in oxygen rich environment ($p < 0.001$). DPA too, caused a decrease, in air by 10%, in high oxygen by 13% ($p < 0.001$). Vitamin E did not affect SOD activity. The effect of MTDQ was highly significantly stronger ($p < < 0.001$), than that of the two other drugs, independently of the oxygen concentration of ambient air.

Discussion

As the results showed, malonyldialdehyde concentration is a sensitive marker of the presence of free radicals liberated under the effect of various concentrations of oxygen. Protracted oxygenation led to a significant increase in the control animals, indicative of an accumulation of free radicals.

Oxygen induced a significant increase in liver and lung SOD activity. MTDQ caused a reduction by 50%; this suggests that the compound may play a role in counteracting the effect of free radicals. DPA caused a 10% reduction which too may be of use, in therapy.

Both MTDQ and DPA have been applied in treatment and we suggest to test their preventive effect against the side-effects of oxygen therapy in premature babies, especially retrolental fibroplasia and pulmonary dysplasia.

Malonyldialdehyde concentration as a marker of the quantity of peroxides liberated in the course of auto-oxidation was chosen by us since we had opportunity to compare this method with the common iodometric and polarometric assays [11, 16, 23, 25]. We have been able to show that the malonyldialdehyde method is most reliable and well-reproducible. We believe that the results can readily be transferred to the therapy of life-threatening conditions caused by elevated levels of free radicals in premature babies.

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D-penicillamine in the prevention of retrolental fibroplasia

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The effect of D-penicillamine on the occurrence of retrolental fibroplasia was analysed in 195 infants born in the years 1977—1980 with birth weights between 700 and 1500 g. Among 109 D-penicillamine treated infants a single one was found to have retrolental fibroplasia whereas of 86 without such treatment 10 developed severe cicatricial retrolental fibroplasia. It seems that in preventing hyperbilirubinaemia and oxygen toxicity the mechanism of action of D-penicillamine is a protection of biomembranes against lipid peroxidation.

Nearly 40 years have passed since Terry [33] had described retrolental fibroplasia (RLF) in premature infants. With the decreasing mortality and increasing survival rates of prematurely born infants, the risk of RLF is still a serious problem. Clinical and experimental evidence has suggested that oxygen toxicity is a major factor in the aetiology of RLF and bronchopulmonary dysplasia [11, 13, 31, 32]. Identification of agents or drugs to protect against oxygen toxicity may provide a means to reduce the incidence of these disorders.

D-penicillamine (DPA) for the control of neonatal hyperbilirubinaemia was introduced in our Department in March, 1973 [18, 19]. This treatment was especially effective in jaundice of haemolytic origin such as ABO or Rh incompatibility, and is used with phototherapy for the prevention of hyperbilirubinaemia of premature infants.

The present study was performed to draw attention to a new possible use of DPA, in the prevention of RLF.

Material and Methods

In a previous retrospective study, 407 premature infants who had received supplemental oxygen therapy during the period May, 1974, to December, 1978, were examined by indirect ophthalmoscopy. Eleven cases of RLF were diagnosed in that study. During the two and a half years prior to the development of our neonatal intensive care unit there was only 1 out of 158 in contrast to the 10 out of 249 cases during the following two years who were found to have cicatricial phase RLF. Every infant who developed RLF had a birth weight under 1500 g and a gestational age of 26 to 33 weeks. It was surprising that among babies treated with DPA to prevent hyperbilirubinaemia in the neonatal period of life there was only one case of RLF. The clinical data of the 193 infant survivors with a birth weight between 700 and 1500 g are shown in Table I. Two aspects have to be considered:

TABLE I

Distribution of very low birth weight infants receiving supplemental oxygen therapy and D-penicillamine treatment in the retrospective screening program

Birth wt g Gestational age, wks	DPA	Intensive care nursery		Total
		Before	After	
		n/RLF		
< 1500	—	46/0	86/10	132/10
26—33	+	25/1	36/0	61/1

n/RLF = Incidence of retrolental fibroplasia in the given groups

TABLE II

Survival rates of infants weighing less than 1500 g, in 1975—1980

	Year					
	1975	1976	1977*	1978*	1979*	1980*
Survival rate per cent	31.5	47.6	60.0	60.6	54.3	60.9

* Treated in intensive care nursery

first, it is obvious that DPA treatment was associated with a decrease in the incidence of RLF; second, only the cases in the marked quadrant can appropriately be compared i.e. the patients discharged after the development of intensive care nursery. The unequal division of cases probably indicates the hesitancy to withhold DPA treatment for premature infants in view of its presumed beneficial influence. So we decided that no additional infants should be placed in the "control" group, and all infants weighing less than 1500 g and requiring oxygen received DPA therapy during the period May 1979 — October 1980 [17a]. Our decision was supported by the following considerations. (i) For infants with respiratory difficulties at any time in the neonatal period, oxygen administration was always indicated. Unfortunately, at the same time we had no possibility to measure the arterial PaO_2 systematically. The method of oxygen treatment has remained unchanged since January 1977. Then, all prematures were assigned to routine (unrestricted) oxygen for the 18 months of the supplemental prospective study. As can be seen in Table II, during the past four years, the survival rate of infants with very low birth weight has improved significantly, largely because of their treatment in the intensive care nursery, and it tends to remain unchanged. (ii) Elevation of the unconjugated bilirubin level is a frequent occurrence in preterm babies. Although DPA itself has a moderate effect against hyperbilirubinaemia of prematures with a short gestational age (15), together with phototherapy it seems to control the plasma bilirubin level satisfactorily in very low birth weight infants too. (iii) Last, but not least, ethical aspects have also influenced our decision, RLF is namely a severe disease resulting in lifelong blindness.

DPA (Metalcaptase® — Knoll AG, Ludwigshafen, FRG) was applied intravenously in doses of 300 mg/kg body weight daily,

divided into 4 equal amounts, starting at 12—24 hours of life. The period of treatment varied between 2 and 5 days. There were 160 preterm infants with very low birth weight admitted to the neonatal intensive care unit from May 1, 1979, to October 31, 1980. All neonates required supplemental oxygen. Of these infants 91 (57%) survived. The infants were examined with regular neurologic and developmental evaluation in our neonatal clinic; 73 (81%) were then examined by indirect ophthalmoscopy with scleral depression. The rest of the patients (n = 18) could not be contacted and did not report at the clinic. Generally, the first fundus examination was scheduled at 3 to 5 weeks of age. If the initial finding was normal, the baby was examined monthly up to 6 months of age. The eye changes were graded according to Reese et al [28]. Gestational age was determined from the menstrual history and confirmed by the clinical criteria of Dubowitz et al [6]. The medical records of the 195 infants, 109 treated with DPA and 86 untreated, discharged between 1977—1980, were reviewed and the systematically tabulated clinical data were analysed by Student's t or the χ^2 test.

Results

The clinical data and neonatal morbidity factors of the 195 survivors with a birth weight between 700 and 1500 g are shown in Table III. These data indicate that the two groups of infants treated with or without DPA were well matched on the basis of birth weight, gestational age, and neonatal morbidity factors. Also, the neonatal factors associated with the inspired amount of oxygen showed no significant difference except for phototherapy in the DPA-treated and not in the control group (Table IV). The data for the 11 infants who developed RLF and survived the neonatal period are summarized in Table V. One case out of 109 DPA-treated infants was found to have RLF, whereas 10 out of 86 without such treatment developed severe cicatricial RLF. The difference was statistically

Table III

Clinical data and neonatal morbidity factors of all infant survivors with a birth weight of 700 to 1500 g, in 1977—1980

Clinical data	DPA-treated	Control	P value*
No. of babies	36 + 73 = 109	86	
Birth weight, g	1261±19.8	1242±20.0	N. S.
Gestational age, wk	32.6±2.8	32.4±2.3	N. S.
Apnoea once	10	8	N. S.
Apnoea several times	56	39	N. S.
Septicaemia	7	4	N. S.
Maximum weight loss, g	121±46	88±7	N. S.

* = Probability value derived from χ^2 test or Student's t test. N. S. denotes $P > 0.05$

TABLE IV

Factors associated with oxygen dose

Factors	DPA-treated (n = 109)	Control (n = 86)	P value
No. of infants treated with O_2. Duration of O_2 exposure (hours)			
$FiO_2 = 1.0$	58	39	N. S.
	2.7±0.5	3.2±0.8	N. S.
$FiO_2 = 0.6$	70	54	N. S.
	77.8±27.8	47.1±6.4	N. S.
$FiO_2 = 0.4$	109	86	N. S.
	61.1±5.9	50.2±5.3	N. S.
Exchange transfusions, (number)	26	25	N. S.
Phototherapy, (number)	56	22	<0.01
Respiratory aids:			
No. of infants treated with CPAP	60	49	N. S.
Duration of O_2 exposure, (hour)	49.1±6.1	51.1±6.5	N. S.
No. of infants treated with O_2 via mask	109	86	N. S.
Duration of O_2 exposure, (hour)	65.3±6.0	49.8±5.3	N. S.

CPAP = Continuous positive airway pressure
FiO_2 = Fraction of inspired O_2

significant by the X^2 test. In addition the DPA-treated RLF infants had more total hours of oxygen treatment than the control RLF babies.

DISCUSSION

The results suggest that DPA treatment in the neonatal period is associated with a marked decrease in the incidence of severe RLF among very low birth weight infants. Such a protective effect of DPA would be consistent with a radical-mediated mechanism of oxygen toxicity.

At present, hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), hydroxyl radical (HO^-), and singlet excited oxygen ($^1O_2$) are all considered to be possible agents of hyperoxic tissue damage. Oxygen radicals, in turn, participate in radical chain reactions causing lipid peroxidation, depolymerization of mucopolysaccharides, protein sulphhydryl oxidation and crosslinking, which lead to enzyme inactivation and damage of nucleic acids [5, 8, 17].

Protective agents act directly to eliminate the formation of toxic substances such as oxygen radicals. Examples of such agents are radical quenchers or antioxidants. A second type of protection occurs with compounds that induce tolerance by increasing the activity of enzymes

TABLE V

Infants with retrolental fibroplasia

Infant No.	Date of birth	Gestational age, wk	Birth wt, g	Cicatricial RLF OD/OS	Total hours O_2 FiO_2			Total hours O_2		Et.	DPA	Comment
					1.0	0.6	0.4	CPAP	Mask			
1	1977	28	1200	O/V	8	100	100	100	108	+	—	IRDS
2	1977	28	950	V/V	6	72	48	72	54	+	—	IRDS
3	1977	31	1500	V/V	—	48	48	48	48	+	—	IRDS
4	1977	29	1400	V/V	—	60	50	60	50	—	—	IRDS
5	1977	30	1200	V/V	—	—	50	—	50	+	—	Tachypnoea
6	1977	32	1420	V/V	6	84	20	84	26	+	—	IRDS
7	1978	26	1150	V/V	6	186	20	180	32	+	—	IRDS
8	1978	33	1300	V/V	—	40	10	40	10	—	—	IRDS
9	1978	32	1250	V/III	—	30	10	30	10	—	—	IRDS
10	1978	27	950	I/II	—	60	70	—	130	+	—	Sepsis
11	1979	29	1100	V/V	4	200	200	200	204	—	+	IRDS

Et = Exchange transfusion

that protect against oxygen. Several studies have provided clinical and experimental data demonstrating that DPA may act by both pathways of the antioxidant defense mechanism.

I. DPA as an oxygen radical scavenger

(i) DPA, chemically dimethylcysteine, like other sulphhydryl compounds, can protect tissues from oxygen toxicity and it is related to its free radical scavenging SH group [7, 21, 27].

(ii) Enzymes which catalyse the dismutation of O_2^- are metallo-proteins termed superoxide dismutases. Lengfelder and Elstner [22] found a superoxide dismutating activity of the red-violet-copper-DPA complex. Recently it has been reported by Robertson and Fridovich [29] that the complex does not catalyse the dismutation of O_2^-. It is, however, not entirely stable. Its gradual decomposition releases Cu(II) or smaller complexes of DPA, and these catalyse the dismutation of O_2^-. In any case, if such copper complexes are formed during DPA treatment, this may offer protection against free radicals. Our previous animal experiments showed that prolonged hyperoxia caused an increase in superoxide dismutase activity while DPA was found to inhibit the inductive effect of oxygen. These data suggest that DPA may provide sufficient antioxidant protection to prevent the usual biochemical response to hyperoxia [14].

(iii) An undefined part of the drug effect might result from its chelating properties since trace metals, especially copper and iron, are potent catalysts of lipid peroxidation [1, 3, 16, 34]. This may be the most probable hypothesis, for it is well-known that the accumulation of trace elements is higher in the newborn's organs than in those of adults [30].

(iv) DPA, as other thiols, has a great tendency to form mixed disulphides with disulphide-containing serum proteins, and through these oxidations it may have a protective, reducing effect on free radicals and act as a hydrogen donor [25].

(v) DPA satisfies several criteria of radioprotection. It is a dose-modifying agent whose dose reduction factor is directly proportional to the concentration of the drug, and it affords protection when administered before, but not after, irradiation [35, 36]. Based on the report of Gerschman et al [9], it has been widely accepted that irradiation and oxygen exposure produce at least some of their lethal effects through one common mechanism, the formation of oxidizing free radicals. In addition, in our experiments the radioprotective effect of DPA was significantly greater in 3—4 days old mice than in adult animals [20].

(vi) In several previous reports we have shown that DPA given intravenously to newborns reduces the plasma bilirubin concentration or prevents the increase usually seen during the first few days of life. There are a number of observations to show that bilirubin is a photodynamic agent, i.e. in the presence of oxygen

and light it produces $^1O_2$ and peroxidation of unsaturated membrane lipids [4, 10, 12].

II. DPA as an inductor of enzymes contributing to antioxidant defense

(i) Administration of DPA may liberate some of the protein-bound glutathione and thus increase the concentration of free glutathione. This thio tripeptide plays an important role in the stability of cell membranes and in protection against damage from superoxides and free radicals [26].

(ii) In a recent study [24] we have examined how in vivo treatment affects the enzymes of peroxide metabolism in the liver of newborn rats and in the tissues of adult rats. It was found that the drug acted in different ways in newborn and adult rats. In both cases DPA caused an increase in the liver microsomal cytochrome P-450 activity and a decrease in lipid peroxidation. As regards the enzyme changes, DPA treatment led to an increase in liver peroxidase and catalase activity only in newborn rats. These findings are in accordance with our previous observation that DPA shortens the hexobarbital sleeping time in newborn but not in adult rats.

The age related differences in the drug effect can well be explained by the data of Maines and Kappas [23]. According to their studies, metal ion induction of haem oxygenase, a rate-limiting enzyme of haem degradation, is accompanied by various disturbances of haem metabolism including alterations in delta-amino-laevulinate synthetase activity, decreased microsomal contents of haem and other haem proteins. When metals are complexed with cysteine or glutathione, their ability to alter the metabolism of haem is totally blocked. The high activity of haem oxygenase in the newborn could reflect the enzyme-inducing action of metals derived from the breakdown of fetal erythrocytes. Chelation therapy in neonates restores the normal activity of enzymes participating in haem metabolism. Briefly, chelating agents facilitate haem synthesis and inhibit haem degradation, thus boosting the newborn's liver to the adult detoxifying level. Since the enzymes which play an important role in antioxidant defense and drug metabolism (peroxidases, catalase, cytochrome P-450) are haem proteins, it is likely that the prevention of hyperbilirubinaemia and of oxygen toxicity by DPA rests on a common mechanism, the protection of biomembranes against lipid peroxidation.

Finally, we wish to emphasize that although DPA was administered to the babies in uncommonly high doses as compared to those applied in adults, no serious side effects have so far been recorded in more than 2000 infants treated in the last eight years. Furthermore, on the basis of a follow-up study carried out at 3—4 years of age we could demonstrate that DPA had no harmful long-term side effects. Neither had the drug a bilirubin displacing effect on the albumin-bilirubin complex [2]. Conse-

quently, concerning risk *vs* benefit of DPA therapy of neonates, the data of the present study provide support for conducting further controlled clinical trials.

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Immunological findings in primary malabsorption

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To evaluate the reactivity of patients affected by primary malabsorptive disorders, circulating and secretory IgA, antibodies to milk and gliadin, and soluble immunocomplexes were studied. Special attention has to be paid to tissue-bound immune complexes detectable by immunofluorescence, as the test may point to an advanced stage of the disease.

In the studies concerning the aetiology of coeliac disease and other malabsorptive disorders of infancy and early childhood, increasing attention has been paid to local and systemic immune responses to nutritive allergens. In this paper the importance of immunological studies in primary and secondary malabsorption has been reviewed. In our patients affected by primary and secondary malabsorption treated during the last ten years, the following tests have been carried out: measurement of immune proteins in serum and jejunal juice; antibodies to cow's milk and gliadin and their relationship to eventual IgA deficiency; measurement of circulating soluble immune complexes; and immunofluorescence of jejunal mucosa of patients affected by coeliac disease.

Materials and Methods

In 121 children, 59 boys and 62 girls aged 8 months to 12 years, affected by malabsorption, and in 70 healthy control children of similar age the quantity of immunoglobulins was measured by the technique of Mancini and values were related to standard values published by Uffelman et al [19] i. e. IgA, 0.72 g/l; IgM, 0.08—1.22 g/l; and secretory IgA in jejunal juice, 0.04—0.44 g/l. Antibodies to cow's milk were estimated by the passive haemagglutination test of Boyden [3] modified by Grenzmann et al [11] as described by Nagy et al [15]. Antibodies to gliadin were measured by immunodiffusion and the circulating soluble immunocomplexes by the polyethylene glycol technique of Digeon et al [9]. For immunofluorescence, the original serum of Hyland dissolved in its solvent was used. Jejunal biopsy specimens were frozen in carbon dioxide and the sections were treated with biopsy buffer; after fixation in ethanol-ether, concentrated FITC serum was applied.

Results

Since in most cases of villous atrophy the serum immunoglobulin level was low [4, 5, 6, 14] and Kaufmann and Hobbe [12] had shown low serum levels of IgA in atopic populations, we performed measurements of

serum and jejunal secretion IgA values in patients affected by coeliac disease and secondary malabsorption. The group of secondary malabsorption comprised patients recovering from acute enteritis, patients affected by dyspepsia caused by giardiasis or protracted antibiotic therapy, and patients with allergic diarrhoea elicited by ingested protein antigens.

Table I shows the results obtained in these patients.

Immunodiffusion revealed low serum IgA levels in a larger proportion of patients affected by coeliac disease than among those with secondary malabsorption. Secretory IgA is frequently absent from the jejunal secretion of patients with coeliac disease. The serum immunoglobulin levels have been described and evaluated in detail in a previous publication [18].

Special attention has been paid to the relationship between decrease or absence of secretory IgA and the presence of antibodies to gliadin since the secretory IgA plays an important role in mucosal defense Those results are presented in Table II.

In patients with coeliac disease having high antibody titres, decreased values or lack of IgA in serum and, more frequently, in the secretions, were encountered especially in the acute phase of the disease. Such a

TABLE I

Secretory and circulatory immunoglobulins of patients affected by primary and secondary malabsorption

	No. of samples examined		IgA			
			in serum		in jejunal juice	
	serum	jejunal juice	low (< 0.68 g/l)	absent	low (< 0.16 g/l)	absent
Secondary malabsorption	91	82	6	—	3	6
Coeliac disease	30	26	12	—	4	22
Total	121	108	18		7	28

TABLE II

Secretory IgA in jejunal secretion and antibodies to gliadin in patients with coeliac disease, cow milk allergy, recovered from acute enterocolitis and in healthy persons

	No. of patients	Jejunal juice secretory IgA		Positive anti-gliadin antibody titre 1/8—1/16
		low (< 0.16 g/l)	absent	
Coeliac disease	32	4	28	22
Cow milk allergy	20	5	2	2
After enterocolitis	24	5	3	2
Healthy	50	6	0	0

TABLE III

Antibody levels to cow milk and gliadin in patients with various forms of malabsorption and in healthy children

	Cow milk protein		Gliadin	
	No.	Positive < 1/16	No.	Positive titre, 1/8—1/16
Healthy children	56	3.6	187	0
Allergic diarrhoea	36	22	36	14
Coeliac disease	18	39	18	83
Giardiasis	17	23	17	6
Other malabsorption	28	11	28	0

TABLE IV

Gliadin antibody titres and circulating immune complex levels in patients with coeliac disease, secondary malabsorption and in healthy children

	No. of patients	Antigliadin antibody titre		Circulating immune complex	
		$\geq 1/8$	$< {}^{1/8}_{1/16}$	≥ 0.12	< 0.12
Coeliac disease	20	8	12	6	14
Other malabsorption	20	16	4	17	3
Healthy children	20	18	2	17	3

high incidence of positivity was not found in the controls or in patients affected by other forms of enteritis. This seems to confirm the assumption that secretory IgA plays an outstanding role in the defense of jejunal barrier function [1, 2, 18, 20].

Table III shows the level of antibodies against cow's milk and gliadin. It can be seen that in some control persons a cow's milk antibody titre exceeding 1 : 32 could be demonstrated. Such a titre was found in a high proportion of the patients affected by either form of malabsorption.

In nearly all patients acutely affected by coeliac disease an antibody titre against gliadin exceeding 1 : 16 was demonstrated. This finding is similar to that encountered by Csorba et al [7] and Rossipal [17], although these authors used different techniques. An elevated level may occasionally be found in enteritis caused by giardiasis and also in other forms. In the secondary forms, however, the phenomenon is only transitory while in gliadin sensitive coeliac disease it is a constant finding and the level of antibody decreases or disappears only after protracted dietary therapy.

Accumulation of deposits containing immunoglobulins in the jejunal mucosa of patients affected by coeliac

disease is a well-documented fact [5, 10, 18]; this has prompted us to extend our studies to immune complexes in the circulation. As can be seen from the data shown in Table IV, there is a numerical correlation between the immune complex content and the anti-gliadin antibody titre of the sera: similar findings have been described by Kávai et al [13]. In our experience a titre higher than 1 : 8 s of diagnostic value; in all cases having a titre as high there was an elevated level of circulating immune complex. In some cases, however, increased immune complex levels were associated with low antibody values.

In most cases the soluble immune complex level was high. Further direct analysis of its composition was not possible, we looked therefore for indirect methods of identification: immune complex deposits in the jejunal mucosa of 30 patients with coeliac

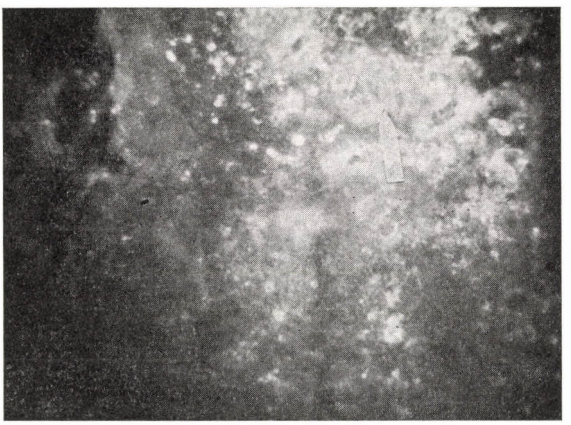

FIG. 1. Immunofluorescent picture of specimen from a 8-year-old boy



FIG. 2. Immunofluorescent picture of specimen from a 2-year-old girl. IgA particles within the duodenal and jejunal mucosa. Magnification, ×720

disease were examined by immunofluorescence. Figures 1 and 2 represent the immunofluorescent picture of specimens obtained from two of our patients. The specimens were tested for the presence of IgA, IgG, IgM and complement. The highest incidence, 30%, was observed with IgA. The next most frequent positive finding was the presence of complement (10%). Nearly all the patients exhibiting immune particles were above two years of age; no immune particles were detectable in infants with gluten sensitive enteropathy. It may thus be concluded that immune particles appear only when the disease has existed for a long time.

Discussion

Several methods are now available for examining the organism's reactivity. In patients affected by primary or secondary malabsorption, determination of serum and secretory IgA and antibodies to cow's milk and gliadin is most rewarding. These methods help much in differential diagnosis and judgement concerning the severity of these disorders.

Absence of secretory IgA in jejunal juice points to a weakening of the local defense of the intestinal mucosa and this may be a pathogenetic factor important in coeliac disease and cow's milk allergy [20]. The simultaneous presence of antibodies against cow's milk and gliadin suggests that gliadin sensitivity may lead to secondary hypersensitivity to cow's milk, in other words, demonstration of antibodies against cow's milk does not exclude the presence of coeliac disease. On the contrary, their coexistence with antibodies to gliadin corroborates the diagnosis.

Our findings confirm the observations of Mietens [14]. In patients with cow's milk allergy, elimination of clinical symptoms and abnormal laboratory findings can be achieved by early institution of an adequate diet for 6—22 months [16].

A titre of 1 : 16 or higher can be found in nearly all patients affected by acute coeliac disease; in addition, it may be encountered in occasional cases of chronic enteritis and with certain intestinal parasites. While in secondary malabsorption the finding is transitory, it is a constant and stable abnormality in patients with coeliac disease, where the antibody level decreases only after prolonged dietary treatment; similar findings have been published by Rossipal [17].

Demonstration of IgA, less frequently IgG or complement in the immune deposits found in jejunal mucosal specimens obtained by suction biopsy may support the pathogenetic role of circulating immune complexes captured by the jejunal mucosa. The level of circulating soluble immune complex is in some degree related to the severity of the disorder or to the remission achieved by treatment. The site of deposition of the immune complex may be specified by immunofluorescence of the jejunal mucosa. In our experience the complex is usually bound to the

glandular substance adjacent to the basal membrane.

Immunological studies are extremely helpful in determining the actual reactivity of patients afflicted by primary malabsorption. In spite of growing insight since the description of the role of gliadin in the pathomechanism of coeliac disease [8], the diagnosis is still difficult. Our findings are believed to have contributed to the better understanding of the immunological aspects of this disorder.

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The effect of caffeine and physical exercise on blood lactate levels of obese children

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The effect of caffeine and physical exercise has been investigated in obese children. The level of free fatty acids, lactic acid and glucose has been observed during physical exercise combined with administration of placebo respectively caffeine. Lactate levels showed a smaller increase in obese children exerting physical work and receiving caffeine than in the placebo groups or in similarly treated children with normal body weight.

Thus, caffeine has been found to increase the working capacity of obese children approaching the level of children under training.

In a previous study we have measured ergometrically the muscle work exerted until exhaustion and found that caffeine increased muscle work in obese and lean children alike. This may be the result of cerebrocortical stimulation or a direct action on muscle. Caffeine is known to promote lactic acid production in muscle tissue.

The aim of the present study was to investigate the effect of caffeine and physical exercise on working capacity and exhaustion; the latter was monitored by blood lactate measurements.

Materials and Methods

Sixteen obese children, aged 10 to 14 years (mean decimal age, 11.85 ± 1.52 years) participated in the study. Six children of normal weight and of similar age (mean decimal age, 11.70 ± 1.41 years) served as controls. The obese children had a mean weight of 67.8 ± 12.8 kg while the children of normal body weight weighed 32.0 ± 7.2 kg on the average.

The trial was performed in three steps. We examined the effect of caffeine alone, of caffeine combined with physical exercise and of placebo combined with physical exercise. The dose of caffeine was uniformly 4 mg/kg calculated for mean body weight corresponding to height. The caffeine was dissolved in 50 ml tap water and administered orally. The placebo consisted of 50 ml tap water. The interval between the steps was 48 hours. In order to exclude psychic effects, half of the children received first caffeine and then placebo, in the others the sequence was reversed. The effect of caffeine was investigated 20 minutes after ingestion of the drug.

Working capacity was measured by a step test. The height of the stair was 45 cm, physical exercise was carried out for 4 minutes. The step count of untreated normal children was 120 ± 4.6, of untreated obese

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children 95 ± 3.2, of caffeine treated children it was 126 ± 5.4 resp. 103 ± 2.9. In all the groups blood lactate (2), glucose (ortho-toluidine) and free fatty acid (5) were determined immediately before and after exercise.

Mean, standard deviation and standard error of the mean were calculated for each experiment, the significance of difference was calculated by Student's one tailed *t* test.

Results

Figure 1 demonstrates the changes in lactic acid level in children of normal weight. Caffeine itself did not induce any change, exercise alone or combined with caffeine led to a significant increase of blood lactate. The height of increase in blood lactate was not influenced by administration of caffeine.

Figure 2 shows the comparable results obtained in obese children. Blood lactate increased in all three sets of experiments, the increase was, however, significantly smaller if physical exercise was combined with administration of caffeine than in children exposed to physical exercise plus placebo.

Table I summarizes the changes in blood glucose and free fatty acids.

No appreciable change was observed in the blood glucose level.

Table I

Free fatty acid and blood glucose level before and after caffeine ingestion, exercise, and their combination ($\bar{x} \pm SE$)

Obese		Free fatty acid mmol/l	Blood glucose mmol/l
Caffeine	before	1.303±0.062	5.25±0.14
	after	1.318±0.061	5.17±0.14
Exercise	before	1.187±0.065**	5.55±0.13
	after	1.051±0.058	5.69±0.25
Caffeine* Exercise	before	1.143±0.065	5.17±0.11
	after	1.103±0.065	4.95±0.10
Controls			
Caffeine	before	0.482±0.065	4.82±0.43
	after	0.580±0.060	4.73±0.12
Exercise	before	0.600±0.100	5.00±0.14
	after	0.455±0.110*	4.95±0.09
Caffeine* Exercise	before	0.766±0.120	4.78±0.20
	after	0.500±0.100*	5.17±0.35

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

A significant change in free fatty acid concentration was seen in both groups when submitted to physical exercise combined with administration of placebo, while the combination of caffeine and physical exercise induced a similar change only in the control group.

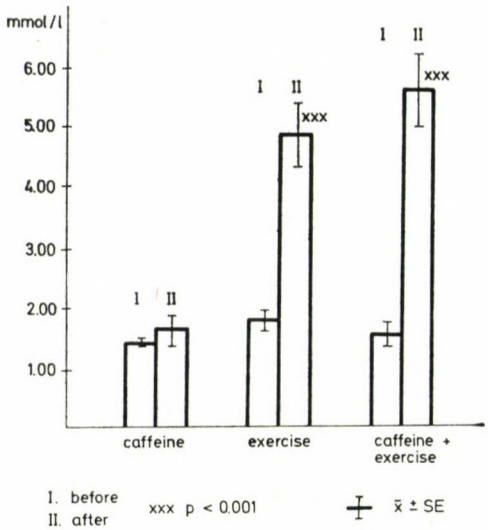

FIG. 1 Lactic acid concentration in the control children before and after caffeine ingestion, exercise and their combination

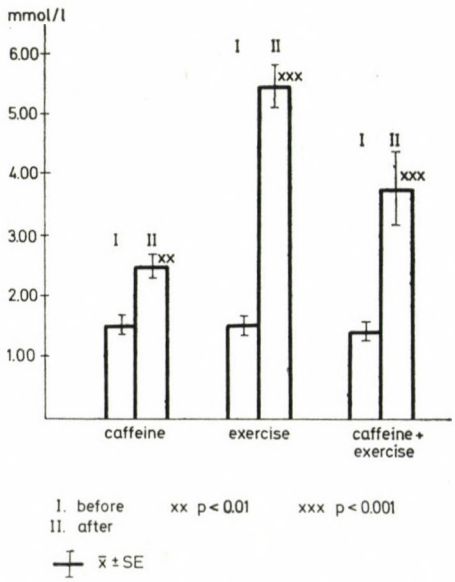

FIG. 2 Lactic acid concentration in the obese children before and after caffeine ingestion, exercise and their combination

Discussion

Work exerted under comparable circumstances (similar stair height, duration, number of steps) resulted in a heart rate of 180—190/min in obese children while of 140—150/min in normal weight children by the end of the fourth minute. Due to their heavier body, the obese children performed more work.

Costill et al [3] have shown that caffeine plus physical exercise increased fat oxidation. Acheson et al [1] found fat oxidation to be less in caffeine treated obese persons than in similarly treated persons of normal body weight. In the experiments of Daubresse et al [4] caffeine did not induce any change in the blood free fatty acid level while Ratzmann et al [9] found caffeine to increase the free fatty acid level in both normal and obese individuals.

The present study showed that caffeine induced a slight increase in free fatty acid levels in normal weight controls but did not significantly alter them in either group. Exercise combined with placebo caused a significant fall in blood free fatty acid in both groups, whereas a similar decrease due to exercise plus caffeine was seen only in the children with normal body weight. Gold et al [8] stated that free fatty acid release stops when lipolysis is inhibited and this depends on the level of blood lactate. Increased free fatty acid release leads to a decrease in the lactate level.

In our experiments caffeine increased blood lactate in obese children, and physical exercise had a similar effect in both experimental groups. The degree of lactate increases in obese children exposed to exercise plus caffeine was significantly smaller ($p < 0.01$) than in similarly treated normal children or obese ones exposed to exercise plus placebo. It may thus be anticipated that although work alone leads to a smaller fat mobilization in obese children than in normal ones, the energy expenditure of the increased work exerted when exercise and caffeine are combined, is covered mainly by fat oxidation. This is in agreement with the findings of Ratzmann et al [9] who observed increased free fatty acid mobilization after administration of caffeine; the increase in free fatty acid levels inhibits lactic acid production, as demonstrated by Gold et al [8]. The combination of physical exercise and caffeine thus produced a pattern similar to that observed by Eichler [6] in children under training.

Flock and Bollmann [7] suggest that the moderate increase in blood lactate during muscle work is due to the improved oxygen and blood supply; this may also explain our results showing a more sensitive reaction to physical exercise plus caffeine in respect of blood and oxygen supply in obese children than in normal controls. This may also be attributed to an altered reaction of the obese organism to caffeine, especially during work. Caffeine may be regarded as a drug in these circumstances.

The idea emerges that under sanatorium conditions a short course of

caffeine treatment may be indicated if at the beginning of a slimming cure, initiation of physical exercise meets with difficulties.

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Use of thrombocytopenia for the early identification of sepsis in critically ill newborns

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The detection of thrombocytopenia seems to have gained widespread use as an early sign of neonatal septicaemia. A prospective study of 78 full term and preterm babies was done to clarify the significance of serial platelet counts for early sepsis identification in critically ill and ventilated newborns. The results show that thrombocytopenia in this special patient group is too unspecific to be associated with septicaemia. Neither sporadic nor serial platelet counts are suitable as a reliable diagnostic test for the early detection of neonatal septicaemia.

The diagnosis of neonatal septicaemia is based on finding a bacteraemia with pathogenic organisms correlated with corresponding clinical symptoms. Because it usually takes at least one to two days for the microbiological detection of bacteria and because of the non-specific clinical manifestations in full term and premature babies [5, 13], simple and rapid auxiliary diagnostic tests have been devised for the early identification of neonatal sepsis. The platelet count seems to have gained widespread use in routine clinical work as a screening test for the early detection of septicaemia [1, 2, 3, 9, 10, 12, 14, 16]. Although the usefulness of this sign is somewhat questionable in non-critically ill newborns [7, 11], more reservations seem to arise in relation to critically ill and ventilated babies [13]. The following prospective study was done in order to clarify the significance of serial platelet counts for early sepsis identification in critically ill newborns.

SUBJECTS AND METHODS

Seventyeight full term and preterm babies with birth weights from 900 g to 4410 g who were admitted to the Intensive Care Unit of the University Children's Hospital Düsseldorf from January to October, 1978, were studied. On a flow sheet clinical data (symptomatology, endotracheal intubation, intravascular catheters, antibiotic therapy), laboratory and microbiological culture results were documented over the two to fifty-two days period of treatment in the Unit. As a routine test, on all babies serial platelet counts were performed daily in a counting chamber according to the method of Feissly—Lüdin [4], and from a peripheral vein 0.5 ml blood was drawn every second day and sent for bacteriological investigation. In case of suspected septicaemia additional cerebrospinal fluid and urine specimens were examined for pathogenic organisms. The diagnosis of sepsis was established according to the following findings [14]:

1. Bacteraemia with an organism considered pathogenic.

2. The same germ was detected in all blood samples and (if examined) in the cerebrospinal fluid and urine cultures.

3. Appropriate clinical manifestations

TABLE I

Clinical data of the 78 children in the study

Patient No.	Normal platelet counts	Chronic thrombocytopenia	Sudden drop in platelet count	Endotracheal tube	Bacteraemia	Adequate clinical symptoms	Other diagnoses	Antibiotic therapy
1	+			+	E. coli	+	HMS	+
2	+		(+)	+	Streptococcus	+	HMS	+
3	+		(+)	+	Listeria	+	AS	+
4		+		+	Serratia	+	HMS	+
5	+		(+)	+	Listeria	+	AS	+
6	+		(+)	+	Serratia	+	AS	+
7	+			+	Staph. aureus	+	AS	+
8	+			+	Staph. aureus	+	HMS	+
9		+		+	E. coli	+	AS	+
10	+			+	E. coli	—	A. S.	—
11			+	+	Ps. aeruginosa (terminal)	—	HMS, IVH	—
12	+			+	Ps. aeruginosa (terminal)	—	HMS, IVH	+
13	+			+	Staph. epiderm.	—	HMS	—
14		+		+	Staph. epiderm.	—	HMS	—
15			+	+	Staph. epiderm.	—	HMS	—
16		+		+	Staph. epiderm.	—	HMS	+
17	+			+	Staph. epiderm.	—	HMS	—
18	+			+	Staph. aureus	—	HMS	—
19		+		+	Staph. aureus	—	HMS	—
20		+		+	—	—	HMS	—
21		+		+	—	—	HMS	—
22	+			+	—	—	AS	+
23		+		+	—	—	HMS	+
24		+		+	—	+	AS	+
25	+			+	—	—	Pneumonia	+
26		+		+	—	—	AS	—
27		+		—	—	—	Ileus operation	+
28		+		+	—	—	Heart disease	+
29		+		+	—	—	Pneumonia	+
30			+	+	—	—	HMS	—
31		+		+	—	—	AS	—
32		+		+	—		HMS	—
33		+		+	—	—	Paroxysmal tachycardia	—
34		+		+	—	—	Meconium aspiration	+
35	+			+	—	—	AS	+
36	+			+	—	—	HMS	—
37	+			+	—	—	AS	+
38			+	+	—	—	HMS	—
39	+			+	—	—	HMS	—
40	+			+	—	—	Heart disease	—
41	+			+	—	—	HMS	—
42	+			+	—	—	HMS	—
43		+		+	—	—	Rh-Erythroblastosis	+
44			+	+	—	—	HMS	—
45			+	+	—	—	HMS	+
46	+			+	—	—	HMS	—
47			+	+	—	—	HMS, IVH	—

TABLE I (cont.)

Patient No.	Normal platelet counts	Chronic thrombocytopenia	Sudden drop in platelet count	Endotracheal tube	Bacteraemia	Adequate clinical symptoms	Other diagnoses	Antibiotic therapy
48		+		+	—	—	HMS	—
49	+			+	—	—	HMS	—
50		+		+	—	—	HMS	—
51	+			+	—	—	HMS, IVH	+
52			+	+	—	—	Diaphragmatic hernia	—
53			+	+	—	—	HMS	—
54			+	+	—	—	HMS	—
55	+			+	—	—	HMS	—
56		+		+	—	—	HMS	—
57	+			+	—	—	AS	—
58			+	+	—	—	HMS	+
59		+		+	—	—	PFC	—
60		+		+	—	—	Heart disease	—
61	+			+	—	—	Heart disease	—
62			+	+	—	—	AS	—
63	+			+	—	—	HMS	—
64			+	+	—	—	AS	—
65		+		+	—	—	HMS	—
66	+			+	—	—	HMS	+
67		+		+	—	—	HMS	—
68		+		+	—	—	HMS	—
69		+		+	—	—	HMS	—
70			+	+	—	—	AS, IVH	—
71	+			+	—	—	HMS	+
72		+		+	—	—	HMS	+
73		+		+	—	—	HMS	—
74	+			—	—	—	Oesophageal atresia	+
75		+		—	—	—	Small-for-dates	—
76		+		+	—	—	HMS	—
77		+		+	—	—	HMS	—
78	+			—	—	—	Ileum resection	+

Abbreviations:
HMS = Hyaline membrane syndrome; AS = Aspiration syndrome; IVH = Intraventricular haemorrhage; PFC = Persistent fetal circulation; Adequate clinical symptoms = clinical manifestations pointing to septicaemia

after exclusion of other, quickly determined causes (i.e. complications of respiratory therapy, anaemia, hypovolaemia, congestive heart failure, hypoglycaemia, acidosis, hyponatraemia).

RESULTS

The main indication for transfer of the 74 babies to the Intensive Care Unit was respiratory failure calling for endotracheal intubation and ventilation. Only 4 of the newborns were treated without any sign of ventilatory disturbance (postoperatively after an ileus operation,; oesophageal atresia; ileum resection; small for date baby of a mother with toxaemia). Sixty-seven babies had an intravascular catheter (umbilical artery, umbilical vein, cutdown) some time during

the observation period, 11 had scalp needles for intravenous fluid therapy. According to the above definition there were 9 full term and preterm babies considered to have sepsis (Nos 1—9). Another 10 (Nos 10—19) had positive blood cultures some time during the treatment, but these were regarded either as contamination or transient bacteraemia. The results of the daily platelet counts could be subdivided into three groups.

1. Normal platelet count (>150,000/mm^3).

2. Chronic thrombocytopenia (<150,000/mm^3) during a period of several days.

3. Sudden drop in platelet count (<100,000/mm^3) within 24 hours.

Table I shows some relevant data of the babies in the study.

The use of antibiotics did not alter the potential diagnostic value of serial platelet counts as can be seen in Table II of the temporal correlations of platelet counts with positive blood cultures, the first observation of clinical manifestations and the first day of antibiotic therapy in the septic babies.

Among the 69 non-septic patients there were only 2 (Nos 25 and 29) with assumed focal bacterial infection (pneumonias). The liberal use of antibiotics in numerous patients without signs of any bacterial infection can only partly be explained by the prophylaxis; it was rather the result of a diagnostic uncertainty in regard to the unspecific clinical manifestations of infections in critically ill newborns.

Besides the chronic thrombocytopenia in 2 cases and the sudden drop in platelet count in 4 cases, another 30 patients with chronic thrombocytopenia and further 14 patients with a sudden drop in platelet count could be observed without any sign of septicaemia. Except patients Nos 45 and 58, all the other 12 babies with a sudden drop were not treated with antibiotics, so that an alteration of the platelet count by antimicrobial therapy could be excluded. In addition, 20 of the patients with chronic thrombocytopenia did not get antibiotics and were not assumed to have any focal bacterial infection.

No further diagnostic tests were performed for the evaluation of thrombocytopenia not associated with septicaemia.

Discussion

Looking at the efforts to find a simple and rapid auxiliary laboratory test for the early detection of septicaemia, one cannot miss to encounter thrombocytopenia [1, 2, 3, 9, 10, 12, 14, 16] especially in relation to Gram negative septicaemias [1, 2, 12]. In a study of postoperative paediatric surgical patients Rowe et al. [12] emphasized the significance of a drop in the platelet count as an early sign of Gram negative sepsis. In their series, 100% of the children with Gram negative septicaemia had platelet counts <150,000/mm^3, 71% had values <100,000/mm^3. A similar optimistic opinion was expressed by Modanlou and Ortiz [10].

TABLE II

Temporal correlation of thrombocytopenia with positive blood culture, the appearance of clinical manifestations and the start of antibiotic therapy

Patient No. (birth date)	Date	Blood culture	Platelet count mm³	First observation of clinical manifestations	Antibiotic therapy
2	7—5	Streptococcus	187,000		—
(7—5—78)	7—6		174,000	++	+
	7—7	—	135,000		+
	7—8		210,000		+
	7—9	—	34,000		+
	7—10		85,000		+
	7—11	—	177,000		+
3	1—13	Listeria	148,000	++	—
(1—13—78)	1—14		151,000		—
	1—15	—	18,000		+
4	1—19	—	74,000		—
(1—19—78)	1—20		76,000		—
	1—21	—	80,000		+
	1—22		83,000		+
	1—23	Serratia	100,000	++	+
	1—24		45,000		+
5	3—17	Listeria	200,000	++	+
(3—17—78)	3—18		115,000		+
	3—19	—	100,000		+
	3—20	—	118,000		+
	3—21	—	98,000		+
	3—22		141,000		+
6	3—26	—	180,000		—
(3—26—78)	3—27		220,000		—
	3—28	—	200,000		—
	3—29		230,000		—
	3—30	Serratia	210,000		—
	3—31	Serratia	120,000		+
	4—1	—	114,000	++	+
	4—2	—	93,000		+
9	9—19	—	82,000		—
(9—19—78)	9—20		54,000		—
	9—21	E. coli	15,000	++	+
	9—22		18,000		+
	9—22		18,000		+

For the often difficult diagnosis of sepsis in critically ill and ventilated newborns [13], such a simple and rapid test would be of great value. There are, however, some theoretical reservations concerning its usefulness. Although there is a pathophysiological association of septicaemia and thrombocytopenia without disseminated intravascular coagulation (DIC), the authors emphasize the combination of thrombocytopenia — DIC and septicaemia [3, 9, 12, 16]. In any case, thrombocytopenia is a criterion of the diagnosis of DIC, so that a drop in platelet count may be considered a sign of DIC, irrespective of the presence of septicaemia. Considering that asphyxia, hypoxia, acidosis and respiratory distress syndrome may also be associated with DIC in the newborn under intensive care [6], the usefulness of the test for the early identification of septicaemia becomes questionable. Another argument for the unreliability of thrombocytopenia in these special patients is the observation of a drop in platelet count in premature babies treated with phototherapy for hyperbilirubinaemia [8]. Finally, thrombocytopenia may also occur in association with intravascular catheterization. The tip of the catheter is always covered with a thrombus. During the procedure of drawing blood from an artery, platelets can be trapped at the tip and dissimulate the real platelet count [15].

These theoretical reservations are supported by practical experience. Serial platelet counts were done in 50 (64%) of the 78 studied patients. An association with septicæmia could be documented in six cases only.

As to the temporal correlation of thrombocytopenia with positive blood cultures and the appearance of clinical manifestations (Table II), one sometimes finds a chronic thrombocytopenia from the beginning (Patients 4 and 9) while in other instances the drop in platelet count occurs late, two to four days after the positive blood culture and the clinical manifestation (Patients 2, 3, 5, 6), an observation already described by other authors [7, 11]. It is therefore concluded that 1) neither sporadic nor serial platelet counts are suitable for use as a diagnostic test for the early detection of neonatal septicaemia; 2) a thrombocytopenia occurring in critically ill and ventilated newborns is too unspecific to be associated with septicaemia; 3) normal platelet counts do not exclude septicaemia.

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Isolation of aerobic bacteria from the placenta

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Cultures for aerobic bacteria were prepared from 353 placentas. Specimens were taken from the chorion after removing the amnion. The specimens were immersed into Stuart transport medium.

Microscopic examination of the placenta and cultures from the throat and ear of newborns were also done. The rate of positive bacterial cultures was 16%. Chorioamnionitis was found in 15%. The proportion of chorioamnionitis caused by aerobic bacteria was 44%. The rate of positive bacterial cultures from the placenta in the group of newborns with clinical signs of intrauterine infection was 63%. Bacteria can be present on the chorionic plate without any histological evidence of chorioamnionitis. Bacteriological examination of the placenta is therefore mandatory when amniotic fluid infection is suspected.

Acute inflammation of the placenta often has no adverse effects on the mother or fetus but in some cases it is associated with early onset of labour, congenital pneumonia and clinical evidence of maternal infection [4, 8, 9, 10]. A few of the infections may be haematogenous but most appear to be initiated by bacteria which have ascended through the cervix [2]. Amniotic fluid infection increases the number of apnoeic episodes of the newborn. In a prospective study of 50,826 neonates, amniotic fluid infection was the underlying disorder in 35% of preterm and 25% of term neonates who had multiple episodes of apnoea [12]. Neonatal bacterial infections may potentiate the neurotoxicity of hyperbilirubinaemia [3, 7, 14]. Amniotic fluid infections increased the frequency of mental motor, visual and hearing impairment [11].

Since demonstration of the organisms is important in infections, we have worked out a method for culturing aerobic bacteria from the placenta.

In this paper we present our experience with isolation of aerobic bacteria from the placenta. We examined the specificity of the method, and the relation between the positive bacterial finding and the clinical evidence of neonatal infection.

Materials and Methods

Before taking material from the placenta we removed the amnion from the chorion. Specimens were taken from the surface of the chorion. For this purpose we used a cotton swab previously boiled in phosphate buffer pH 7.4 and treated with 2% active charcoal in water. After obtaining the test material, the swabs were immersed into Stuart transport medium modified by Abdou [1]. In this medium the specimens could be preserved without overgrowth of

non-pathogenic organisms. The specimens were then inoculated into broth and blood agar. In the case of bacterial growth further identifications were made.

Specimens were taken within one hour after birth. The placentas were kept at room temperature, under sterile conditions. For the examination of specificity [6] we have chosen cases where on the basis of history, the clinical appearance of the newborn and histological examination of the placenta no bacterial growth could be expected. In this group we included newborns with the criteria as follows. (1) The membranes had not ruptured prematurely; (2) the cervix was not dilated before the beginning of labour; (3) delivery occurred without any instrumental aid; (4) the mother was not toxaemic and the amniotic fluid was not meconium-stained; (5) the mother had no urogenital infection; (6) there was no inflammation in the subchorionic plate of the placenta; (7) the mother and her baby had no fever before and after the delivery. We believe that under such conditions the chorion must be free of bacteria.

An infiltration by neutrophils of the subchorionic plate of the placenta was considered an evidence of chorioamnionitis [11].

Immediately after birth, throat and ear swabs were also taken.

The rate of bacterial growth from the placenta was examined whenever some clinical evidence of neonatal infection could be discovered. In this group we included newborns with the symptoms of intrauterine acquired infection: apnoea, respiratory distress, tachypnoea, fever, distension, hyperbilirubinaemia [5]. The placentas were examined unselected. The birth weight of the newborns ranged from 980 g to 4200 g.

Results

Specimens for bacterial culturing were taken from 353 placentas. Bacterial growth could be observed in 57 cases (16%). The organisms isolated were Escherichia coli in 16, cases, Pseudomonas aeruginosa in 14 Streptococcus beta haemolyticus in 14, Staphylococcus aureus in 9, and Klebsiella pneumoniae in 4 cases.

Chorioamnionitis was found in 52 cases (15%).

In the group of healthy newborns, specimens were taken from 54 placentas. Bacteria could be isolated from 2 placentas (4%). In 96% of the cases the chorionic plate was free of aerobic bacteria and on the basis of the history, the appearance of the newborn and the histological findings the presence of organisms was not expectable.

In 52 cases with chorioamnionitis, bacterial growth was seen in 23 cases (44%).

In the group of placentas with a positive culture, chorioamnionitis has been found in 23 cases (40%) and 22 newborns showed clinical evidence of intrauterine infection (38%).

In 35 newborns with clinical evidence of intrauterine infection, bacteria were isolated from the placenta in 22 cases (63%).

The same organism could be isolated from the ear and throat of 10 newborns.

Discussion

Pathogenic bacteria may be cultured from many placentas [9, 10]. In our study the rate of positive cultures of aerobic bacteria amounted

to 16%. Naeye et al could identify congenital pneumonia in 76% of their cases with placentitis; pathogenic aerobic bacteria were present in the lungs of 63% of those with, and 14% of those without, congenital pneumonia [8].

Specificity is the ability of a test to identify correctly all of the non-diseased patients as non-diseased [6]. Therefore we examined the rate of positive bacterial cultures in healthy placentas. The placenta passes the birth canal and during this process the surface can be contaminated with bacteria, so it senseless to take specimens from the maternal or fetal surfaces. Therefore we removed the amnion and the specimens were taken from the chorion. We did not find bacterial growth from the placentas of healthy newborns in 96% of the cases.

The sensitivity of the method could not be determined. Sensitivity is the accuracy of a test in correctly identifying all of the diseased patients [6]. The non-specificity and wide range of signs and symptoms that may be observed in neonates with intrauterine infection make the correct diagnosis difficult [5]. Cultures of gastric aspirate, of throat and ear swabs or of cord blood may be misleading [5].

In our material the proportion of chorioamnionitis caused by aerobic bacteria was 44%. This can be explained by the fact that chorioamnionitis can be caused by organisms other than aerobic bacteria, i.e by anaerobic bacteria, viruses, mycoplasma, etc. [9, 13].

Positive bacterial cultures were found in 57 cases. In this group chorioamnionitis was found in 40%. According to this finding, bacteria can be present on the chorionic plate without causing leucocytic infiltration. Hence the absence of chorioamnionitis does not rule out the presence of bacteria on the chorionic plate. The organisms can spread to the fetus, causing infection.

Conclusions

Bacteriological examination of the placenta is mandatory if amniotic fluid infection is suspected. Bacteria can be present on the chorionic plate without any evidence of chorioamnionitis. Owing to the small percentage of false positive cases, the method seems to be suitable for isolation of aerobic bacterial from the placenta.

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Long-term prognosis of asphyctic neonates from an intensive care unit: Intrauterine retarded infants at high risk of cerebral palsy

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The surviving asphyctic infants born during the three-year period 1977 to 1979 and admitted to the regional neonatal intensive care unit were prospectively followed up to 2 and 4 years of age. According to outcome the children were divided into three subgroups:

1 who did not show any consequence of asphyxia;
2 who showed syndromes classified as cerebral palsy;
3 whose psychomotor functions were found to be retarded.

The cerebral palsy group mainly consisted of children who were born with a weight deficit after 37 weeks gestation. Only 4 out of the 15 children were premature and among them only 1 was of very low birth weight. Thus, dysmaturity was the main somatic characteristic of the severely handicapped children. In addition to intrauterine growth retardation, the high rate of congenital anomalies was a further feature of the cerebral palsy group.

Gestational complications (toxaemia, haemorrhage, threatening abortion or premature delivery), as well as the distortions of the most important biochemical variables (pH, calcium, bilirubins and glucose level) either in themselves or in combination had a similar incidence in the three subgroups. Among postnatal conditions, only the incidence of convulsions seemed to be important prognostically.

On the basis of the great difference in outcome it is concluded that there is no simple relationship between asphyxia and eventual handicap. There are certainly some other noxious factors (e.g., dysmaturity, congenital anomalies, etc.) associated with, or related to asphyxia which can be of great aetiological significance.

The present study deals with the follow-up of asphyxiated newborn infants admitted to the regional neonatal intensive care unit of the Department of Paediatrics in Pécs. The main purpose was to analyse the different prenatal, natal and postnatal characteristics of newborns and their relationship to the neurological and developmental outcome. In fact, a comparison was made between three subgroups of children, namely normal, developmentally retarded and mentally and physically severely damaged children. The study is intended to serve as a basis for evaluation of further follow-up studies of critically ill newborns admitted to our neonatal intensive care unit in the future. Such a feedback is expected to be helpful in judging the adequacy of intensive care and treatment on the one hand and to identify the various preventable risk factors of asphyxia on the other hand.

Material and Methods

The surviving asphyctic newborns admitted to the regional neonatal intensive care unit were followed prospectively. Selection was based on symptomatic asphyxia with its major or minor manifestations. Apnoea at birth needing resuscitation, ventilation either by mask or intubation, meconium stained amniotic fluid, metabolic acidosis, the immediate response to asphyxia as measured by Apgar score < 6, oxygen and bicarbonate therapy in the delivery room, poor condition with cerebral symptoms, cardiomegaly, clinical and radiological signs of massive aspiration syndrome, cardiac decompensation, cyanosis, repeated apnoeic attacks, ventilation support on admission were regarded as major asphyctic and post-asphyctic signs. Apathy syndrome, absent sucking and swallowing response, gestational and perinatal history such as haemorrhage, abruption of placenta, toxaemia and umbilical cord anomalies were considered to be minor criteria. In all cases a combination of major and minor signs was observed showing that the patients had suffered significant asphyxia and acute sequelae constituting the so-called postasphyctic syndrome. The selected neonates did not only need immediate resuscitation as a preventive measure against acute emergencies such as anoxia and acidosis, but also transfer to the intensive care unit. The children born during the three-year period from 1977 to 1979 were followed-up to the end of 1980, that is to 1 to 4 years of age. During the first year the infants suspected of some damage were examined monthly and those who appeared normal, at intervals of three months. After the first year the handicapped children were seen every half year until the age of six, and those without clinically manifest sequelae at three, five and six years of age. The follow-up examinations included anthropometric measurements, standardized neurological and developmental evaluation based on status of motor functions, behaviour, ophthalmological and audiological screening. At the age of 3 and 5 years an intellectual test was performed. Those whose psychomotor function based on the developmental quotient was found to be within the range of 70 to 90 were regarded as developmentally retarded.

The children were divided into three subgroups. 1. Those who did not show any consequence of asphyxia throughout the follow-up period and appeared normal as far as physical and mental development were concerned. 2. Those with syndromes classified as cerebral palsy due to severe cerebral damage. 3 Children whose psychomotor function based on the developmental quotient was found to be in the range of 70 to 90 were regarded as developmentally retarded. These three subgroups of children were compared in order to explore the somatic, gestational, perinatal and postnatal characteristics at birth, and their relationship to the late sequelae of asphyxia.

The consequences of a major cerebral damage were classified as cerebral palsy which is not a single handicap but involves different syndromes. Among the 15 cases followed-up in the present survey, 7 spastic tetraplegia, 2 ataxic syndromes, 4 spastic diplegia, 1 hemiparesis and 1 monoparesis were seen. In addition, 13 children were slightly and 2 severely retarded mentally, 4 showed microcephaly, 3 had epilepsy, 1 sensorineural hearing loss, and 10 various minor ophthalmological defects.

The terms minor handicap or physical and mental retardation are very broad and encompass a great variety of conditions which are not all pathological in the classical sense. They can be defined in many ways, and therefore variations in their incidence may be expected. In the present study the so-called retarded group represents such a heterogeneous collection of minor long term consequences and mainly developmental retardation.

Within these three groups, the children were also classified according to birth weight gestational age and percentile position on

our local weight for gestational age chart. Gestational age less than < 37 weeks and a percentile position ≤ 10 were regarded as the criteria of prematurity and intrauterine growth retardation, respectively.

Results

Birth weight, gestational age and intrauterine growth rate of the total material

Table I shows the birth weight, gestational age and percentile distribution of the total population. In 13 infants weighing less than 2500 g, and 6 infants above 2500 g, the gestational age was unknown. According to the percentile position of weight for age, 77.4% of the newborns were below 50, and 27.4% below the 10 percentile. Thus the population showed a shift towards the lower percentiles.

Weight, gestational age and weight for age in the three subgroups

From Table II it is evident that 67% of the children with cerebral palsy were born with a birth weight less than 2500 g. The majority of these low birth weight children (7 out of 9) weighed between 2001—2500 g. This distribution already suggests that the cerebral palsy group consisted mainly of children who were born with a weight deficit after 37 weeks gestation. Gestational age and weight for age classification undoubtedly confirms this suggestion. Only 4 out of the 15 children were prematures according to gestational age and among them only one was of very low birth weight. No doubt, dysmaturity was the main characteristic of the severely handicapped children.

In contrast, the majority of mentally and developmentally retarded children were true preterm newborns (Table III): of the 37 low birth weight infants (<2500 g) 33 were born earlier than at 37 weeks (in 4 cases the gestational age was unknown) and 10 were of very low birth weight, 9 out of 45 classifiable children were below the 10th percentile at birth.

The majority of the children, 155 out of 218, had no handicap and represented the normal group. According to weight and gestational age distribution (Table IV), roughly 40% were of term weight (>2500 g) and gestational age (>37 weeks). The shift towards the lower intrauterine growth rates was also striking in this group of children, which, as already pointed out, turned out to be a general somatic characteristic of the population studied.

Incidence of congenital abnormalities in the three subgroups

It is known [11] that dysmaturity is often associated with minor or major congenital abnormalities which might be directly or indirectly related to permanent cerebral damage. Hydrocephaly, microcephaly, meningocele, Klippel—Feil-syndrome, cleft palate, oesophageal atresia and three minor abnormalities occurring in combination were classified as minor congenital anomalies. The incidence of

minor and major abnormalities found in the present material is shown in Table V. It is interesting that the occurrence rate (major and minor) in the total population as well as in the subgroups was found to be high. Particularly high (80%) was it in the group with severe cerebral sequelae.

TABLE I

Birth weight, gestational age and percentile distribution of the total children population followed

Birth weight, g	1500	1501—2000	2001—2500	2501—3000	3001—3500	3501—4000	4000
No. of children	32	62	45	36	27	14	2
Gestational age, weeks	30	31—33	34—36	37—41	42—43	Unknown	
No. of children	27	36	58	66	12	19	
Weight for gestational	10	10—50	50—90		90	Unknown	
No. of children	57	104	42		5	10	

TABLE II

Birth weight, gestational age and percentile distribution of the cerebral palsy groups children

Birth weight, g	1500	1501—2000	2001—2500	2501—3000	3001—3500	3501—4000	4000
No. of children	1	2	7	2	3	—	—
Gestational age, weeks	30	31—33	34—36	37—41	42—43	Unknown	
No. of children	1	1	2	8	1	2	
Weight for gestational age, percentiles	10	10—50	50—90	90			
No. of children	*9	4	2	—			

* Two neonates whose gestational age was unknown showed the typical somatic signs of dysmaturity and were therefore included in the group weighing $\leq$ 10 percentile

TABLE III

Birth weight, gestational age and percentile distribution of the mentally and developmentally retarded groups of children

Birth weight, g	1500	1501—2000	2001—2500	2501—3000	3001—3500	3501—4000
No. of children	9	19	8	6	3	3
Gestational age, weeks	30	31—33	34—36	37—41	42—43	Unknown
No. of children	13	10	10	8	1	6
Weight for gestational age, percentiles	10	10—50	50—90		90	Unknown
No. of children	9	26	8	2	3	

TABLE IV

Birth weight, gestational age and percentile distribution of children with normal outcome

Birth weight, g	1500	1501—2000	2001—2500	2501—3000	3001—3500	3501—4000	4000
No. of children	22	41	30	28	21	11	2
Gestational age, weeks	30	31—33	34—36	37—41	42—43	Unknown	
No. of children	14	24	46	50	10	11	
Weight for age, percentiles	10	10—50	50—90		90	Unknown	
No. of children	39	74	32		3	7	

TABLE V

Frequency of congenital anomalies in the three subgroups of children

	Major congenital anomalies		Minor congenital anomalies		Total anomalies	
	Number	Percentage	Number	Percentage	Number	Percentage
Normal children	8	3.8	49	22.4	57	26.2
Children with cerebral palsy	7	46.6*	5	33.3	12	80
Retarded children	5	10.4	16	33.3	21	43.7

* Significantly different from that of normal children ($p < 0.001$) and from that of the retarded children ($p < 0.02$)

Incidence of clinical signs of asphyxia

The frequency of meconium stained amniotic fluid, Apgar score less than 6 and the therapeutic measures pointing towards the poor postasphyctic condition necessitating admission immediately after birth are shown in Table VI. It can be seen that meconium stained amniotic fluid was more frequently encountered in the severely handicapped children. Still, the incidence of an Apgar score less than 6, and the number of newborns needing resuscitation or bicarbonate therapy did not differ between the three subgroups.

Prenatal and postnatal events and complications

Although the population followed up was selected by different criteria of asphyxia as the principal cause of serious mental and physical impairment, we also looked for other events and complications operating over a period of time during gestation,

TABLE IV

Frequency of clinical indications of perinatal asphyxia

	Meconium stained amniotic fluid		Apgar score < 6		Resuscitation		Bicarbonate therapy	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Children with normal outcome	23	14.8	93	58.7	65	41.9	148	99.3
Children with cerebral palsy	5	33	9	60	7	46.6	13	86.6
Retarded children	6	12.5	36	75	23	47.9	47	98
Total population	34	15.1	138	63.3	95	43.5	208	95.3

TABLE VII

Frequency of toxaemia and threatening abortion or premature delivery

	Toxaemia		Threatening abortion or premature delivery	
	Number	Percentage	Number	Percentage
Children with normal outcome	64	41.3	80	51.6
Children with cerebral palsy	4	26.6	7	46.6
Retarded children	23	47.9	30	62.5
Total population	91	41.7	117	53.6

TABLE VIII

Incidence o the relevant postnatal manifestations and therapeutic interventions

	IRDS		Recurrent apnoea		Convulsions	
	Number	Percentage	Number	Percentage	Number	Percentage
Children with normal outcome	10	6.9	44	28	15	9.6
Children with cerebral palsy	4	7.3	11	23	4	26.6
Retarded children	—	—	5	33	4	8.3
Total population	14	6.4	60	27.5	23	10.5

	Other cerebral symptoms		Ventilation		O_2-therapy	
	Number	Percentage	Number	Percentage	Number	Percentage
Children with normal outcome	90	58	24	15.5	144	99.3
Children with cerebral palsy	30	62.5	7	14.4	47	98
Retarded children	9	60	1	6.6	12	80
Total population	129	59	32	14.6	203	93.1

around or after birth. These pathological conditions might represent antecedents or consequences of asphyxia and more or less contribute to the handicap.

In Table VII the frequency of toxaemia and threatening abortion or premature delivery can be seen. Both complications were frequent in every subgroup with no significant difference. Thus, a large proportion of the pregnancies were of high risk.

The incidence of the most relevant postnatal events and manifestations as well as therapeutic interventions is shown in Table VIII. The occurrence rate of IRDS was lower than 10% in the normal and retarded group, and it was not observed at all in the CP group. The proportion of infants affected by recurrent apnoea exceeded, however, 20% in every group. Cerebral symptoms such as convulsions, increased tremor, abnormal reflex responses and motor behaviour were frequently seen quite independently of the outcome of the asphyctic insult. Prognostically it appears important that the incidence of convulsions in the CP group exceeded that observed in the retarded and normal children.

Neonatal biochemical distortions potentially relevant to the outcome of asphyxia

Neonatal biochemical factors which might add to the brain damage incurred during perinatal asphyxia have also been explored in the population studied. In Table IX, the mean blood glucose, calcium and pH values

TABLE IX

Mean (± SD) postnatal blood glucose, pH, serum calcium and bilirubin levels of the three subgroups of children

	Blood glucose mg/dl	Serum calcium mg/dl	pH	Maximum serum bilirubin, mg/dl
Children with normal outcome	50.4±19.5 n = 119	8.2±1.2 n = 102	7.3±0.10 n = 138	12.3±6.8 n = 133
Children with cerebral palsy	46±19 n = 10	7.6±1.4 n = 10	7.25±0.09 n = 15	12.6±6.6 n = 9
Retarded children	51±13 n = 35	7.8±1.3 n = 38	7.3±0.08 n = 41	13±5.9 n = 41

at admission, and the mean maximum postnatal serum bilirubin levels can be seen. Neither mean value distinguished between the children with different outcome. It is interesting that the frequency of hypoglycaemia was practically the same in the normal children and in those with cerebral palsy (27.7% vs. 30%). The incidence of calcium and pH values lower than 7 mg/dl and 7.20, respectively, was somewhat but not significantly higher in the CP group in comparison to the retarded and normal children. The mean maximum serum bilirubin level exceeding 15 mg/dl was not significantly different in the three groups.

DISCUSSION

The question that often arises in connexion with the outcome of an asphyctic insult is, what constitutes a clinically significant episode of asphyxia? The duration and degree of asphyxia are those criteria whose knowledge would be important to avoid the inconsistencies in the definition of asphyxia in follow-up studies. Since these criteria are either lacking or not precisely recorded we have used our own criteria given in the section of Materials and Methods. We believe that these minor and major asphyctic features constitute a reliable guide to the presence of asphyxia and its acute sequelae in the immediate postnatal period which, in fact, necessitated admission to the intensive care unit for further treatment and observation.

Somatic, gestational age and intrauterine growth characteristics

The distribution of intrauterine growth rates of the three groups of children showed a shift to the lower weight for gestational age zones. Growth retardation defined as birth weight $\leq 10\%$ is a striking feature of the children suffering from cerebral palsy. The majority of low birth weight CP children (weight <2500 g) were full term infants (gestational age ≥ 37 weeks). Only one severely handicapped child had a very low birth weight.

This suggests that it is the dismaturity associated with asphyxia which should be regarded as a major risk factor of cerebral palsy rather than prematurity with very low birth weight (<1500 g). Although several previous follow-up studies [2, 9, 10, 16, 22, 23, 24] undoubtedly show that growth retardation is an important determinant of severe handicap, the single cerebral palsy patient with very low birth weight found in this study does not diminish the aetiological role of immaturity associated with perinatal asphyxia in the pathogenesis of severe brain damage. The actual prevalence of major handicaps in very low birth weight infants depends on several factors such as the frequency of high risk pregnancy, length and severity of asphyxia, peri- and neonatal care, and what is very important, the ultimate prevalence at different ages will depend on the survival of asphyctic very low birth weight infants. It is reasonable to suggest that a high mortality in this group of neonates would lead to a low incidence of severe permanent handicap, since the majority of critically ill and seriously damaged asphyctic infants by peri- and intraventricular haemorrhage would not survive. Our regional neonatal intensive centre is a referral unit, and the mortality of seriously affected premature infants weighing less than 1500 is rather high. This can be one of the reasons that in the present survey only one spastic child of very low birth weight was encountered. The large proportion of premature infants weighing less than 1500 g who showed minor, mostly developmental consequences of asphyxia points, however, towards the possibility that the outcome of acutely asphyctic immature infants is seldom characterized by cerebral palsy syndromes; it mainly threatens more mature, but intrauterine retarded infants suffering from chronic or subacute asphyxia. This would support the conclusion of several surveys that the improved quality of perinatal management largely contributes to the improved outcome of very low birth weight infants. The different sensitivity to asphyxia of the immature and more differentiated brain could also explain the considerably higher frequency of cerebral palsy in small for gestational infants than in those of very low birth weight. Since severe handicap usually results from more than one gestational or perinatal factor, it is also possible that the combination of asphyxia and intrauterine malnutrition is associated with a more unfavourable outcome than the combination of immaturity and asphyxia.

The significance of intrauterine growth retardation

The high proportion of intrauterine growth retardation in the CP group of children observed in the present survey confirms previous observations. Hagberg et al [16] found that in a cohort of 357 CP children, weight for age beyond 35 weeks of gestation was much less than the normal average for respective age. The sizable

portion of twins in this group also points to the aetiological role of dysmaturity whose importance considerably increases during the second part of the third trimester. From this survey it also follows that the frequency of cerebral palsy increases with increasing weight deficit for gestational age. Sabel et al [22] and Scherzer and Miké [24] also stressed the importance of smallness for dates. According to Hagberg et al [16] it appears that fetal deprivation of supply in the aetiology of cerebral palsy has recently become a more important factor in Sweden.

In an epidemiological study of cerebral palsy in Western Australia Dale and Stanley [10] also found that intrauterine growth retarded infants were high risk of spastic cerebral palsy. In their survey only 2.9% of the control population was small for dates compared with 17.8% of the cases.

In contrast Ellenberg and Nelson [14] found that the birth weight for gestational age was within normal limits in full term infants suffering from CP. They regard low birth weight and short gestation as the most important risk factor of cerebral palsy. Drillien [11] examining infants weighing less than 2000 g observed no difference in the incidence of cerebral palsy among small and large for dates newborns in comparison to true premature infants. She stated that some cases of mental and neurological deficit were due to hypoglycaemia, but the most striking feature was the much higher incidence of congenital anomalies (both minor and major) in those who were markedly underweight for gestational age. Drillien is inclined to believe that in some children the handicap is due to some developmental malformation. The global incidence of major and minor abnormalities in the three subgroups of the present survey was also rather high, particularly in the children who had been small for dates. This might support the contention that congenital developmental anomalies could be a further factor contributing to the severity and possibly type of handicap.

The significance of intrauterine retardation as a risk factor should also be kept in mind when the outcome of low (2500 g) or very low ($<$1500 g) birth weight infants is to be explored. Commey and Fitzhardinge [9] examined the neurodevelopmental state of 149 newborns of very low birth weight at two years of age. Thirty per cent had a major handicap, and the second highest risk were infants with evidence at birth of severe intrauterine growth retardation. Twelve of 28 malnourished babies of less than 33 weeks gestation were handicapped compared with only three of 28 preterm babies of appropriate weight for gestation and with similar neonatal course. Thus the growth retarded preterm infants showed a significantly higher incidence (49%) than that seen in very low birth weight but appropriate for gestational age. Commey and Fitzhardinge's survey in fact confirmed the conclusion of Hagberg et al. [16] that cerebral palsy increases progres-

sively with increasing fetal malnutrition and decreasing gestational age. As a result, the very small for gestational age prematures have a fifteenfold greater risk of CP. In the present survey only 4 of the CP children were premature and among them one had a very low birth weight and two were small for gestation.

In contrast to these findings Ellenberg and Nelson [14] reported that the risk of CP was highest among very low birth weights appropriate for gestational age and it was also higher among infants weighing 1501 to 2500 g if they were true prematures than if they were small for dates. This discrepancy and uncertainties in the prevalence of a history of low birth weight in children with cerebral palsy either appropriate or retarded for age are probably due to the biases in the selection of patient population as it has been pointed out by Brown[4].

Despite some inconsistencies in the observations cited it appears justified to conclude that smallness for date both in preterm and term infants represents a high risk factor of cerebral palsy. On the basis of the present study we agree with Hagberg et al. [16] that "fetal growth retardation and other factors summarized in the term fetal deprivation supply, stand out as particularly important, probably both as predisposing and directly damaging factors". This brings into focus the prenatal factors operating during a certain period of time, causing cerebral palsy, or making the brain more vulnerable to asphyxia, or other perinatal complications.

The significance of asphyxia in premature and mature infants

During the last decade great efforts have been made to decrease the mortality of very low birth weight infants [6, 13, 17, 18, 20, 21, 27, 29]. As to the aetiology of late handicaps in this group of neonates, the potential factors are difficult to evaluate. The question which often arises is whether prematurity, immaturity or asphyxia are directly related to brain damage or developmental and intellectual retardation. Although asphyxia can be regarded as the single most important adverse condition causing long term sequelae the great difference in outcome (intact survival, impaired survival with different neurological, developmenta[1] and behavioural sequelae, death) makes it difficult to attribute the handicap only to asphyxia [1, 5, 25, 26]. It should always be kept in mind that the majority of asphyctic newborns show no sequelae and even a severe asphyctic insult must not always have grave consequences. Mulligan and Shennon [19] reported that gestational age was not related to the incidence of sequelae of asphyctic neonates and the severity of impairment was similar in mature and premature infants. Does this mean that the risk factors of cerebral damage following an asphyctic insult are independent of gestational age and birth weight? We have no evidence to settle this problem.

The present study has confirmed the previous observation [1, 5, 25, 26]

that the majority of neonates affected by an asphyctic insult show no long term abnormalities. Out of 218 asphyctic newborns 15 developed major handicaps and 48 were found to show some neurodevelopmental disability and intellectual performance. The remainder were found to be normal. This strikingly demonstrates that there is not a simple relationship between asphyxia and eventual handicap. Scott [25] found that even infants whose respiration could be restored within the first 20 minutes may be normal at follow up. Out of the acute consequences of acute asphyxia (metabolic acidosis, meconium stained amniotic fluid, the immediate neonatal status as judged by the Apgar score, and resuscitation either by mask or intubation), only intrauterine meconium passage occurred more frequently in the cerebral palsy group, but the difference was not significant.

There are certainly other noxious factors associated with or related to asphyxia which can be of a great aetiological significance. It is therefore difficult to ascribe a causal significance to prematurity or asphyxia as a single factor leading to various long term effects. The prevention of either one of the two important conditions by adequate care of the pregnant mother and modern management of the perinatal period can be expected to lead to a marked reduction in the incidence of major handicaps simply because it also prevents those secondary conditions which directly or indirectly lead to long term neurological abnormalities. A recent review [28] of the reported data appears to support the suggestion that increased survival of the very low birth weight in infant is not associated with an increased incidence of brain damage.

The prognostic value of pre- peri- and postnatal factors in asphyxiated neonates

In order to correlate pre- peri- and postnatal factors and complications with the outcome of high risk neonates one has to find reliable predictors of the late status which can be of help in establishing a better management and successful prevention.

In the present survey gestational complications such as toxaemia and haemorrhage indicating threatening abortion or premature delivery had the same incidence in the three subgroups of children. It would, of course, be important to know additional variables such as the duration and degree of asphyxia, severity of toxaemia and postnatal condition as well as the biochemical abnormalities potentially capable of causing cerebral damage. Among the postnatal conditions only the incidence of convulsions seemed to be important prognostically which according to Volpe has a high predictive value for an unfavourable outcome [8, 30].

Some authors [3, 7, 12] reported that transient neurological signs in high risk neonates can be predictive of less severe developmental abnormalities or schooling difficulties.

Therefore they regard transient poor conditions and transient neurological signs important which may pinpoint to later sequelae. In the present study cerebral symptoms other than convulsions were frequently seen in the three groups, showing that asphyxia was often associated with neonatal cerebral morbidity which, however, was not predictive of the outcome. The same applies to the distortions of the important biochemical variables (pH, calcium, bilirubin, glucose) which either by themselves or in combination do not seem to be helpful in identifying those asphyctic newborns who are at high risk of major sequelae. In connexion with hypoglycaemia, <30 mg /dl in full term, and <20 mg/dl in preterm neonates, it should be stressed that in neither case was it judged as symptomatic hypoglycaemia, which is known not to be followed by cerebral damage.

Although the neonatal conditions and isolated cerebral signs have no great predictive value, one should not disregard the potentiality of certain signs, for example of neonatal convulsions.

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Book reviews

Fetal growth retardation. Edited by F. ANDRÉ VAN ASSHE and WILLIAM B. ROBERTSON, Churchill Livingstone, Edinburgh 1981. 265 pages Price: £ 20

This book presenting the papers given by a number of well-known authors at a symposium on fetal growth and growth retardation is aimed not only at obstetricians with special interest, but also at neonatologists and paediatricians who are involved in newborn care. A multidisciplinary and multifactorial approach to the physiology and pathophysiology of intrauterine growth is a characteristic feature throughout. It is a great pity that the neonatal aspects and consequences are not dealt with, since they are so closely linked with prenatal pathophysiology. That is why I feel that this book gives a somewhat unbalanced account of intrauterine growth retardation.

The six sections of the book are logically arranged and cover most aspects of the subject indicated by the titles. The section on Fetal metabolism and energetics and Insulin and fetal growth are particularly valuable. Brief and concise accounts are given on recent findings and concepts of fetal substrate utilization, energy metabolism and the significance of the endocrine pancreas in intrauterine metabolism and growth.

The section entitled Fetal growth and growth retardation deals with aetiological, clinical, nutritional, social and uteroplacental issues of prenatal growth. These presentations will be of great interest for clinicians, especially perinatologists whose everyday task is to select, assess and manage neonates who are born too small. It is disappointing that S. Campbell's presentation Assessment of fetal growth has been omitted. How could it happen that such an important subject given by a well-known investigator is represented by title only? A book dealing with fetal growth retardation cannot be complete without reviewing the assessment of fetal growth and anthropometry by ultrasound. A further striking omission is the lack of discussion of the clinical management of pregnant mothers with growth retarded fetuses and the optimum time of delivery.

In spite of the omissions mentioned, the book can be recommended to obstetricians, perinatologists and paediatricians. The authors are all experts, their presentations are well readable, concise, well illustrated and up-to-date.

J. MESTYÁN

P. CATZEL, R. OLVER: *The Paediatric Prescriber*. Fifth edition. Blackwell Scientific Publications, Oxford 1981. 433 pages. Price: £6.20.

After a seven-year interval we have a new edition of the Prescriber in our hands. It is bulkier than its predecessors as its chapters have been revised and some seem to be completely rewritten. Apart from a new and very good chapter on paediatric anaesthesia, there are many up-to-date additions such as cimetidine, the recently

described hypothalamic hormones, the monocomponent insulins, steroids for inhalation, a revised chapter on the antibiotics in present-day use, etc., etc. The ethical principle emphasized in every previous edition persists: "the writing of a medical book intended as a guide to other doctors is indeed a responsible task, for incorrect advice may entail repercussions on a large and unsuspecting public".

As in the earlier editions, with every drug we find the non-proprietary and some of its proprietary names, a short note concerning its use and mechanism of action, the basic adult dose and finally the possible side-effects. As to conversion of the adult dose to infants and children, the surface area method is recommended vs. the mg/kg one.

The book ends with a practical appendix containing useful tables of measurements and units and their conversion factors. A list of the SI prefixes is added, unfortunately without conversion tables but with the only error (μl instead of ml) in the book.

The excellence of this little book, constructed on entirely Anglosaxon lines, is well-reflected by its international usefulness. It is characteristic that it has been translated into Greek and Spanish but could be used everywhere by paediatricians quite irrespective of their nationality.

ANNEMARIE GEGESI

H. OLBING und H. U. EICKENBERG: *Nephrologie und Urologie* Band 1 der Serie Fortbildung in der Kinderheilkunde. XII + 252 Seiten mit 98 Abbildungen und 25 Tabellen. Georg Thieme Verlag, Stuttgart 1981. Preis: DM 48,—

Als erster Band dieser neuen Serie haben wir eine ausgezeichnete Arbeit vor uns, die die nephrologischen Beziehungen der pädiatrischen Diagnostik und Therapie behandelt. Der Zielsetzung entsprechend ist der Text verhältnismäßig kurz, soz. prospektartig gefaßt, wobei in erster Linie Definitionen, therapeutische, anatomische und histologische Grundbegriffe äußerst korrekt erörtert werden. Der didaktische Effekt wird von einer großen Anzahl von meist farbigen Abbildungen und Tabellen gefördert. Das Buch befaßt sich nicht mit strittigen oder noch der Lösung harrenden Fragen der pädiatrischen Nephrologie und Urologie, sondern — dem Wesen der Aufgabe entsprechend — faßt die in der täglichen Praxis erworbenen Erfahrungen der Autoren zeitgemäß und korrekt zusammen.

Der vom didaktischen Standpunkt aus so vorteilhafte Stil mit den scholastischen Definitionen birgt jedoch mitunter die Gefahr des Doktrinärtums; so lautet z. B. die Definition des Morbus Berger: Rezidivierende renale Makrohämaturie mit diffusen mesangialen IgA Immunkomplexen,- wogegen das Krankheitsbild gelegentlich mit isolierten Proteinurien einhergeht und seine Existenz überhaupt zweifelhaft sein dürfte. Auch könnte man in Frage stellen, ob die Klassifikation der histologischen Bilder aufgrund der ausgezeichneten, doch individuellen Nomenklatur von Thoenes, oder aber eher auf der klassischen WHO Nomenklatur erfolge. Diese und ähnliche unwesentliche Einzelheiten benehmen des Wertes des Buches aber nichts, es ist ein hervorragender Wegweiser für den in der Praxis tätigen Kinderarzt.

M. MILTÉNYI

G. JUN: *Kinder, die anders sind. Ein Elternreport.* 181 Seiten. Verlag Volk und Gesundheit, Berlin 1981. Preis: 6,— M

Das Buch befaßt sich mit behinderten Kindern, also solchen, die deshalb anders sind, weil sie z. B. Down—Kranke oder infolge einer Prä- oder postnatalen Schädigung mental oder auch in ihrer Bewegung behindert sind. Es handelt sich nicht um ein Fachbuch; das Ziel der Autorin war, mit einer Vorstellung solcher behinderten

Kinder und ihrer Familie das Mitleid, Verständnis und die Toleranz der Umwelt zu gewinnen, die Vorurteile der Gesunden gegenüber Behinderten zu beseitigen.

Die Arbeit besteht aus 3 Teilen. In dem ersten findet man Interviews mit 8 Müttern und 3 Vätern von behinderten Kinder, die über ihre Erfahrungen mit der Umwelt berichten. Es kommen solche Probleme zum Ausdruck, wie die Eltern bei der Pflege und Erziehung ihrer Kinder die emotionale Belastung ertragen, wie sie ihr psychisches Gleichgewicht bewahren bzw. zurückgewinnen, wie ein harmonisches Familienleben gestaltet werden kann.

Der zweite Teil befaßt sich mit den einzelnen Krankheitsformen; hier werden die Grundkenntnisse hinsichtlich der Ursache, Häufigkeit, therapeutischen Aufgaben in einer auch für Laien verständlichen Form erörtert. In einer Tabelle wird sogar das entsprechende oder falsche Verhalten der Eltern dargestellt.

Der dritte Teil ist den sozialen Maßnahmen gewidmet, welche in der DDR in den vergangenen 30 Jahren zur Hilfe der Behinderten getroffen wurden.

Das Büchlein erfüllt eine doppelte Aufgabe: es vermittelt einerseits emotionale Impulse und anderseits entsprechende Sachinformation. Deshalb soll es all jenen empfohlen werden, die im Laufe ihrer Arbeit mit behinderten Kindern in Kontakt kommen: Ärzte, Schwestern, Psychologen, Heilpädagogen, soziale Fürsorgerinnen und schließlich auch Eltern derartiger Kinder.

M. Ladócsy

M. Burdelski und H. Huchzermeyer: *Gastrointestinale Endoskopie im Kindesalter.* XI + 138 Seiten mit 46 teilweise farbigen Abbildungen, 2 Farbtafeln und 37 Tabellen. Springer-Verlag, Berlin—Heidelberg—New York 1981. Preis: DM 98.—

Die im Kindesalter angewandte gastrointestinale Endoskopie hat sich in Europa nach entsprechender Miniaturisierung der Instrumente vor etwa 10 Jahren verbreitet, als diese Untersuchungen mit seitenoptischen Instrumenten mit kleinem Durchmesser auch im Neugeborenen- und Säuglingsalter ermöglicht wurden.

In der vorliegenden Monographie finden wir eine ausführliche Zusammenfassung der Probleme der Endoskopie, Information über die spezifischen Indikationen, die Technik bei den verschiedenen Altersgruppen und die Auswertung der Befunde. Mit der im Kindesalter vorgenommenen Endoskopie können chronische entzündliche Darmerkrankungen, Colitis ulcerosa, Ileocolitis granulomatosa, die Crohnsche Krankheit sehr früh erkannt werden.

Das in 7 Kapitel gegliederte Buch demonstriert mit vielen Abbildungen, unter denen man neben endoskopischen Bildern auch die Röntgenaufnahmen findet, die Untersuchungsergebnisse beim Verdauungstrakt, bei den Gallenwegen und der Bauchspeicheldrüse. Das Buch eignet sich hiermit auch zum Studium der Gastroenterologie. Die pädiatrische Bedeutung einiger mit oberflächlicher Schleimhautveränderung einhergehenden Krankheiten (Reflux, akute gastrointestinale Läsion, Blutung, entzündliche Dickdarmerkrankungen im frühen Stadium) ist erst mit Hilfe der Endoskopie völlig erkannt worden.

Zur Zeit haben sich die technischen Standarde für die Untersuchung der Säuglinge und Kleinkinder nicht ausgestaltet, doch zum gesicherten Vorgehen liefert das vorliegende Werk wichtige Richtlinien. Auf die Frage, ob man bei dieser Altersgruppe bei dem heutigen Stand der technischen Entwicklung ohne Gefahr zur Endoskopie greifen darf, ist die Antwort der Verfasser eindeutig ja. Der Eingriff darf jedoch nur in endotrachealer Narkose zur Anwendung kommen, um eine eventuelle Aspirationskomplikation zu vermeiden.

Der mit dem Verfahren noch nicht vertraute Leser erhält ausführliche Information über die Ausstattung eines endoskopischen Laboratoriums, den Personalbedarf

und über die korrekte Dokumentation und das Archivieren.

Das Buch wird für den sich für dieses Untersuchungsverfahren interessierenden Arzt ein unentbehrliches Handbuch, für den auf diesem Gebiet Gewandten eine wichtige Hilfe sein.

I. BÁLINT

Therapie der Krankheiten des Kindesalters. Herausgegeben von G.-A. v. HARNACK. 2., völlig neubearbeitete Auflage. XIII + 991 Seiten mit 15 Abbildungen und 203 Tabellen. Springer-Verlag, Berlin—Heidelberg—New York 1980. Preis: DM 128.—

Die zweite, neubearbeitete Auflage des Werkes hat weiterhin die Zielsetzung, dem Kliniker und praktizierenden Arzt ein hilfreicher und kompetenter Ratgeber in allen therapeutischen Fragen der Pädiatrie zu sein. Ein Therapiebuch muß den neuesten Stand des Wissens widerspiegeln, und deshalb wurden sämtliche Kapitel überarbeitet, manche völlig umgestaltet. Das große Material machte die Mitwirkung von etwa 63 Autoren notwendig, dennoch ist die Verfassungs- und Darstellungsweise einheitlich und übersichtlich. Die Pathogenese der Erkrankungen ist meistens nur soweit angegeben, wie es das Verständnis für die Therapie unbedingt erfordert. Die bewährten Behandlungsmethoden werden ausführlich und klar geschildert. Randgebiete der Pädiatrie wurden soweit miteinbezogen, daß die Grundsätze der Therapie erkennbar werden. Von den seltenen Krankheitsbildern wurden nur jene berücksichtigt, bei denen therapeutische Möglichkeiten bestehen; seltenere Malformationen mit nur symptomatischer Therapiemöglichkeit wurden weggelassen.

Das Buch gliedert sich in 24 Kapitel. Es beginnt mit den Neugeborenerkrankungen, wonach Körperform- und Stoffwechselstörungen, Erkrankungen der endokrinen Drüsen, Infektionskrankheiten, rheumatische Erkrankungen, Immunkrankheiten und jene der blutbildenden Organe folgen. Hiernach werden die Krankheiten der einzelnen Organe und Organsysteme, die psychogenen Störungen und die Verletzungen besprochen. Ein gesondertes Kapitel ist den besonderen Therapiemaßnahmen wie z. B. Reanimation, Schockbekämpfung, parenterale Ernährung, Bluttransfusion, Steroidbehandlung usw. gewidmet. Abschließend findet man ein ausführliches Arzneimittelregister.

Für die wertvolle Hilfe, die das Buch dem Kinderarzt bei seiner täglichen Arbeit bietet, soll den Autoren nur Dank gebühren.

K. SCHMIDT

H. MAU und I. GABE: *Die sogenannte Säuglingsskoliose und ihre krankengymnastische Behandlung.* 2., überarbeitete Auflage. VIII + 90 Seiten mit 63 Abbildungen und 8 Tabellen. Georg Thieme Verlag, Stuttgart 1981. Preis: DM 54,—

Das Büchlein befaßt sich mit einem der schwierigen Probleme der Orthopädie. Die Definition der Säuglingsskoliose ist unsicher, ihre Prognose unbestimmt, und die Prinzipien ihrer Behandlung sind auch nicht eindeutig. Im ersten Lebensjahr ist die Krümmung der Wirbelsäule meistens eine Folge des schiefen Liegens, was teilweise mit der asymmetrischen Empfindung des Gehirns im Zusammenhang steht. Aufgrund der Literaturdaten und ihres Krankengutes gelangen die Autoren zu dem Schluß, daß sich die strukturellen Veränderungen der Wirbelsäule graduell am Ende des ersten Lebensjahres oder noch später entwickeln, sodaß es am richtigsten erscheint, von einer "sogenannten" Säuglingsskoliose zu sprechen. Zur Zeit ist es noch nicht entschieden, ob die exogenen oder aber die endogenen Ursachen die größere Rolle spielen.

Hinsichtlich der Behandlung dürfte die Entlastung des Thorax von der einseitigen Belastung das wirksamste Mittel zu sein. In diesem Sinne müssen die laterale Verbiegung behoben und die Verschluß-

platten durch Reklination der Wirbelsäule entlastet werden; die Bauchlage hat sich zu diesem Zweck bewährt, da dadurch die ungünstigen Reflexe reduziert, die Muskelkraft zur Korrektion aktiviert und die Schrumpfungen an der konkaven Seite gedehnt werden. Die gezielte Gymnastik trägt zur Behebung der ungewünschten Reflexaktivität und Kontrakturen und zur Aktivierung der Muskulatur bei. Wichtig sind auch die eine entsprechende Lage fördernden Hilfsgeräte, später Gipsverbände und Korsette. Der zweite Teil des Buches ist der Heilgymnastik gewidmet. Ein gesonderter Abschnitt befaßt sich mit den Fragen der passiven (Massage, passive Bewegung, Liegen) und aktiven (Bewegung in Bauch-, Rücken-, Seiten- oder Sitzlage) Behandlung. Diese Übungen werden mit Hilfe von guten Abbildungen illustriert.

Das kleine Buch ist nicht nur eine ausgezeichnete Zusammenfassung der vieldiskutierten Probleme der Säuglingsskoliose, sondern bietet viele nützliche und praktische Kenntnisse hinsichtlich der Behandlung.

GY. LÉNÁRT

M. HERTL und R. HERTL: *Das kranke Kind.* Ratgeber für die Pflege zu Hause und bei Krankenhausaufnahme. X + 308 Seiten mit 69 Abbildungen und 15 Tabellen. Georg Thieme Verlag, Stuttgart 1981. Preis: DM 14,80

Das in der Reihe "Ärztlicher Rat" veröffentlichte Buch wendet sich an Patienten, "die viele Fragen haben" und wird von Ärzten empfohlen, die "wenig Zeit haben". Es richtet sich also an besorgte Eltern und will ihnen Rat und Hilfe bieten, wann und was sie bei ihren Kindern bei leichteren Krankheitszeichen von sich aus tun und wie sie dem kranken Kind mit Selbsthilfe beistehen können. Sie werden jedoch auch informiert, welche Erscheinungen sie bedenklich machen sollen und wann sie den Arzt in Anspruch nehmen müssen.

Einige herausgegriffene Themen aus dem Inhalt des Buches: Gesundheit und Krankheit als Schicksal, was hilft und was schadet; Grundlagen zum medizinischen Verständnis der Krankheiten: Begriffe, Ursachen, Organreaktionen, Diagnose-Therapie; Eltern als Hilfe des Kindes und des Arztes: Beobachtung, häusliche Pflege, Essen-Trinken-Diät, Medikamente und deren Eingabe, Desinfektion, Verbände; Krankheitszeichen, deren Bedeutung, Folgerungen in etwa 70 verschiedenen Körperregionen bzw. Situationen; ungefähr 50 Krankheiten mit besonderen Problemen; Impfungen; Urlaub im Inland und Ausland mit mehrsprachigem medizinischen Dolmetscher; wenn das Kind in ein Krankenhaus muß: positive und negative Seiten, Vorbereitung des Kindes auf das Krankenhaus. Abschließend findet der Leser ein Sachregistes und Erklärungen von medizinischen Fachausdrucken.

Das von zwei anerkannten Fachärzten verfaßte kleine Buch ist allgemeinverständlich und bietet alle pflegetechnischen und psychologischen Informationen, die zur Heimbehandlung des kranken Kindes nötig sind.

ANNEMARIE GEGESI

V. HOFMANN: *Ultraschalldiagnostik (B-Scan) im Kindesalter.* 174 Seiten mit 160 Abbildungen. G. Thieme Verlag, Leipzig 1981. Preis: M 30,—

Heute verfügen wir bereits über mehrere Publikationen in bezug auf die Ultraschalldiagnostik im Kindesalter. Die vorliegende Monographie ist in der Hinsicht eine bahnbrechende Unternehmung, daß der Autor es versucht hat, das ganze Spektrum der pädiatrischen Anwendungsmöglichkeiten zusammenzufassen.

Nach einem einleitenden Kapitel, in dem die historischem, physikalischen und technischen Grundlagen der Ultraschalldiag-

nostik erörtert werden, wird kurz über die pränatale Diagnostik, Erkrankungen des Gehirns, der Schilddrüse und des Thorax berichtet. Der größere Teil des Buches ist der Beschreibung und Demonstration der Veränderungen der abdominalen Organe gewidmet. Die gesonderte Schilderung der Echographie von Niere, ableitenden Harnwegen und Nebenniere ist durch deren praktische Bedeutung begründet, doch ist es nicht ganz klar, warum die Tumoren in einem separaten Kapitel besprochen werden, wenn das Buch nach Organen gegliedert verfaßt wurde. Die Proportion der einzelnen Teile ist nicht immer optimal; dies dürfte sich daraus ergeben, daß der Verfasser womöglich alle Anwendungsmöglichkeiten, mitunter als Schlagworte, anführen wollte.

Die Echogramme wurden mit dem Vidoson Typ 635 real-time und dem Sonodiagnost Typ B 62 — mit wechselnder Qualität — verfertigt. Infolge der unbegründet reduzierten Bildgrößen bzw. der schwachen Reproduktion sind einige Aufnahmen nicht entsprechend demonstrativ, — dies bezieht sich hauptsächlich auf die real-time Bilder. Das sich den einzelnen Kapiteln anschließende Schrifttum ist sehr nützlich und enthält alle bedeutenden Literaturdaten.

Die pädiatrische Echographie ist eine der wichtigsten Gebiete der Ultraschalldiagnostik, da es sich um ein nichtinvasives Verfahren handelt, welches nach heutigem Wissensstand kein genetisches Risiko hat. Unser Bestreben ist also der weitläufige Enisatz des Ultraschalls auf allen Gebieten, wo damit andere riskante Methoden ersetzt werden können. Das vorliegende Buch kann in der Verbreitung der diesbezüglichen Kenntnisse gute Dienste leisten, denn der Einbau einer neuen Methode in eine gewohnte Untersuchungsroutine erfordert gewisse Veränderungen der Ansichten.

Die Arbeit wird einerseits jenen empfohlen, die in der Ultraschalldiagnostik die grundlegenden Kenntnisse und Praxis schon erworben haben und sich zur Einleitung des Verfahrens bei Säuglingen und Kinder entscheiden und anderseits jenen Kinderärzten und Kinderchirurgen, die sich über den Wert und die Grenzen der Methode informieren möchten. Die Leistungsfähigkeit und Technik und somit der klinische Wert stehen noch unter ständigem Wandel, wir verfügen jedoch schon heute über Geräte, die die entsprechende Untersuchung von Kindern sichern.

Z. HARKÁNYI

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CONTENTS

Down syndrome: correspondence of clinical diagnosis and karyotype. *M Osztovics, P Kiss* 261

Unusual chromosome aberrations in 3 children with Down syndrome. *M Osztovics, S Tóth, O Wilhelm* 283

Familial occurrence of partial trisomy 4q and probable monosomy 5p due to 4q/5p translocation. *A Gencik, A Genciková, A Pálová* 291

Developmental changes in lymhocyte surface markers. *E Walcz, I Seri, D Kuti, T Révész, D Schuler* 299

Hormonal control of sodium content in human milk. *T Ertl, E Sulyok, M Németh, I Tényi, I. F. Csaba, F Varga* 309

Damaging effect of free radicals liberated during the reduction of oxygen: its influencing by drugs. *G Balla, A Makay, Z Pollák, B Matkovics, L Lakatos, L Karmazsin* 319

D-penicillamine in the prevention of retrolental fibroplasia. *L Lakatos, I Hatvani, G Oroszlán, L Karmazsin, B Matkovics* 327

Immunological findings in primary malabsorption. *B Szabó, J Jezerniczky, M Kávai, A Nagy, B Nagy* 337

The effect of caffeine and physical exercise on blood lactate levels of obese children. *L Barta, M Pena, M Tichy, A Regöly-Mérei, G Koltai, M Bedő* 343

Use of thrombocytopenia for the early identification of sepsis in critically ill newborns. *W Storm* 349

Isolation of aerobic bacteria from the placenta. *L Kovalovszki, Z Villányi, I Pataki, I Veszelovszky, Z. B Nagy* 357

Long-term prognosis of asphyctic neonates from an intensive care unit: Intrauterine retarded infants at high risk of cerebral palsy. *J Storcz, J Mestyán* 361

Book reviews 375

Index: 26.021

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Assessing obesity with body weight and height

M. Amador, C. Rodriguez, María E. González, J. Bacallao

Department of Paediatrics, Department of Physiological Science and Centre of Cybernetics, Higher Institute of Medical Sciences of Havana, and Department of Physical Development, Institute of Sports Medicine, Havana, Cuba

Anthropometric measurements in one hundred girls and one hundred boys from 4.6 to 5.5 years of age were carried out in order to study the correspondence between the criteria of obesity according to weight for stature (BW–HT) and according to the magnitude of fat body mass (FM calculated of two different regression equations, one developed starting from body weight (BW), height (HT) and two skinfolds, and another one, using only BW and HT.

Employing the criterion of obesity based upon BW–HT above 120%, a number of heavy but not fatty subjects (mainly boys) were included as obese, and three of the girls studied were fatty but not overweight. Conversely, if we calculate FM using the regression equation based on BW and HT, and the criterion of obesity based upon body fat percent, the incidence of obesity could be underestimated. The best results were given by regression equations which include BW, HT and two skinfolds (triceps and subscapular).

Though the usefulness of BW and HT in nutritional assessment is not discussed, the diagnostic limitations of these measurements used alone or in combination are evident and therefore it is necessary to include skinfold measurements if we wish to obtain a correct diagnosis of obesity.

Since body weight (BW) can significantly be influenced by body length or stature (HT), the use of weight for age for the assessment of the nutritional status in children has been progressively substituted by indices which combine BW and HT in different manners [21, 23, 34].

The most commonly used indices are weight for length or stature (BW–HT), which is expressed as the percentual value of expected weight for actual length [7, 9, 11, 21, 23, 34]; the ratio BW/HT [1, 26], or quantity of body mass per unit of linear dimension; the Ponderal Index, which converts BW in linear dimension [1, 32]; and those indices which establish an age independent relation between BW and height, like Rao's [30] and Dugdale's [12] indices.

Though all these indices are based upon simple measurements, some of them introduce mathematical procedures which complicate the determinations without a significant increase of diagnostic accuracy. None of these methods is capable to express the variations in body composition; they only show the changes in whole body mass, that in a strict sense is not enough to appraise the magnitude

of malnutrition and often leads to confusion and misunderstanding of the actual nutritional status [5].

Obesity is the type of malnutrition characterized by an increase of fat body mass (FM) [24], though in most children with hyperplastic forms of obesity there is also an increase of fat-free mass (FFM) [16]. Exogenous obesity is characterized by a tendency of the stature to be in the upper percentile fields [19].

The increase of FM and to a lesser extent of FFM determines the increase of BW in obese subjects, but the increase of BW not always means an increase of FM, especially when the overweight is slight or moderate. Hence, the study of body composition is indispensable for differentiating obese subjects from those who are constitutionally heavy because of their better developed FFM.

In many but by no means in all people a moderately satisfactory estimate of the body fat content can be obtained from BW and HT. For a more precise evaluation several methods are available which give a reasonably accurate measure of FM both in normal subjects and in individuals of unusual body build [14].

Body composition can be assessed by determining FM by linear regression equations developed for the purpose. Dugdale and Griffiths [13] determined several of these equations starting from combinations of anthropometric measurements: BW, HT and skinfolds, and have proved that combining BW, HT and two fatfolds, triceps fatfold (T) and subscapular fatfold (SS), the degree of accuracy compared with the methods using K^{40} is higher than when one or two skinfolds are used alone or combined with BW.

The correspondence between the criteria of obesity according to BW–HT and to the magnitude of FM calculated on the basis of regression equations which use only BW and HT has not been studied. The purpose of the present paper was to examine whether, if only those two measurements are at disposal, it is advantageous to use in the diagnosis of obesity the regression equation instead of BW–HT.

Materials and Methods

One hundred girls and one hundred boys aged from 4.6 to 5.5 years were studied. This sample of two hundred children was selected from among those who attended to polyclinics for periodic health surveillance. None of them had symptoms or a history of chronic disease or malformation.

Data of identity and measurements were obtained by skilled personnel trained at the Department of Physical Development of the Institute of Sports Medicine. These data were:

- Name of the subject,
- Sex,
- Date of birth and date of recording,
- Body weight (BW) in kg, to the nearest 0.1 kg,
- Stature (HT) in cm, to the nearest 0.1 cm,
- Triceps skinfold (T) in mm, to the nearest 0.1 mm,
- Subscapular skinfold (SS) in mm, to the nearest 0.1 mm.

Anthropometric measurements were obtained employing the instruments and

following the procedure recommended by the International Biologic Program (36) as described in a previous paper (2).

Decimal age was obtained from the date of birth and the date of recording, following Tanner (36).

Body weight for stature (BW–HT) was calculated according to Ounsted and Simons (27) as follows

$$BW-HT=\frac{A}{B}\times 100$$

where

$$A=\frac{\text{actual weight (kg)}}{\text{actual stature (cm)}}$$

and

$$B=\frac{\text{50th \% expected BW for age (kg)}}{\text{50th \% expected HT for age (cm)}}$$

Expected values for weight and stature corresponded to the 50th percentile of the Cuban National Child Growth Study (22) for the sex and the age of the subject.

Fat body weight (FM) was obtained from the linear regression equations developed by Dugdale and Griffiths (11). We employed two groups of equations: one, which uses BW, HT and two skinfolds (T and SS) as follows:

For boys: FM_1 (in kg) = 1.753 + 0.304BW — — 0.064HT + 0.187T + 0.140SS

For girls: FM_1 (in kg) = 7.259 + 0.647BW — — 0.150HT — 0.027T + 0161SS

The other equations use only BW and HT according to the expressions:

For boys: FM_2 (in kg) = 5.244 + 0.380BW — —0.085HT

For girls: FM_2 (in kg) = 7.869 + 0.650BW — —0.151HT

Body fat percent (or relative fat body weight) (%BF) was obtained from FM by the equation

$$\%BF=\frac{FM\times 100}{BM}$$

Criteria of obesity were established according to BW–HT if the actual BW was 20% or more above the expected BW for stature. According to %BF, obesity was considered if relative fat body weight was 25% or more or 30% or more in boys and in girls, respectively, according to Bray (8).

Mean values and standard deviations for FM_1 and FM_2 in each sex, and for BW–HT were obtained. Means were compared by Student's t test. Correlations between $\%BF_1$ and BW–HT $\%BF_2$ and BW–HT, FM_1 and BW–HT and FM_2 and BW–HT and between FM_1 and FM_2 were calculated and linear regression equations were drawn.

All the subjects considered obese by the BW–HT criterion were cross-classified as obese and non-obese according to their relative fat body weight (%BF) determined by the two previously described regression equations ($\%BF_1$ and $\%BF_2$). A McNemar test was then carried out with the purpose of establishing whether the two methods led to the same conclusion concerning obesity or non-obesity. This test was performed for both boys and girls.

A paired-sample test was performed to compare both methods of determination of %BF for both boys and girls.

Finally, a simple linear regression model was fitted by the least square procedure to the value of $\%BF_1$ and $\%BF_2$ in boys as well as in girls. The statistical hypothesis that b (the slope) was one and that a (the intercept) was zero, was also tested

Results

Mean values for each variable were obtained as shown in Table I. No significant differences were found between FM_1 and FM_2 in boys, while in girls differences were slightly above the significance level ($\alpha = 0.05$). FM_1 as well as FM_2 values were significantly higher in girls, but while in females a slight difference between means could be found, in boys no significant differences were obtained.

TABLE I

Mean ±SD weight for stature and fat body weight in 100 boys and 100 girls aged 5 years

	Mean ± S.D.	Student's *t* test
Boys		
FM_1, kg	3.06± 1.22	5.310 ($p < 0.001$)*
FM_2, kg	3.05± 0.90	1.929 ($p < 0.05$)**
BW–HT	103.91±12.57	3.539 ($p < 0.001$)*
		0.134 (n.s.)*
Girls		
FM_1, kg	4.16± 1.66	
FM_2, kg	3.71± 1.62	0.066 (n.s.)**
BW–HT	106.31±12.59	

FM_1 = Fat body weight obtained by regression equations using body weight, stature and two skinfolds.
FM_2 = Fat body weight obtained by regression equations using body weight and stature.
BW–HT = Body weight for stature.
* Differences in females.
** Differences between FM_1 and FM_2.

Values for BW–HT were similar in both sexes.

Correlation studies are summarized in Table II. All combinations proved to be highly significant, with the *r* values were higher in girls.

Figures 1 and 2 show the dispersion diagram, correlation study and regression line between BF_1 and BW–HT in boys and girls respectively. Cut-off points were drawn defining the limits for the criteria of obesity according to BW–HT (120% of expected weight for stature) and according to %BF (25% for boys and 30% for girls). In Fig. 1 it is possible to observe that, though in 14 subjects BW–HT was above 120%, only 4 of them showed a proportion of body fat exceeding the limit established for the employed criterion of obesity. In girls, the discrepancy was not so manifest, and from 14 overweights (above 120% BW–HT), eleven were also fatty (more than 30% BF), as shown in Fig. 2.

Table III shows the cross-classification of male and female children as obese and non-obese according to both methods of determination of relative fat body weight ($\%BF_1$ and $\%BF_2$). The results of the McNemar test also appear in Table III.

Table IV contains the mean ±S.D. percentage of fat body weight by the two methods and the value of the

TABLE II

Correlations between anthropometric indices of fatness of overweight in 100 boys and 100 girls aged 5 years

x	y	Regression equation $y = bx + a$	Correlation coefficient r*
Boys			
FM_1		$y = 8.84x + 70.87$	0.859
FM_2	BW–HT	$y = 12.78x + 64.92$	0.917
FM_1	FM_2	$y = 0.65x + 1.06$	0.878
$\%BF_1$	BW–HT	$y = 2.02x + 70.48$	0.730
$\%BF_2$	BW–HT	$y = 1.93x + 58.22$	0.757
Girls			
FM_1	BW–HT	$y = 6.30 + 80.71$	0.878
FM_2	BW–HT	$y = 8.37x + 7.541$	0.967
FM_1	FM_2	$y = 0.85x + 0.16$	0.71
$\%BF_1$	BW–HT	$y = 1.87x + 63.57$	0.930
$\%BF_2$	BW–HT	$y = 2.08x + 60.43$	0.823

* All correlation coefficients were significant at the level of $\alpha = 0.001$.
FM_1 and $\%BF_1$: Fat body weight and body fat percent obtained by regression equations using body weight, stature and two skinfolds.
FM_2 and $\%BF_2$: Fat body weight and body fat percent obtained by regression equations using body weight and stature.
BW–HT: Body weight for stature.

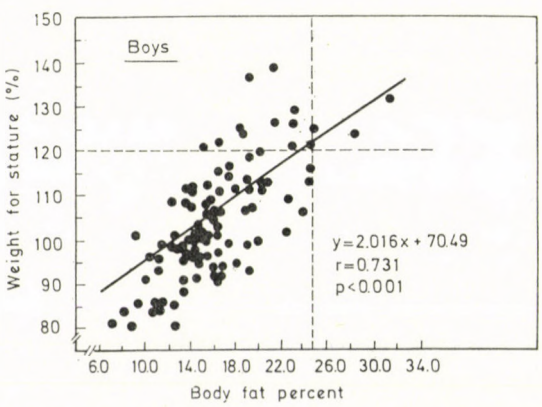

FIG. 1. Correlation between body fat percent obtained from fat body mass calculated from body weight, stature and two skinfolds and weight for stature in 100 five-year old boys. Note that from 14 overweight (above 120% BW–HT) children, only 4 show overfatness

paired sample test in boys and girls.

Finally, Table V shows the values of the parameters and the correlation coefficient corresponding to the fitted linear regression model for each sex.

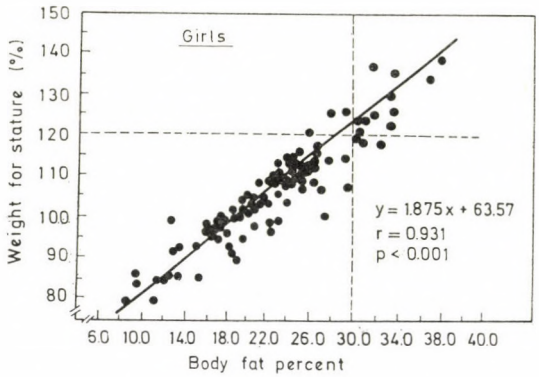

FIG. 2. Correlation between body fat percent obtained from fat body mass calculated from body weight, stature and two skinfolds, and weight for stature in 100 five-year old girls. Note that from 14 overweight (above 120% BW–HT) children, 11 are actually fat and another three fat girls are not overweight

TABLE III

Cross-classification of 14 boys and 14 girls with weight for stature above 120% according to their relative fat body weight (%BF) obtained by two different regression equations

Boys		Relative fat body weight ($\%BF_1$)	
		<25%	≥25%
Relative fat body weight ($\%BF_2$)	<25%	10	4
	≥25%	0	0

McNemar test = 2.25 (n. s.)

Girls		Relative fat body weight ($\%BF_1$)	
		<30%	≥30%
Relative fat body weight ($\%BF_2$)	<30%	3	5
	≥30%	0	6

McNemar test = 3.20 (n. s.)

$\%BF_1$: Relative fat body weight calculated from body weight, stature and two skinfolds.
$\%BF_2$: Relative fat body weight calculated from body weight and stature.

TABLE IV

Paired sample test: Mean ± SD body fat percent obtained by two different methods in 5-year old children

Sex	$\%BF_1$ Mean ± S.D.	$\%BF_2$ Mean ± S.D.	Paired sample test
Girls			
(n = 100)	22.79±6.25	20.34±5.49	11.216 ($p < 0.001$)
Boys			
(n = 100)	16.57±4.56	16.57±2.60	0.004 (n.s.)

$\%BF_1$: Relative fat body weight (Body fat percent) calculated from body weight, stature and two skinfolds.
$\%BF_2$: Relative fat body weight (body fat percent) calculated from body weight and stature.

TABLE V

Parameters of the fitted linear regressions to the values of body fat percent obtained by two different regression equations in 5-year old children

Parameter	Boys (n = 100)	Girls (n = 100)
Slope (g)	1.30	1.07
Intercept (a)	−5.07	1.04
Correlation Coefficient r	0.74	0.94
Test value for slope ($b = 1$)	2.54 ($p < 0.05$)	1.78 (n.s.)
Confidence interval for a	from: −9.03 to −1.13	from: −0.43 to 0.43

DISCUSSION

Because BW and HT are easily obtainable measurements, many authors stress the importance of these anthropometric indicators in nutritional assessment [31], and many different definitions, equations and formulas for weight for height have been employed [25, 29, 33, 35]. Poskitt and Cole [29] used both the weight/height ratio and the weight-for-height standards to assess obesity in children, arriving at contradictory conclusions when comparing both methods. Hermelo et al. [20] correlated weight for height with weight/height in 184 healthy infants and children, finding a highly significant correlation coefficient (0.931) for the differences between both methods apparent for underweight children, but not for overweight ones. Weight/height has been widely used in adults for assessing obesity [10].

According to Dugdale and Griffiths [11], the estimation of FM based on BW and HT produces highly signif-

icant correlations in both sexes. The addition of triceps skinfold to the regression equation meant only a slight improvement that did not reach statistical significance, while the inclusion of further skinfolds to these equations made almost no difference in the percentage of variance. Finally, these authors concluded that the addition of triceps skinfold reduced the number of large errors in the estimates, especially in children who are unusually stocky or "long and lean".

If we take into account the values of FM_1 and FM_2 obtained in boys and in girls, we should assume that, as the differences are very slight or do not exist, and the correlation coefficients are so highly significant, the calculation of fat body mass does not need skinfold measurements and for practical purposes can be done easily by regression equations including only BW and HT. Still, as fat body mass increases, the correspondence between the two methods decreases (Amador et al., unpublished data).

It has been widely demonstrated that, using weight for stature above 120% as the criterion of obesity, the number of "obese" subjects is overestimated, because it includes overweight subjects who are not fat. Regarding the methods for calculating body fat by regression equations, the results of the McNemar test show no significant difference between both methods of determination as regards the classification of obese and non-obese. The discrepancy appears to be high especially in girls, but is not sufficiently so for significance. However, the mean values of the two methods differ significantly in girls, being higher for the method taking into account the skinfolds.

The fitted regression models show very strong correlations between the two methods for $\%BF_1$ and $\%BF_2$ as we found between FM_1 and FM_2. This correlation is higher in girls where the two parameters do not differ significantly from 1 and 0, respectively. In boys, however, the regression line shows that for values of $\%BF_2$ above roughly 17 (in fact, 16.842) $\%BF_1$ tends to be higher than $\%BF_2$ while the relation is reversed for values below 17.

These results led us to assume that the two methods are not indistinguishable even in girls in spite of the fact that b and a do not differ significantly from 1 and 0, respectively. The values of b and a however show that $\%BF_1$ tends to be systematically higher than those of $\%BF_2$ for any value of the latter and this is reflected in the significant differences obtained by the test for comparison of means.

Taking into account the above considerations, we could assume that the use of BW and HT for assessing obesity leads to an overdiagnosis if we use the weight for height criterion, and probably leads to underdiagnosis if we apply the regression equations for calculating FM without including skinfolds.

According to our findings, if we wish to assess fatness, we must include some fat measurement to

increase the sensitivity and reliability of the method employed, and the higher the number of fatfolds we include, the higher the accuracy of the method, though for practical purposes two skinfolds seem reliable enough [13, 25].

Though the quantity of subcutaneous fat related to the total body fat [17] is lower in girls than in boys, the r coefficients obtained when correlating FM or %BF with BW–HT were higher in females, the opposite to what we had expected. One explanation of this controversial finding could be that boys are more likely to exhibit an overweight due to the increase of fat-free mass, while in girls the increase in BW is frequently related to fatness. In three of our girls $\%BF_1$ was above 30% and BW–HT was under 120% (Fig. 2), a fact that supports our opinion that it is necessary to include fatfold measurements in the assessment of obesity, because in some cases body tissues can be replaced by fat and obesity may be present without any significant change in body mass [18].

Although we arrived at the conclusion that the definition of childhood obesity is a difficult task [6], we believe that the main fact is to keep in mind the limitations of the methods [4]. The diagnostic limitations of using BW and HT grow with the age of the child, and if we are dealing with a moderately overweight patient it is very important to define precisely how fat in fact he or she is before making a hasty diagnosis of obesity. For this purpose, it is evident that we cannot trust parameters like body weight and height alone.

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Diagnosing and misdiagnosing malnutrition

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With the purpose of validating new tools as AKS and Energy/Protein Indices for a more precise diagnosis of malnutrition, specially in the case of constitutionally thin or constitutionally heavy subjects which could mistakenly be interpreted as undernourished or obese, two hundred healthy childen, one hundred girls and one hundred boys 4.6 to 5.5 years of age, were studied.

The limitations of weight for stature and the advantages of indicators which determine body composition as accurate measures of malnutrition were demonstrated. In a group of lean individuals with weight for stature under 90%, if E/P figures are under the 10th percentile and AKS is under 1.12, the subject is very likely to be undernourished. In contrast, if the subject, though underweight, shows an E/P Index above the 10th percentile and an AKS over 1.12, constitutional thinness should be considered. In overweight subjects, E/P figures above the 90th percentile point to obesity, while children with E/P between the 10th and 90th percentiles are probably stocky but not fatty subjects.

The usefulness of the combination of the above mentioned criteria in cases suspected to be malnourished, and its introduction in daily practice, will prevent from iatrogenic practices due to erroneous diagnosis.

The problem of diagnosing malnutrition in an individual is very closely linked to the diagnosis of adequate nutrition. Although theoretically the adequacy of nutrition is defined as an equlibrium between needs and intake of energy, the assessment of unbalance is not easy when signs of deficiency are lacking in the case of undernutrition; or when an evident increase of body fat cannot be detected with accuracy if obesity is suspected. The problem has a particular significance when using methods which are not entirely precise to inform about the variation of body composition in patients with early malnutrition [10, 23, 48].

The main effect due to undernutrition on body composition is the loss of fat-free body mass (FFM) [20, 44, 50], while in obesity the outstanding feature is the increase of fat body mass (FM) [26, 43]. These changes cannot be measured by obtaining body weight (BW) alone, because this indicator can be influenced by the variations of body components other than fat and muscle, i.e., water. Furthermore, a light or heavy body constitution could be an individual feature because of minimal adiposity or greater muscularity (the latter can also be acquired by exercise), without meaning either undernutrition or obesity [28, 46, 48].

The development in recent years of regression equations through which it is possible to calculate FM and FFM [10, 11] or indices like the Energy/Protein (E/P) Index [1, 3], capable of reflecting the relationship between FM and FFM starting from single measurements obtained at the middle third of the upper arm, have introduced new tools for the use of clinicians and field workers in individual and community nutritional assessment.

Moreover, some other indices commonly used in anthropologic studies, sports medicine or related sciences are scarcely applied to nutritional assessment though they are able to give good information about body composition. This is the case with the AKS Index, which expresses the weight of FFM contained in a cubic centimeter of body mass [47].

The purpose of this paper is to show the results of the application of some of these methods to nutritional assessment in children in which the diagnosis of malnutrition is controversial when using other anthropometric procedures common in clinical nutrition.

Material and Methods

Two-hundred children, one hundred girls and one hundred boys from 4.6 to 5.5 years of decimal age were studied.

The sample was selected from children attending two Teaching Polyclinics in the city of Havana for periodic health surveillance. Children with chronic diseases or malformations were excluded.

The data were obtained by a measuring team composed of skilled personnel previously trained at the Department of Physical Development of the Institute of Sports Medicine in Havana. These data were:

- Name of the subject,
- sex,
- date of birth and date of recording,
- body weight (BW), in kg, to the nearest 0.1 kg,
- stature (HT) in cm, to the nearest 0.1 cm,
- mid-arm circumference (MAC) in cm, to the nearest 0.1 cm,
- triceps skinfold (T) in mm, to the nearest 0.1 mm,
- subscapular skinfold (SS) in mm, to the nearest 0.1 mm.

BW, HT, MAC and skinfolds were obtained employing the instruments and following the procedures recommended by the International Biologic Program of the United Nations [51], as described elsewhere [3].

Decimal age was obtained from the date of birth and the date of recording according to Tanner [51]. Body weight for stature (BW/HT) was calculated according to Ounsted and Simons [32]. The standards for BW and HT were those corresponding to the 50th percentile of the charts of the Cuban National Child Growth Study [25] for each sex, at the age of the subject.

The E/P Index was calculated from the equations $E/P = \frac{TT}{\log 10\, MAMC}$ where TT is the transformed T [14], and MAMC the mid-arm muscle circumference, obtained according to Jelliffe from the equation $MAMC = MAC - \pi T$ [24].

Reference values for E/P were obtained from the percentile distribution previously reported by us [3].

Fat body weight (FM) was obtained from the linear regression equations developed by Dugdale and Griffiths [12], for BW, HT and two skinfolds (T and SS), as follows.

For boys under 150 cm stature:

$$FM = 1.753 + 0304BW - 0.064HT + + 0.187T + 0.140SS$$

For girls under 140 cm stature:

$$FM = 7.259 + 0.647BW - 0.150HT - - 0.027T + 0.161SS$$

From FM given in kg, and BW, body fat percent (%BF) and FFM could be calculated.

The AKS Index was obtained according to Tittel and Wutcherk [47] as

$$AKS = \frac{FFM\ (\text{in g}) \times 100}{HT^3\ (\text{in cm})}$$

Mean values and standard deviations for each variable were calculated, and t tests for comparison of means were performed. Simple linear regression equations were also found when correlating pairs of them.

Results

Mean values and standard deviations for the different variables studied are shown in Table I. Significant differences between sexes were found for E/P, FM and %BF, being higher in girls in all cases.

All correlation studies but one proved to be significant (Table II). Figs 1 and 2 show the correlations between %BF and E/P Index in boys and girls respectively. Regression lines were also drawn and in both cases significant r values were obtained, being higher in boys. In these figures two other aspects must be pointed out: one of them is that in the scatter diagram we divided the individuals into four groups according to BW/HT as follows. Obese

Table I

Mean and standard deviations for several indicators used in nutritional assessment of children 4.6 to 5.5. years old

Indicator	Girls		Boys		t value p
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	
A.K.S. index	1.19	0.08	1.22	0.09	0.589 n.s.
Energy/protein index	1.697	0.124	1.638	0.129	3.269 <0.005
Body fat per cent	22.79	6.25	16.58	4.55	7.980 <0.001
Kilograms of body fat	4.16	1.66	3.06	1.22	5.291 <0.001
Weight for stature	106.31	12.59	103.91	12.57	1.338 n.s.

Level of significance: $\alpha = 0.05$.
Number of subjects: Girls: N = 100
Boys: N = 100

TABLE II

Correlation studies between pairs of indicators in children 4.6 to 5.5 years old

Girls (N = 100)		Regression equations	r	p
x	y			
AKS index	E/P index	$y = 0.676x + 0.89$	0.414	<0.001
%BF	E/P index	$y = 0.014x + 1.38$	0.706	<0.001
AKS index	BW/HT	$y = 72.066x + 20.17$	0.436	<0.001
Boys (N = 100)		**Regression equations**	**r**	**p**
x	y			
AKS index	E/P index	$y = 0.221x + 1.37$	0.164	n.s.
%BF	E/P index	$y = 0.024x + 1.23$	0.857	<0.001
AKS index	BW/HT	$y = 91.525x + 7.58$	0.701	<0.001

Level of significance: $\alpha = 0.05$.
E/P = Energy/Protein index
%BF = Body fat per cent
BW/HT = Body weight for stature

(BW/HT $>$ 120%); overweight (BW/HT 111–120%); mean weights (BW/HT 91–110%); and underweight (BW/HT $\leq$ 90%). The other aspect is that the cut-off lines for the 90th and 10th percentiles for E/P and for the conventional limit of obesity (20 and 30 %BF) [4, 7] were drawn in the Figure.

It is evident that an important number of subjects classified as overweight or obese according to BW/HT

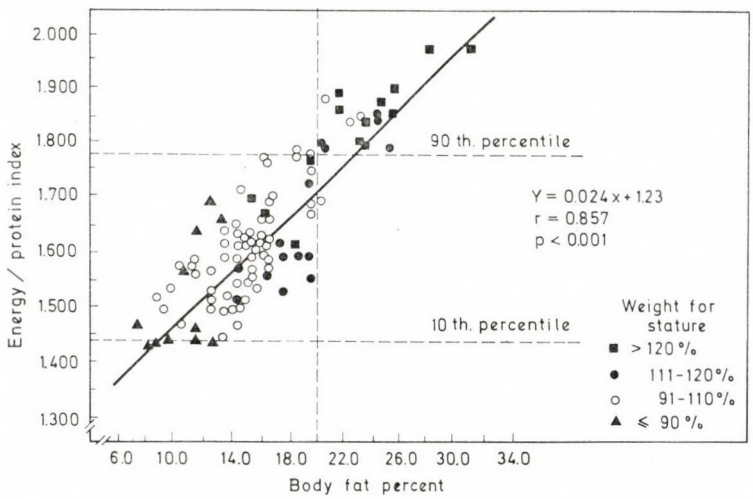

FIG. 1

were not actually obese according to their FM, showing E/P values under the 90th percentile; and the opposite situation, few fatty individuals were not overweight, showing E/P values above the 90th percentile. On the other hand, children classified as underweight (BW/HT $\leq 90\%$) were neatly grouped into two categories: those who are very near or below the cut-off line of the 10th percentile for E/P, and those who are clearly above this line.

Figures 3 and 4 show the correlations between the two indices AKS and E/P. The results differed from one sex to the other, the r values being significant only in girls, although the distribution of the subjects was much more disperse than in Figure 2. The dispersion was also notable in children over 120% BW/

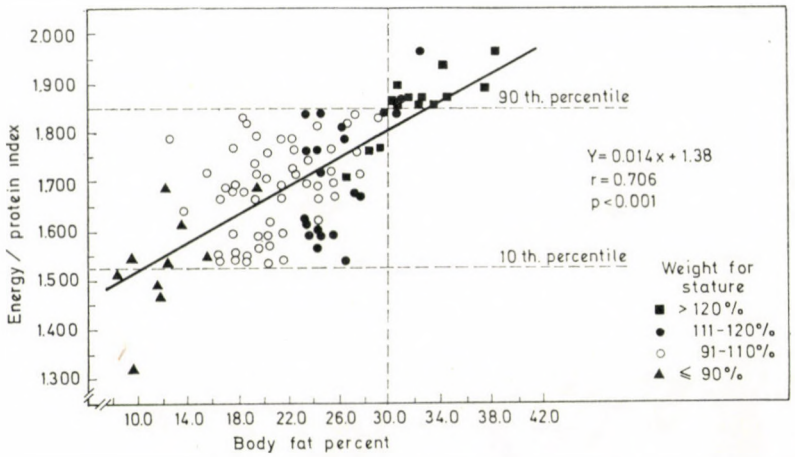

FIG. 2

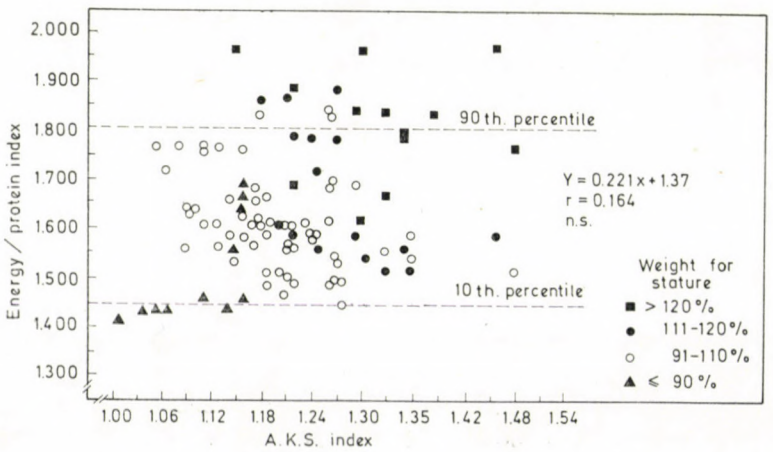

FIG. 3

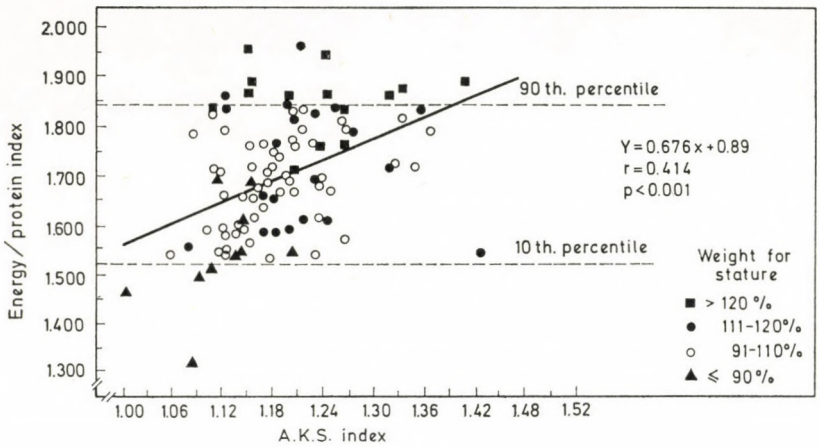

FIG. 4

HT, with a very wide range of values for AKS, especially in boys. Once again we have focussed our attention on the thin children previously reported in Figs 1 and 2. We could find a correspondence between low E/P and low AKS figures. Most of the children, boys as well as girls, with AKS under 1.12 had E/P values at or below the 10th percentile.

DISCUSSION

Nutritional adequacy is now considered an important factor in the life of an individual from the time of gestation to the time of his acceptance of full responsibility as a socially functioning adult [11]. This adequacy is evaluated according to the equilibrium between energy and nutrient needs and intake. We could say with Greaves and Berry [18] that "nutritional status is that condition of health of people that can be attributed to the foods they habitually consume". Traditionally, concern with nutritional status has been focussed chiefly on the identification and measurement of undernutrition, but the definition suggested clearly implies that problems arising from overnutrition should also be considered.

The limitations of HT and BW as indices of growth performance are well recognized, yet they continue to be used because of ready availability and ease of measurement [15]. Every physician on the other hand recognizes the fact that children differ in body build as do adults, and although it is true that generally an increase of BW expresses an increase of fat, in other cases there is an increase in BW at muscle expense, and this means not to be more fatty but more muscular [16].

If we consider body mass to be distributed into two compartments of relatively constant composition: FM

and FFM [13], it is evident that the increase of both components with age in children does not contribute in the same proportion to the increase of overall size of the body. These changes are influenced not only by age and sex, but also by constitutional peculiarities [19], and variations in balance between input and output of energy (physical activity included), have an influence upon these changes [35, 40]. For this reason, our sample was classified according to sex and our study was restricted to children in the range from 4.6 to 5.5 years of age in order to avoid two factors of variation.

Though the limitations of regression equations in predicting FM and FFM are well-known, they have proved to reflect with more accuracy the degree of fatness or leanness than those indicators which use only BW and length or stature [8, 17, 31]. Recent studies by Hermelo et al. demonstrated that there is a highly significant correlation between two different criteria of classification of malnutrition which use weight for height or the ratio weight/height [23]. We have also found a significant correlation between %BF and BW/HT ($r = 0.931$ in girls and $r = 0.731$ in boys of preschool age) [6], but we could demonstrate that overweight is not always coincident with fatness. Peña et al. [39] demonstrated that in obese children the quantitative information offered by BW/HT is not always proportional to the actual fat mass, and may even be paradoxical. This discrepancy could be demonstrated in the present paper too, and not only among overweight or obese children, but also among lean or underweight ones.

As we have no own figures for our population, we have used Dugdale and Griffiths's regression equations for BW, HT and two skinfolds (T and SS) [12]. The results obtained are satisfactory enough for our purposes and they are comparable to those obtained by other authors using different methods [9, 27, 45], though there are not many reports about body composition in preschool children.

We agree with Dugdale and Griffiths [12] that, combining BW, HT and fatfolds, the possibilities of error arising from the use of regression equations based upon skinfolds alone or using BW and skinfolds [27], are quite low.

Once again, the usefulness of the E/P Index for nutritional assessment has been demonstrated, and a close correspondence could be found between %BF and E/P, similar as that obtained in trained, non trained and obese schoolboys [5]. Though highly significant in both sexes, r figures were higher in boys, exactly opposite to our previous findings, when correlating %BF with BW/HT [6]. These variations could be attributed to the sexual differences in fat distribution and FFM development present since early childhood [29, 33, 34, 37, 42].

It is clear that, according to our results, there are two groups of underweight children who differ in

body composition: one of them showing E/P figures above the 10th percentile and an AKS Index above 1.12; and another group with E/P figures below the 10th percentile and an AKS below 1.12. In other words; both groups are composed by lean children, but in the second one, the FFM is significantly lower.

The correlation between AKS and E/P is not significant in boys; in girls, though significant, the values show a fair scattering. The constitutional differences in the development of FFM seem to be responsible for these findings, more evident in boys who, at the age studied, differ from girls in their rapid growth of muscle mass [37, 38]. Though in severely undernourished children BW decreases significantly at the expense of FFM, in mild or moderate forms there is no such a correspondence [21], meanwhile AKS seems to show a higher sensitivity, proving useful in the determination of the degree of development of FFM, when the decrease of muscle mass due to undernutrition results in a low AKS figure. Thus, this Index is applicable not only in sports medicine but also for nutritional assessment.

If we accept the reliability of the calculation of FFM through the regression equations and we take into account our previous results validating the E/P Index as a useful tool in the evaluation of the nutritional status [2, 4], we may conclude that the diagnosis of malnutrition can be made with more accuracy if we include these indicators (E/P and AKS Indices) as complementary tools among those commonly used in field studies or in clinical practice.

This is particularly important when, after an initial screening based upon body weight, we segregate groups of "atypical" subjects among whom it is necessary to determine exactly which of them are actually malnourished. Thus, in a group of lean individuals it is important to know which are undernourished and which are constitutionally thin, and, on the other hand, in a group of stocky subjects, who are obese and who are constitutionally heavy. Thus at least in five-year old children, in the first case those who have E/P figures below the 10th percentile and AKS under 1.12 are very likely to be undernourished, because they combine three criteria: underweight, low fat/muscle ratio (as expressed by E/P), and low FFM (as expressed by AKS). Therefore, those with an E/P over the 10th percentile and AKS above 1.12 are probably not undernourished, but constitutionally thin or "light".

In the second case, "heavy" or overweight children with E/P values between 10th and 90th percentiles are very likely not to be obese.

Several authors have been working in the same direction. Reiken et al [41] developed a somatogram combining HT, BW and three skinfolds which permits to differentiate between overweight and overnutrition or obesity. Mueller and Wohlleb [30] have made an analysis of body build and fat distribution which seems to be useful in the assessment of obesity. We

ourselves have proposed to combine several non-costumary criteria for the diagnosis of malnutrition, especially in borderline cases.

Recently, Habicht [22] commenting on a paper by Trowbridge and Staehling [49] said that the main fact to have in mind when assessing the nutritional status is that the indicators must be chosen according to the type and degree of malnutrition, time of evolution, and several other factors, and therefore previous to any study it is necessary to establish what we are looking for. If we apply schematically the same pattern to every condition, the possibility of misdiagnosing malnutrition will highly increase.

Coincident with this point of view, we have focussed our attention on a group of children prone to be involved in inadequate management because of a peculiar body constitution misdiagnosed as malnutrition. The possibility of giving a reliable definition to an anxious family worried about a thin or heavy child suspected to be malnourished, makes worthy the effort in using the proposed additional criteria.

Acknowledgments

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Effect of exercise on the lipid parameters in the fasting state and under glucose load

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The lipid parameters were studied in the course of exercise in six children in the fasting state and in six under a glucose load. During the test the serum triglyceride level did not change notably, FFA values decreased significantly under the glucose load, most likely due to insulin mobilisation. The total cholesterol/HDL-cholesterol ratio underwent a significant change only in the course of physical work done in the fasting state.

In the treatment of childhood obesity of non-endocrine origin, in addition to the diet [2, 10] the augmentation of the working capacity [8, 9] is of outstanding importance. A number of papers have reported on the increase of the HDL-cholesterol (HDL–C) level which is a well-known protective agent against arteriosclerosis [4, 5].

The aim of the present study has been to follow the parameters of lipid metabolism in obese children who performed physical work in the fasting state or under glucose loading.

Material and Method

Twelve children of both sexes, three girls and nine boys of 13.2 years mean age were included in the study. At five sites on the right side of their body the skinfold thickness was determined using a Holtain caliper. The body fat per cent in the children was over 35% as estimated according to Parižkova [6]. Endocrine obesity was excluded in the subjects.

In all patients glucose loading was carried out under the same conditions. Then the children were divided into two groups. The first group of six children performed two-step tests, first between 20 and 40 min, then two days later between the 20–40 and 90–120 min of the glucose loading. The second group of six children performed the two-step exercise on two occasions. The points of time were similar but this time the children were fasting.

In order to examine the physical working capacity (PWC_{170}) the children had to move up and down on two 45 cm high steps under ECG control with the leads attached to their chest. The test was done in the morning hours, lasting 1–3, 3–6 and 6–9 min for 10, 15 and 20 periods per min. One period consisted of two paces up and two down. In the last 30 s of each min the heart rate was estimated by ECG. The PWC_{170} value was determined by plotting the regression line of the performance attained by the child before the heart rate had risen to 170/min.

Blood was obtained at zero and 180 min for the estimation of cholesterol (Chol),

according to Rappaport–Eichhorn, triglyceride (tg) according to Laurell, FFA according to Dole, HDL–C after phosphotungstic condensation by the enzymatic method of Chole. Differences between the zero min and 180 min findings were compared with the t test. Blood sugar was estimated by the o-toluidine method. Results are shown diagrammatically in the Tables.

Results

Blood sampling at zero and 180 min was done during dextrose loading (D) when work was done once (D + 1m) and twice (D + 2m), and in the fasting state when work was performed once (1m) and twice (2m). The means and standard deviation of FFA, tg, Chol/HDL–C are summarized in Table I.

FFA values at zero and 180 min decreased under the effect of glucose loading especially when no work was performed. The decrease was less under the effect of work done once (D + 1m) and twice (D + 2m) while in the fasting state without dextrose loading the exercise did not affect the FFA level. The ratio total Chol/HDL–C and its values at zero and 180 min declined under the effect of work done during glucose loading. But when work was performed in the fasting state, the ratio total Chol/HDL–C increased significantly. In the case of work done in the fasting state the changes were significant statistically: for 1 m, p was < 0.05, and for 2 m, $p < 0.02$.

The graphs show that the patients' dextrose tolerance improved significantly. The free fields seen in Figs 1 and 2 under the graphs I, II and III indicate how sugar tolerance improved

Table I

Values for FFA, tg, and the ratio Chol/HDL–C during sugar loading and, in the case of work done once and twice after sugar loading, further in the case of work done once and twice after fasting

	D	D + 1m	D + 2m	1m	2m
FFA, mmol/l					
0 min	0.684±0.185	0.585±0.151	0.554±0.196	0.652±0.198	0.587±0.261
180 min	0.268±0.044	0.471±0.198	0.486±0.176	0.559±0.129	0.594±0.300
tg, mmol/l					
0 min	1.10 ±0.52	1.22 ±0.50	0.98± 0.15	1,32 ±0.83	1.16 ±0.47
180 min	0.81 ±0.27	0.85 ±0.29	0.84 ±0.26	1.22 ±0.77	1.09 ±0.41
Chol/HDL–C					
0 min	4.06 ±0.87	4.38 ±1.12	3.98 ±0.96	4.92 ±2.25	4.76±1.00
180 min	4.07 ±1.00	4.59 ±1.10	4.15 ±0.89n	*4.50±1.81	**4.24±1.08

In the case of work done once $p < 0.05$
In the case of work done twice $p < 0.02$

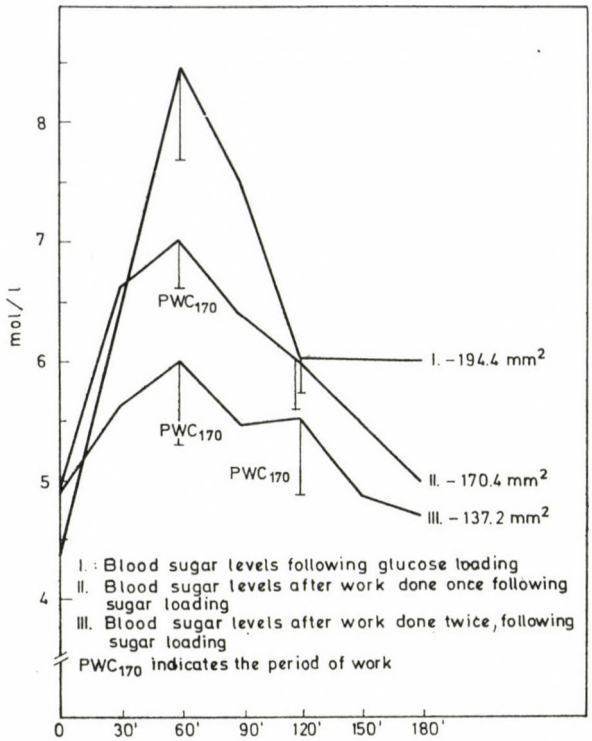
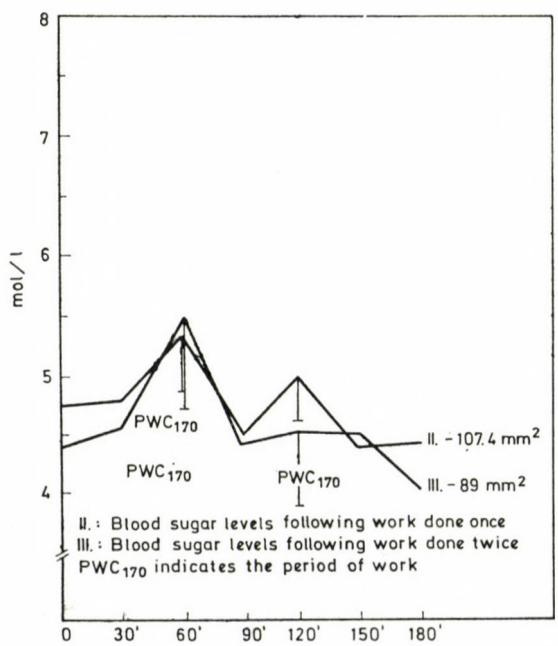

mol/l
8
7
6
5
4
PWC170
PWC170
PWC170
I. – 194.4 mm²
II. – 170.4 mm²
III. – 137.2 mm²
I. : Blood sugar levels following glucose loading
II. Blood sugar levels after work done once following sugar loading
III. Blood sugar levels after work done twice, following sugar loading
PWC170 indicates the period of work
0
30'
60'
90'
120'
150'
180'

FIG. 1


mol/l
8
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6
5
4
PWC170
PWC170
PWC170
II. – 107.4 mm²
III. – 89 mm²
II. : Blood sugar levels following work done once
III. : Blood sugar levels following work done twice
PWC170 indicates the period of work
0
30'
60'
90'
120'
150'
180'

FIG. 2

TABLE II

PWC_{170} indicates the increase (+) and decrease (—) between two performed works

Exercise after sugar loading		
Case No.	Body weight kg	PWC_{170}
1	98	— 0.3
2	77	+15.3
3	105	+14.9
4	66	+11.3
5	73	+11.4
6	64	+22.1
1	104	+17.9
2	87	+18.8
3	78	+20.4
4	57	+20.0
5	92	+ 5.0
6	66	+ 6.7

when loading occurred during exercise done once (II) and twice (III). The PWC_{170} arrows show the periods of physical work.

Finally, the improvement in PWC_{170} was obvious in both groups (Table II).

DISCUSSION

In the prevention of cardiovascular diseases the search for risk factors and their control in children would mean an important progress. As risk factors in children, obesity, hyperlipoproteinaemia and diabetes mellitus are of particular importance [3, 9].

In the treatment of childhood obesity it is important that the weight loss caused by the diet and physical exercise should not harm the formation of lipid parameters. In this relation the atherogenic effect of tg and Chol and the protective effect on the blood vessels of HDL–C are well-known [4, 5, 11]. From this aspect the ratio total Chol/HDL–C is of special interest. This ratio can be improved by decreasing the serum cholesterol level and/or by increasing the concentration of HDL–C. The increase of HDL–C plays a decisive role in the prevention of vascular diseases while its low value in itself represents a risk factor.

In our examinations the work performed after sugar loading resulted in an improvement of the sugar tolerance but this was not associated with an improvement of the lipid parameters. At the same time, however, work done in the fasting state improved the lipid parameters; with an unchanged FFA value the ratio total Chol/HDL–C decreased, and this change has to be considered favourable for the future vascular occurrences in obese children. The improvement of the ratio was naturally due to the significant increase in HDL–C.

The administration of sugar obviously caused an insulin mobilisation in the patients; this was proved by the decrease in the serum FFA level. So, during sugar loading without physical work (D) the FFA concentration decreased considerably but during work (D + 1m and D + 2m) the decline became less and less. Taking into consideration that during exercise the utilization of fat increases,

the mobilisation of lipid must have been significant, in conformity with the rise of the cortisole and noradrenaline levels [1]. Thus, work performed in the fasting state definitely improved the lipid parameters and the FFA value, which indicates an improvement of fat mobilisation. Therefore in the treatment of obese children it is considered important to take into account the changes of lipid parameters in addition to the weight loss and the improvement of the physical condition.

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Ring chromosome 15

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Ring chromosome 15 was found unimodally in the blood cells and in mosaic form in the skin fibroblasts of a female patient. Observations made in the reported case supported the assumption that the instability of ring formation is responsible for the most common clinical symptoms, the intrauterine and postnatal growth retardation.

Ring chromosome 15 is a rare chromosome aberration. To our best knowledge only 18 patients with r(15) have been described in the literature after banding techniques had allowed an exact individualization of chromosomes in the human karyotype [1–7, 10, 11, 13–19]. In this paper we report an additional case with r(15) aberration and the findings are compared with those occurring in the cases published in the literature.

Report of a Case

The female patient T. J. was born in the 39–40th gestational week from the 2nd pregnancy when the mother was 31 years and the father 32 years old. The parents were healthy and unrelated. From the first and the third pregnancy healthy boys were born.

The birth weight was 2500 g (small for gestational age), body length was 55 cm, head circumference was 35 cm. No perinatal problem occurred.

The patient was seen first at 2 months of age. At that time her weight was 3000 g, body length 55 cm, head circumference 35 cm. These parameters remained below the 25th and 3rd percentile values throughout her life.

Clinical examination revealed a delay in motor development, generalized muscular hypotonicity and grand mal type epilepsy with corresponding EEG alterations. Only a few signs of craniofacial dysmorphism could be observed; they were a high forehead and triangular face, narrow palpebral fissures, bulbous tip of the nose, thin lips, high arched palate and sparse hair. There were a coloboma of the iris on the right side and coloboma of the retina and choroid on both sides. The body shape was disproportionate to the long upper extremities and trunk. The hands and feet were small (Figs 1 and 2).

Urography, ECG and laboratory examinations did not reveal any pathological changes.

After gradual deterioration, the patient died with bronchopneumonia in the 36th month of life. Necropsy

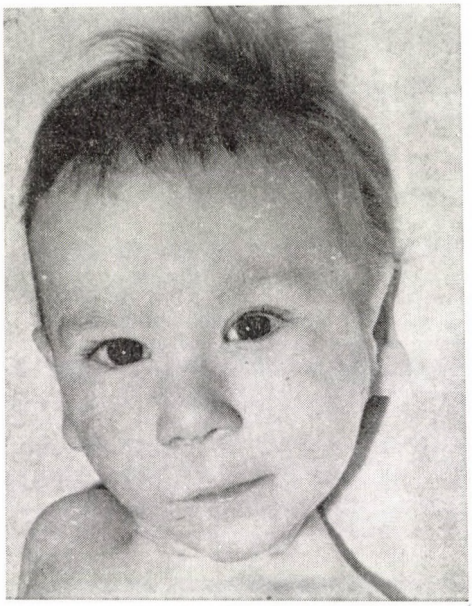

FIG. 1. The patient at 1 year of age

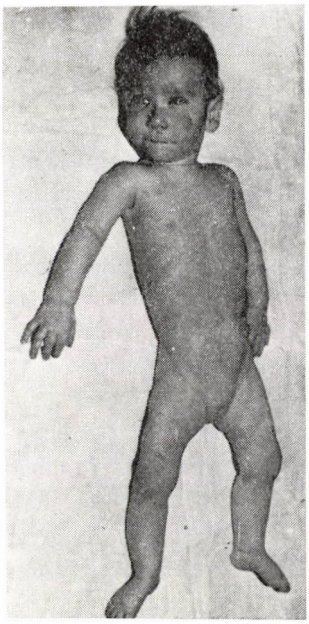

FIG. 2. The patient at 1 year of age

showed unilobar lungs on both sides and microcephaly accompanied by striking microgyria and moderate dilatation of the ventricles (Fig. 3).

Cytogenetic studies. In routinely cultivated blood cells, ring formation of chromosome 15 was found (Fig. 4). In 50 investigated metaphases neither a derivate structure nor loss of r(15) chromosome could be observed. In cultivated skin fibroblasts, out of 66 metaphases 6 displayed the loss of the ring chromosome. On the basis of these findings, a mosaicism was stated.



FIG. 3. Unilobar lungs of the patient

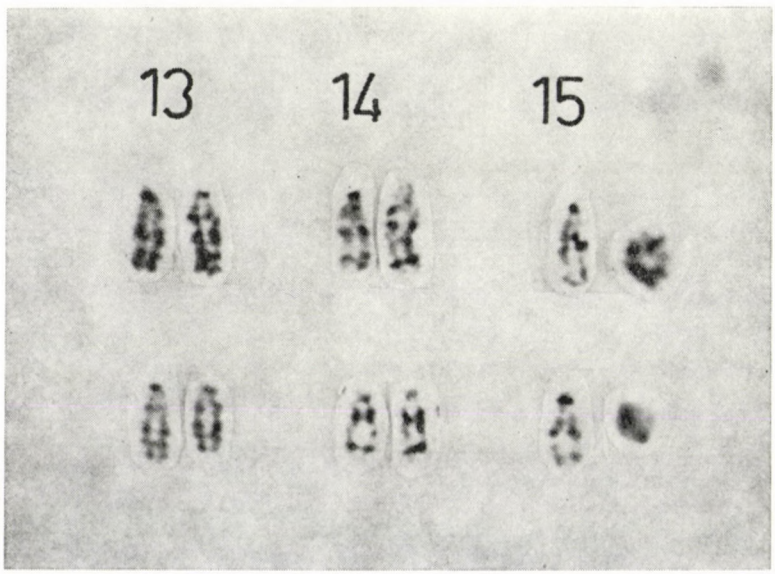

FIG. 4. Ring chromosome 15 with GAG banding

The karyotype of the parents as well as that of the two other children proved to be normal.

Discussion

Ring chromosomes are produced by breakage in each of the two arms of chromosomes and a subsequent reunion of the broken ends. The loss of genetic material depends on the site of breakage. The exact identification of the break points in cell samples during postnatal life is usually difficult; it did not succeed in most of the reported cases of r(15) including the present case. Short arm deletion was identified only in two cases with r(15) chromosome [16, 18] by NOR staining. From the practical point of view, a deficiency of the short arm is without importance, since a monosomy of the short arms of acrocentric chromosomes does not affect the phenotype. Long arm deletion in r(15) chromosome could also be evidenced [16, 4]. An explanation for the failure of exact identification, even in the best banded metaphases, lies probably in the circumstance that there is no significant loss of genetic material in most of the cases [18].

In spite of the minimum deficiency of chromosome material, the phenotype is usually seriously involved and there seems to be no correlation between the clinical findings and the primary deletion. Several investigators emphasized that the phenotypic

Table I

Clinical data and symptoms

Data and symptoms	Emberger et al [1]	Ferrante et al [2]	Forabosco et al [3]	Fryns et al [4] 1	Fryns et al [4] 2	Jacobsen et al [7]	Ledbetter et al [10]
Sex	M	F	F	F	F	M	F
Age	8.5y	15m	16m	18m	6.5y	4ly	15m
Birth weight			2300	2200	2700	1800	
Growth retardation	+	+	+	+	+	+	+
Mental deficiency	+	mild	mild	mild	mild	mild	+
Microcephaly	+			+	+		+
Triangular face	+	+	−	−	+	−	−
High forehead	−	−	−	−	−	−	−
Hypertelorism	−	+	−	+	−	−	−
Epicanthus	−	−	−	−	−	−	−
Microor retrognathia	−	+	−	+	+	−	−
Small hands and feet	−	+	+	+	+	−	
Finger anomaly	−	+	+	+	+	+	+
Major malformation	−	−	+	−	−	+	−

manifestation and variation could rather be connected to the structural instability of ring formation [9, 8]. The instability of ring chromosomes is due to the specific behaviour in the mitotic anaphase. A ring formation with minimum primary deficiency can result in daughter cells with a severe unbalanced genome. Firstly, the ring chromosome can be lost and the daughter cells will be monosomic. Secondly, ring formation can be recombined and daughter cells will contain different derivates that mean partial duplication and/or deficiency in their genomes. Derivates of a ring chromosome have excellently been demonstrated by Niss and Passarge [12]. As a result of these events, mosaicism arises and a certain proportion of the cells will probably be lost at cell division and a significant reduction in the number of viable cells will occur prenatally and postnatally alike. Kjessler et al. (8) assumed that the presence of a ring chromosome per se can predispose to a significantly retarded intrauterine and postnatal growth reflected in low birthweight, growth retardation, delay of development and mental retardation. In the light of this hypothesis, one may understand why all cases with ring chromosome 15 have a low birthweight and postnatal delay in growth and mental development (Table I).

In addition, it is likely that difficulties occur in the anaphase and, as a consequence, the proportion of unviable cells varies from case to

of patients with r(15)

Meinecke and Koske–Westphal [11]	Pfeiffer et al [13]	Rumenic et al [14]	Scheibenreiter and Frisch [15]	Schmid et al [19]	Stoll et al [17]	Yunis et al [18]	Wisniewski et al [19]	Present case
M	F	F	M	F	F	F	M	F
37y	3y	11y	5.5y	14y	2.2 y	46m	19m	⊞ 36m
	2420			2250	2310	1800	2460	2500
+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+
+	+			+	+	+	+	+
−	−	+	+	−	+	−	−	+
−	−	−	−	−	+	−	−	+
−	−	+	+	+	−	+	−	−
+	−	−	−	−	−	+	−	−
−	−	−	+	+	+	−	−	+
+	+	+	+	+	+	−	−	+
+	+	−	−	+	−	+	−	−
−	−	−	+	−	−	−	−	+

TABLE II

Cytogenetic data in patients with r(15)

	Material	Number of cells investigated					Size of r(15)
		all	with r(15)	without r(15)	with derivate	normal	
Emberger et al [1]	blood	151	138	13	0	0	variable
Forabosco et al [3]	blood	80	79	1	0	0	constant
Fryns et al 1. [4]	blood	41	39	0	0	2	constant
2.	blood	60	29	4	27	0	variable
Jacobsen et al [7]	blood	76	76	0	0	0	
Meinecke and Koske–Westphal [11]	blood	45	41	4	0	0	variable
Pfeiffer et al [13]	blood	50	30	0	0	20	variable
	fibro-blasts	50	26	0	0	24	
Rumenic et al [14]	blood	50	50	0	0	0	variable
Schmid et al [16]	blood	200	197	0	3	0	variable
Stoll et al [17]	blood	100	58	42	0	0	variable
Wisniewski et al [19]	blood	216	207	0	9	0	variable
Present case	blood	50	50	0	0	0	variable
	fibro-blasts	66	60	6	0	0	

ase. The variation in ring stability can then cause variations in the phenotype of the patient. It is not only the degree of the somato-mental retardation that varies from patient to patient, but the cranio-facial dysmorphism, too. Signs of dysmorphism such as a narrow forehead, mongoloid slants, hypertelorism, epicanthus, flattened nasal root, micro and retrognathia, etc., have been mentioned in some cases with r(15) but, except for the triangular face and small hands and feet with finger anomaly, none of them can be regarded as a characteristic dysmorphic symptom. Gross internal anomalies are not frequent. Out of 16 patients, 5 had heart malformations; in one case it was accompanied by renal anomaly. In the present case, the necropsy revealed a pulmonary anomaly.

Constancy of ring formation in 50–100 cells investigated in postnatal samples could hardly be a convincing proof against a mosaicism in the patient; it only means that no mosaicism could be detected. Therefore, it is not possible to evaluate the cytogenetic findings of the reported cases. (Table II.) It is, however, remarkable that mosaicism could be observed in many patients.

Another object of further studies is the dominance of females. Out of 16 patients, there were only 5

males. A female excess could be found in other chromosome aberrations, too, e.g. in the 4p and 5p deletion syndromes, but it seems that the sex ratio in the case of r(15) is much more overbalanced.

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Abnormal dermatoglyphics in absence of thumb

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Dermatoglyphics of six patients with absence of thumb are described. Two specific abnormal configurations were seen in the palmar area. In the first, there was no axial triradius and the course of the ridges in the proximal part of the palm was transversal. In the second, a peculiar distal loop on the radial border was present with a palmar triradius. These abnormal patterns are probably due to the absence of a thenar volar pad or the presence of an abnormal one when the ridges are formed.

Holt [4] found in the absence of the thumb a special dermatoglyphic pattern characterized by transversality of the ridges in the proximal part of the palm, without an axial triradius. We [1] observed a different abnormal pattern in the same anomaly; it consisted of an atypical large radial loop on the radial border of the palm with a triradius that could be interpreted as being the axial triradius. These two peculiar dermatoglyphic patterns seem to be specific in absence of the thumb and are probably due to two different mechanisms which produce the effect. To support this statement, the dermatoglyphic findings in six aetiologically different cases with no thumb are reported.

MATERIAL AND METHODS

The dermatoglyphics of six patients with absence of thumb were collected from among 3153 palm prints. Prints from the patients were made by the ink method and read with the aid of a stereoscopic microscope by the same observer. The methodology of Cummins and Midlo [2] and the International Nomenclature [6] were used to evaluate the prints. The six cases were unrelated and had the following clinical conditions.

Case 1. A 14-year-old boy, was born at term after an uncomplicated pregnancy. At the time of his birth the mother's age was 16 years and the father's age 22 years. In the family history no malformation occurred. He had bilateral absence of thumb and balanic hypospadias. His intelligence was normal.

Case 2. A 4-year-old boy was born with a shorter left upper extremity with a specially short forearm and limited extension of the elbow. The left hand was clubbed and no thumb was present. A protosystolic murmur was heard over the pulmonary area and an angiocardiographic study revealed a tetralogy of Fallot. Radiologically a shorter left radius and ulna with radioulnar synostosis were seen. He was diagnosed as having a Holt–Oram syndrome. The case was sporadic.

Case 3. A 12-year-old girl was born after uneventful pregnancy. At the time of her

birth, the mother was 35 years of age and the father was 47-year-old. She was the 8th child in a sibship of nine. She had a short stature (height, 117 cm) and a severe dorsolumbar kyphoscoliosis which had begun in the 1st year of life. Extension of the right elbow was limited and a very short right forearm with antecubital pterygium was present. Both hands were ectrodactylic. On the right hand the thumb was missing and syndactyly was present between the 4th and 5th fingers. On the left hand, the thumb was hypoplastic and there were only two contracted fingers. No heart defect was found and haematological studies were normal.

Radiologically, hemivertebrae and multiple anomalies of the ribs were seen. X-rays showed synostosis of metacarpals of the 4th and 5th fingers and no thumb on the right hand. On the left hand, a hypoplastic thumb with short metacarpal and phalanges were seen and only two fingers with their three corresponding phalanges. Her intelligence was normal. The anomalies of the vertebrae and ribs suggested a spondylocostal dysplasia.

Case 4. A 6-year-old boy was born at term after a normal pregnancy and normal delivery. The mother was 19 years old and the father 20 years old at the time of birth. He was the only child. He had microcephaly, heavy eyebrows, long curly lashes, short nose with anteverted nostrils, absent philtrum, small upper lip, fish mouth, low set ears, micrognathia, cleft palate with no uvula, bilateral ectrodactyly, the right hand had four brachydactylic fingers without a thumb and on the left hand there were only three short fingers with a hypoplastic thumb. The neck was short, the left nipple was inverted and the first right toe very small without a nail. Mental and motor development was retarded. He was diagnosed as having the Cornelia de Lange syndrome.

Case 5. A 3-year-old male was born after a normal 40 week pregnancy. The mother was 18 years old and the father 22 years old. He was the 2nd child. At birth, a left micromelia was found with the upper left extremity being much shorter than the right. The left hand was extremely small and there were only four very short fingers with no thumb. The mother had urinary infection and had taken chloramphenicol and phenobarbital during the first trimester of gestation. There was threatened abortion in the 2nd month of pregnancy. This case was sporadic.

Case 6. A 7-month-old female was the product of a seven months pregnancy and normal delivery. She was the 4th child of a mother of 27 years and the first child of a 24-year-old father. She had a short neck with right torticollis, hypoplasia of the sternocostal head of the right pectoralis major muscle, ipsilateral absence of the thumb, right hypoplastic nipple, slight pectus excavatum and dorsolumbar scoliosis. A systolic cardiac murmur and diminution of breath sounds were found over the right hemithorax. A bronchographic study showed agenesis of the right lung. The angiocardiogram revealed a ventricular septal defect. X-rays showed cervicodorsal hemivertebrae, absence of the first right rib, the second rib was bifid and situated transversally. She had also malrotation of the intestine with the stomach on the right side. This case was also sporadic.

Results

The dermatoglyphics of these patients showed the following pattern.

Case 1. Left hand, digital patterns: –UUUA; on the palm a distal axial triradius t''. Main lines terminations: 10. X.6.1. An abnormal large radial loop opens on the radial border of the palm. Right hand: digital patterns: –UUUU; On the palm, a distal triradius t''. Main lines terminations: 11.9.7.5'. A large radial loop is present on the radial side of the palm.

Case 2. Left hand: digital patterns: –UWUW. On the proximal part of the palm, there is a triradius t', most radially situated. Main lines terminations: 7.5''.3.3. Termination of the main line B is infrequent in position 3.

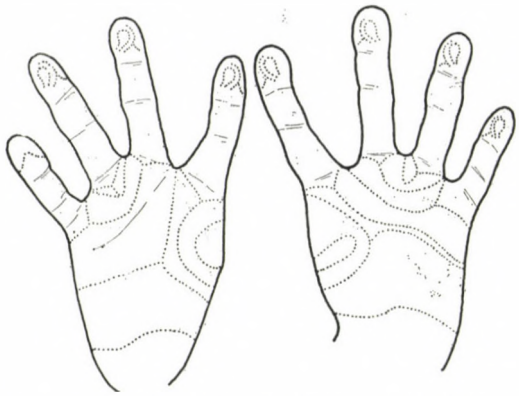

FIG 1. Case 1. An abnormal distal radial loop is present in the thenar area with a distal triradius on both palms, with bilateral absence of thumb

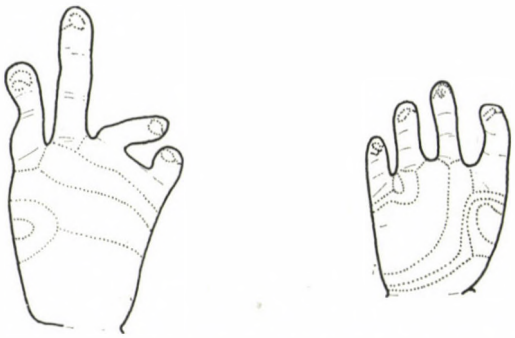

FIG. 2. On the right the same abnormal configuration is seen as in Case 1. The same pattern appears on the left palm of Case 2 and on the right palm of Case 3

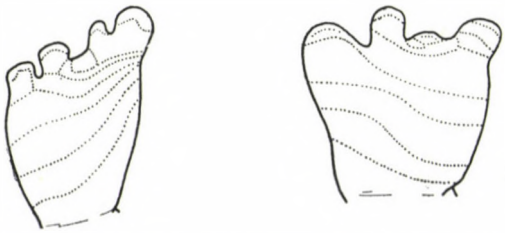

FIG. 3. Grossly malformed hands of Case 4. on the right and Case 5 on the left side. There is no axial triradius on the palms and there is a transverse course of the ridges on the proximal part

A large radial loop similar to those described in the first patient, opens in the radial side of the palm.

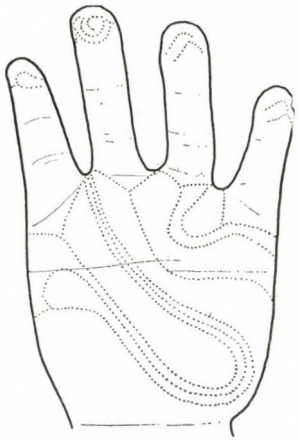

FIG. 4. Case 6. Right hand with absence of thumb. On the radial border of the palm, there is a distal triradius with a carpal loop and a distal loop

Case 3. Right hand: digital patterns: –WUUR; on the palm, there is a radially situated distal triradius t''. Main lines terminations: Oid0.5''.5''. A large radial loop is seen on the radial border. Triradii c and d are absent.

Case 4. Right hand: digital patterns: –AAAA; there is no axial triradius on the palm and the ridges have a transversal course. Only two main lines are seen, D and C. Main lines terminations: 11.9.0.0.

Case 5. Left hand: digital patterns: –AAAA, there is no axial triradius and the ridges have a transversal course across the proximal part of the palm. There are two main lines C and D and a zygodactylous pattern ab. The main line C and main line D, end in 13 and AB in 9.

Case 6. Right hand: digital patterns: –UWUU, there is a distal triradius t' on the radial border of the palm and a complex true pattern, a carpal loop with a distal loop, radially situated on the palm. Main lines terminations are: 7.5''.5'.11. The main line A ends in position 11 and forms the distal loop. There is no axial triradius.

DISCUSSION

The dermatoglyphics of these six patients with no thumb showed clearly two specific abnormal configurations. The pattern reported by Holt [4] was present in cases 4 and 5; the other, described by us [1], was seen in cases 1, 2 and 3. In one case the pattern was different, as there was a distal triradius on the radial border of the palm. Herrmann et al [3] reported two patients with no thumb; in one of them the first abnormal pattern was present and in the other the second configuration.

Patients 4 and 5 had very short fingers with arches, a fact observed by Holt [5] in brachydactyly.

Holt [4] suggested that the abnormal palmar configuration with no triradius and transversal alignment of the ridges on the proximal part of the palm with absence of thumb were due to an absence of a volar thenar pad, at the time of ridge formation. We think that the other abnormal pattern, when there is no thumb, is the result of a different intrauterine defect. It is possible that there was a thenar volar pad at the time of ridge formation, but an abnormal one.

The two patients reported by Herrmann et al [3] were sibs and autosomal recessive inheritance was suggested as the cause. The cases described by Holt [4] and our patients had, however, different entities and it seems that these two different abnormal patterns in absence of the thumb are related with the fetal hand morphology, affected in the same way by different aetiological agents.

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Antenatally diagnosed thanatophoric dysplasia

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A case of antenatally diagnosed thanatophoric dysplasia is described. Other syndromes accompanied by chondrodysplastic tetramicromelia were excluded and diagnosis was based on the narrow thorax, secondary pulmonary hypoplasia and macrocephaly detected by ultrasound and on radiological findings of disturbed bone formation. At the mother's request labour was induced and radiological, anatomic and histological examination of the newborn confirmed the prenatal diagnosis. On basis of the literature, the possible aetiology of the disease is discussed and autosomal recessive heredity is suggested. Attention is focussed on the significant hydramnios which led to the suspicion of fetal malformation. The importance in pregnancy of routine ultrasound screening is emphasized, since such malformations can be detected as early as in midtrimester pregnancy.

Thanatophoric dwarfism or dysplasia is one of the twenty-one basic types of osteochondrodysplasia detectable in the newborn period. It has been suggested that instead of the term dwarfism which seemed somewhat offending to the patients or their families, dysplasia should be used [16]. Thanatophoric dysplasia was distinguished from other types of congenital short-limb conditions, especially from classical achondroplasia [10]. The name is derived from the Greek thanatophoros meaning death-bearing because the affected infants die a few hours or days after birth.

The characteristics of the disease are a short stature with markedly shortened extremities (tetramicromelia) and relatively normal trunk length, a narrow thorax with hypoplastic lungs. Respiratory distress which may be caused by the chest deformity, is a prominent feature and so are the comparatively large head (macrocephaly) with depressed nasal bridge, and death in early infancy. Reviewing the literature we found that despite the classical X-ray signs only one case was correctly identified prenatally. This was a thanatophoric dwarf born after another pregnancy that directed attention to the possibility of recurrence, also combined with hydramnios [5]. "Prenatal diagnosis" in the title of publications accessible to us proved to refer to X-rays evaluated retrospectively in the postnatal period [1, 4, 9, 13, 18, 20]. The malformation may not be suspected prenatally due to its rarity, and may be difficult to differentiate from other types of chondrodysplasia.

Radiologically only achondroplasia has been diagnosed in utero, and prenatal diagnosis of this condition

compatible with life has not influenced obstetric management. Therefore we feel justified in reporting a case of thanatophoric dysplasia, a lethal condition, diagnosed prenatally by ultrasound and amniofetography.

Report of a Case

A 23 year old woman, with one healthy child, was admitted at 30 weeks gestation with hydramnios and poor fetal movements. The husband was 25 years old.

There was no family history of dwarfism, no consanguinity nor any history of exposure to teratogenic agents. On examination the uterus was large for dates, and marked hydramnios was present. Ultrasound examination showed a single fetus with 7.5 cm biparietal diameter (mean for 30 weeks gestation = 7.0 cm, range 6.4–7.8 cm), no dilation of the cerabral ventricles, a protuberant fetal abdomen containing a full bladder, and extreme hydramnios.

Transabdominal amniocentesis yielded 400 ml clear fluid containing 2.5 μg/ml (normal) alphafetoprotein. The morphological characteristics and percentile distribution of non-cultured amniotic fluid cells were normal, thus excluding a neural tube defect. The lecithin/sphingomyelin ratio was very low (0.8).

Repeated ultrasound examinations showed remarkably short limbs larger than normal in diameter, extending away from the trunk in central position (Fig. 1). The thorax was depressed, its contours enlarging in cone shape towards the protuberant abdomen (Fig. 2). The abdominal diameters were markedly larger than the thoracic ones. At each examination the fetus was in the supine position.

Amniofetography: following an abdominal X-ray, transabdominal amniocentesis was performed and 10 ml of Lipiodol (lipid-soluble) and 10 ml of 60 % Uromiro (water-soluble) radiopaque dye was injected and 8 and 24 h later X-rays were taken repeatedly. They showed shortened, slightly bowed and broad long tubular bones. The metaphyses were irregular and cupped. The skin of the extremities was folded like an accordion and the many folds could well be discerned by the lipo-soluble contrast medium adhering to the vernix caseosa. No discontinuity of the skin was detected. The skull, though somewhat larger than normal, had a regular bone structure with a protruding forehead and a depressed nasal bridge. The thorax was narrow and the ribs were short. The vertebral bodies were small in vertical diameter and their posterior part was well ossified. The iliac diagonal diameter was larger than usual, the pubic bone and the ischium were broad and short. 24-hour X-rays revealed no obstruction of the gastrointestinal tract, so there was no sign of atresia (Fig. 3).

On the basis of the above findings, the intrauterine fetus was diagnosed as a thanatophoric dwarf. Chondrodysplasia, tetramicromelia, brachydactyly, macrocephaly without hydro-

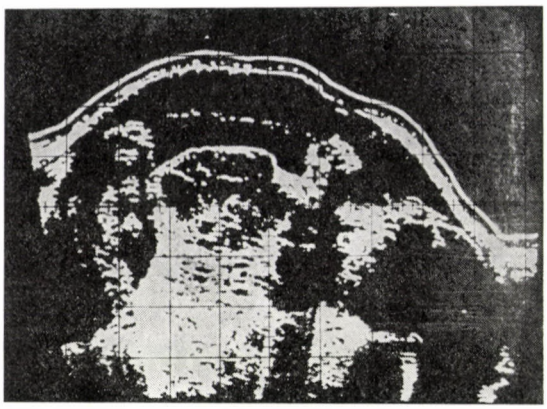

FIG. 1. Ultrasound scan showing the thick limbs and the large head

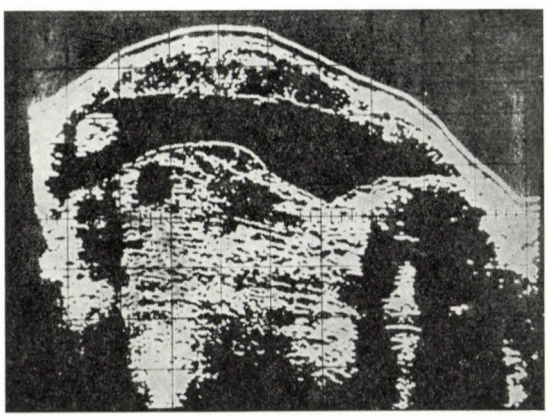

FIG. 2. The narrow thorax assumes a cone shape toward the protuberant abdomen

cephalus and the narrow thorax were the positive diagnostic features. Although prenatal examinations showed a lethal condition, the pregnancy could not be terminated at that time. Two weeks later, however, despite repeated removal of excess amniotic fluid, labour was induced on the basis of the mother's cardiovascular and psychological condition. A female infant weighing 2050 g was delivered; she died of respiratory and cardiovascular failure 12 h after birth (Fig. 4). At post mortem the placenta weighed 500 g and showed no gross change. The head was disproportionally large, with a protruding forehead, a depressed nasal bridge and a large tongue. The thorax was narrow and dilated in cone shape towards the protruding abdomen. The lungs were hypoplastic with patchy areas of atelectasis. The left lung had 3 lobes. The right heart ventricle was dilated. The umbilical cord contained 3 vessels. The tubular bones were all short



FIG. 3. X-rays taken 24 h after injection of contrast medium into the amniotic cavity



FIG. 4. The newborn infant with short hydropic limbs, protuberant abdomen and depressed thorax

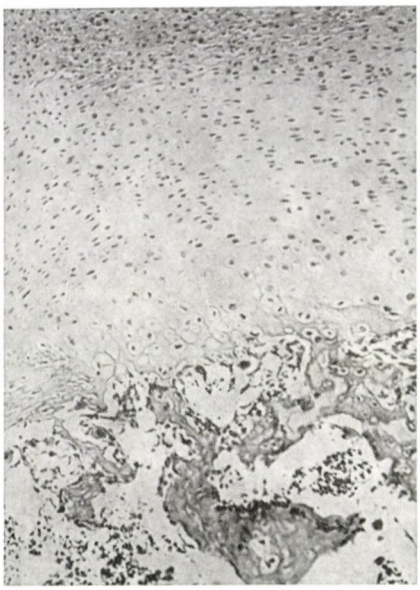

FIG. 5. Irregular columnarization of chondrocytes in the epiphyseal growth zone of the humerus

and slightly bowed, the skin of the limbs displayed many deep folds. Histological examination showed that the basic defect was a disturbance of endochondral ossification. In the epiphyseal growth zone the columnarization of chondrocytes was irregular (Fig. 5). The cartilage matrix was irregularly calcified, the number of bone trabeculae was small and they did not run parallel with the longitudinal axis of the bone as in a normal epiphysis. Periosteal bone formation was well developed. On the proximal epiphyseal side of the humerus the periosteal connective tissues extended over the epiphyseal plate to the cartilage. Electron microscopically, most of the resting chondrocytes resembled the principal cells of normal hyaline cartilage [7].

The majority of vertebral bodies consisted of hyaline cartilage but the ossification centre could be seen and ossification developed well. The skull showed a normal periosteal bone formation and the cartilage of the ribs, larynx, trachea and bronchi displayed a regular hyaline cartilage structure. In the lungs partial atelectasis and passive hyperaemia were noted. The heart, kidneys, spleen and thymus were normal for gestational age. In the liver extramedullary haematopoiesis was seen. Microscopic findings excluded the two types of chondrodysplasia since in achondroplasia the epiphyseal cartilage is well developed and regular [17], while in achondrogenesis the principal cells of the hyaline cartilage are absent thus preventing the formation of a

growth plate [8]. Death was due to acute dilatation of the right ventricle in the postnatal period resulting from an extreme pulmonary hypoplasia.

Discussion

The aetiology of thanatophoric dwarfism has not been clarified. The majority of the reported cases were males [6]. In the present case the neonate was a female. The shift in sex ratio could be explained by assuming that most female fetuses die in the early prenatal period rather than by supposing a sex-linked inheritance. Some authors argue that, as in classical achondroplasia, an autosomal dominant mutation would be responsible for the condition [15] and in support of this theory is the usually advanced paternal age [22]. Still, several authors oppose this view [6].

Discordant twins have been described and these are against the aetiologic role of external factors [11]. Pena and Goodman [15] reviewed the cases reported in the literature and concluded that polygenic inheritance was the most likely mechanism and suggested an empiric recurrence risk in sibs of 2%. As expected, no chromosomal aberration could be detected.

The most probable mechanism, at least in the great majority of cases, seems to be an autosomal recessive inheritance, and this has been suggested by the familial occurrence [2, 5, 6, 14, 15, 19]. If this is accepted, the risk of recurrence of the disease is 25%. This risk was considered lower by some authors [11] but they estimated the predisposition to spontaneous abortion to be higher. This was based on the high proportion of abortions occurring in the gestational history [2, 15].

The use of prenatal diagnostic methods is justified (i) in the case of pregnancy following the birth of an affected child; (ii) when hydramnios suggests a fetal malformation; and (iii) when a reduction of normal fetal movements suggests an abnormality of the limbs.

Hydramnios was observed in 70% of the reported cases [5, 12, 22], although the association has not been fully explained. Defective swallowing by the malformed fetus has also been reported [22]. In our case, the quantity of contrast medium swallowed and its progress through the gastrointestinal tract were monitored, and no important difference could be seen compared with our previous experience with amniofetography. An alternative explanation would be the pulmonary hypoplasia, since the fetal bronchopulmonary circulation has an important role in the circulation of amniotic fluid, affecting both its formation and resorption.

The maternal appreciation of reduced fetal movements might have been due to the hydramnios or to the abnormal extremities. In the absence of real-time ultrasound, fetal movement could not be analysed reliably, but it was remarkable at each examination to find the fetus in exactly

the same position with the extremities leaning against the uterine wall, and the thick bones fixed in an abducted position.

In differential diagnosis, several diseases had to be excluded, since most types of chondrodysplasia with tetramicromelia are compatible with life up to two decades. Heterozygous achondroplasia shows similar osseous changes but they are less severe than those found in thanatophoric dysplasia, only the vertebral bodies are not so flat and the tubular bones not so short, curved and cupped. Homozygous achondroplasia is nearly as severe as thanatophoric dysplasia, but can be excluded on the basis of the parents' bone structure. In both types of achondroplasia the trunk is very short, the ribs are short and horizontal, and there is impairment or complete absence of ossification in the vertebral bodies and tubular bones, thus making the differentiation comparatively easy. In severe hypophosphatasia ossification of the skull may not take place, so a proper calcification of the skull excludes this possibility. Asphyxiating thoracic dysplasia (Jeune syndrome) was excluded by the macrocephaly and the severe dysplasia of the vertebrae and the tubular bones. Chondroectodermal dysplasia (Ellis—van Creveld syndrome), Majewski and Saldino–Noonan type chondroplasias can be excluded by the absence of postaxial polydactyly. Metatrophic dwarfism could be excluded by the normal length of the thorax and limbs. Pathologic head size excludes chondrodysplasia punctata and diastrophic dwarfism.

Differentiation between the different types of dwarfism depends on the exact knowledge of the differences existing between them. Subtle radiological details are of extreme importance in diagnosis. At present, therefore, ultrasound and X-rays play the main roles in the diagnosis of dwarfism. The ultrasonic findings which led to further investigation were a large head, small thorax, protruding abdomen and short extremities. Abnormalities of ossification revealed by X-rays confirmed the exact type of chondrodysplasia. The prenatal diagnosis was further supported by postnatal X-rays and the ossification disturbances characteristic of thanatophoric dysplasia revealed by necropsy and proved by the histological examination of the epiphyseal cartilage. This is important since in contrast with previous suppositions [21] the differences in bone formation in achondrogenesis, thanatophoric dysplasia and achondroplasia are not exclusively qualitative but also quantitative [3].

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Congenital postural deformity association

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The so-called congenital postural deformities were evaluated in the material of the Hungarian Congenital Malformation Registry, 1970–1976. Seven categories of postural deformities: dislocation of the hip, clubfoot, torticollis, musculoskeletal, other limb, face-nose and auricular were separated. The occurrence of the combination of dislocation of the hip, clubfoot and torticollis is manifold of random combination. These three postural deformities, however, are not associated more often with other nonpostural type abnormalities. Thus the congenital postural deformity association composed of two or more combinations of dislocation of the hip, clubfoot and torticollis without other major congenital abnormalities is treated as a provisional entity. Its birth prevalence is 0.4 per 1000 total births thus after Down syndrome it is the second common type of multiple congenital abnormalities. The aetiology and its higher sib-occurrence are explained by intrauterine maternal moulding factors.

Recently some new classifications of congenital abnormalities have been suggested (Fig. 1). The so-called deformations or deformities arise in late fetal life and they are alterations in the shape or structure of a previously normally formed part of the body [19]. The great majority of deformations involves the musculoskeletal system; they are called congenital postural deformities [11–17]. The ancient hypothesis of Hippocrates of these deformities being caused by mechanical factors acting during fetal life was confirmed by many authors [1, 2, 3, 4, 11–17, 20]. The highly significant phenotypic combination among congenital postural deformities is well known; it is explained by their common mechanical origin. Our point is how to classify and register the combinations of congenital postural deformities. If they have a common aetiology then they represent a nosological entity and so a separate registration of each congenital postural deformity would be misleading. The purpose of this study was to define the association of congenital postural deformities on the basis of the material of the Hungarian Congenital Malformation Registry (HCMR) 1976, involving 38,023 births with congenital abnormality for 1,188,529 total births [8].

BASELINE DATA

As a first step, all index patients with congenital postural deformities were selected from the material o

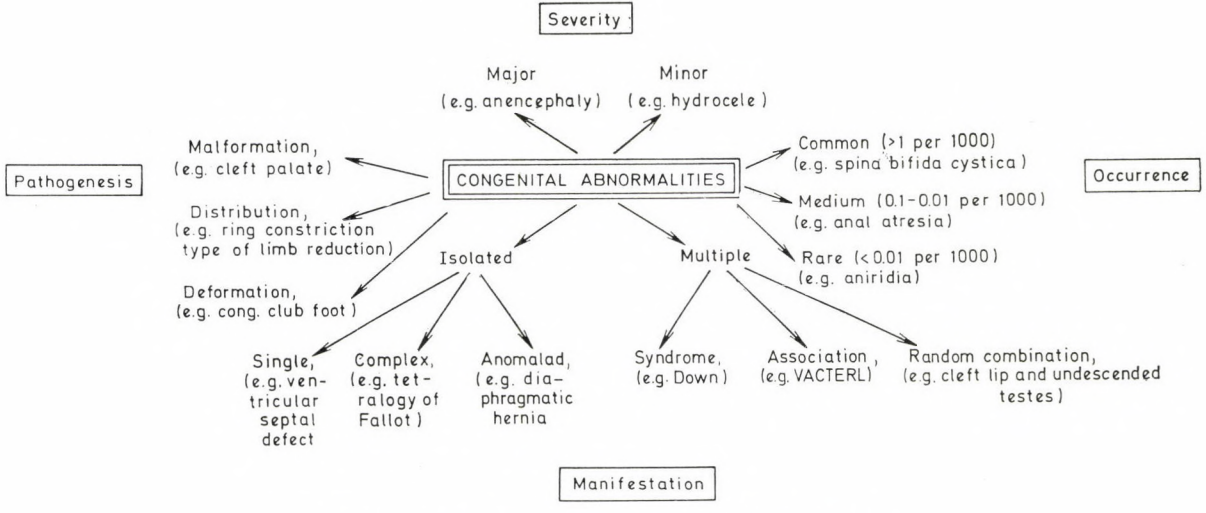

FIG. 1. Classification of congenital abnormalities

TABLE I

List of congenital postural deformities (modified after Dunn (17)) and their number in the material of the HCMR, 1970–1976

Part of body	Congenital postural deformities	No. of index patients
Skull	Dolichocephaly	0
	Plagiocephaly	0
	Depressions in skull	4
	"Skull deformities"	20
Face	Nasal and oral deformities	4
	Mandibular asymmetry	6
	Retrognathia	36
	Auricular deformities	351
	"Facial deformities"	116
Neck	Sternomastoid contracture; torticollis	399
Upper limb	Dislocation of shoulder	0
	Clubhand	1
	"Upper limb deformities"	39
Trunk	Pigeon chest	2
	Pectus excavatum	44
	Postural scoliosis	2
	"Trunk deformities"	184
Lower limb	Dislocation of hip	7760
	Bowing of long bones	1
	Genu recurvatum	2
	Talipes equinovarus	2437
	Talipes calcaneovalgus	103
	Metatarsus varus	2
	"Lower limb deformities"	88
Whole	Arthrogryposis multiple, congenital	21

the HCMR, 1970–1976. The list of congenital postural deformities and the number of index patients with each deformity are shown in Table I. On account of inaccurate notifications, groups of unspecified skull, facial, trunk and limb "deformities" had to be set up. Potter's syndrome, Potter's facies and Potter's hand were excluded because Potter's syn-

TABLE II

Distribution of congenital postural deformities within the categories studied

Category of postural deformity		Abbreviation		Distribution of number of postural deformities					
				1	2	3	4	5–7	Total
*D*islocation of *h*ip		DH	No.	7,035	638	68	12	7	7,760
			per cent	90.6	8.2	0.9	0.2	0.1	100.0
*C*lub *f*oot	talipes equinovarus talipes calcaneovalgus metatarsus varus	CF	No.	1,955	435	101	28	23	2,542
			per cent	76.9	17.1	4.0	1.1	0.9	100.0
*T*orticollis		TC	No.	146	211	35	5	2	399
			per cent	36.6	52.9	8.8	1.2	0.5	100.0
*M*usculoskeletal	All skull deformities All trunk deformities	MS	No.	133	66	33	12	12	256
			per cent	51.9	25.8	12.9	4.7	4.7	100.0
*O*ther *l*imb	All upper limb deformities All lower limb deformities excluded DH and CF	OL	No.	91	27	8	3	2	131
			per cent	69.5	20.6	6.1	2.3	1.5	100.0
*F*ace-*n*ose	All face deformities excluded AU	FN	No.	67	34	30	16	15	162
			per cent	41.4	21.0	18.5	9.9	9.2	100.0
*Au*ricular		AU	No.	225	45	36	27	18	351
			per cent	64.1	12.8	10.3	7.7	5.1	100.0
		Total	No.	9,652	1,456	311	103	79	11,601
			per cent	83.2	12.5	2.7	0.9	0.7	100.0

drome is caused by renal agenesis and the above-mentioned deformities are secondary consequences.

There are three shortcomings of these data from the point of view of registration.

1. Congenital postural deformities frequently occur in specified syndromes of known aetiology (e.g. fetal alcohol syndrome) or unknown aetiology (e.g. caudal regression), but in general they are not mentioned separately. Thus, specified syndromes were excluded. Multiple congenital arthrogryposis as a well-known clinical entity was not evaluated, either.

2. Notification is far from complete in most cases of congenital postural deformity.

3. Congenital postural deformities show a wide spectrum of severity in clinical manifestation, thus it is difficult to differentiate between normal variation and minor anomalies and between minor anomalies and major abnormalities.

Owing to the low number of certain kinds of cases and the inaccuracy of diagnosis, some types and groups of congenital postural deformity were drawn together and only 7 categories were evaluated (Table II).

Classification

The rates of isolated (one) and multiple (two or more combinations) congenital postural deformities showed a significant deviation from each other ($\chi^2_6 = 14.92$; $p \ll 0.001$) in the studied categories (Table II). Furthermore, the distribution of associated other congenital abnormalities (two, three, four and five-seven) also differed significantly ($\chi^2_{18} = 342$; $p \ll 0.001$). Thus these categories of congenital postural deformity do not seem to be homogeneous.

Table III shows the number of index patients affected by multiple congenital abnormalities, i.e. a concurrence of two or more different major congenital abnormalities in the same individual [10] and the distribution of congenital postural deformities in index patients having two or three and more congenital abnormalities.

In an effort to increase phenotypic and perhaps aetiologic homogeneity, the index patients were divided into two groups.

Group 1 consisted of 519 index patients who were not known to have other congenital abnormalities. 490 of them had two congenital postural deformities. Their distribution is shown in Table III below the diagonal. 27 index patients had three congenital postural deformities, their distribution was as follows:

DH–CF–TC	23
DH–CF–AU	2
CF–MS–OL	1
CF–FN–AU	1

Finally 1 index patient each had four (DH–CF–TC–OL) and five (DH–CF–MS–FN–AU) congenital postural deformities.

Group 2 consisted of 567 subjects who had postural and non-postural type congenital abnormalities. Within

TABLE III

Number of index patients affected by two (below the diagonal) and at least three (above the diagonal) congenital abnormalities

Postural deformities	DH	CF	TC	MS	OL	FN	AU	Total
DH		48	37	4	1	2	9	101
CF	244		28	6	5	12	12	63
TC	189	19		1	1	1	3	6
MS	8	7	0		3	14	5	22
OL	7	6	0	1		0	1	1
FN	1	2	0	0	0		15	15
AU	2	2	0	2	0	0		(208)
Total	451	36	0	3	0	0	(490)	—

this group 81 index patients had two congenital postural deformities, in the following distribution:

DH–TC	9	CF–TC	2	TC–MS	1
DH–CF	19	CF–OL	2	OL–MS	1
DH–MS	3	CF–MS	3	OL–AU	1
DH–AU	3	CF–FN	8	MS–FN	12
		CF–AU	6	MS–AU	3
				FN–AU	8

Ten index patients had three congenital postural deformities:

DH–CF–TC	2	CF–MS–OL	1
DH–TC–AU	2	CF–FN–AU	2
DH–FN–AU	1		
FN–AU–MS	1		
FN–AU–TC	1		

The 476 cases with two combinations of single congenital postural deformities and non-postural type congenital abnormalities are summarized in Table IV. In comparison to the observed and expected number of different groups of non-postural type abnormalities their occurrence might be explained by chance, i.e. they were random combinations. The expected number of a congenital abnormality is $\frac{p_i \cdot 476}{\Sigma p_i}$, where p_i is the true birth prevalence of the congenital abnormality. Thus it seemed wortwhile to exclude the index patients affected by a combination of postural and nonpostural type abnormalities.

ANALYSIS

The first task was to confirm the non-random combinations of congenital postural deformities. The principles of stochastic syntropology [26, 21] were adopted (Table V). All syntropy indexes were significantly exceeding 1.0 showing that they were not random combinations. This approach was not, however, amenable for our purpose because almost all

TABLE IV

Combinations of single congenital postural deformities with one non-postural type congenital abnormality

Congenital abnormality	DH	CF	TC	MS	FN	OL	AU	Total observed number	Expected number	χ^2
Cleft palate	2	8	0	2	5	2	2	21	4.2	11.5*
Syndactyly	2	22	0	1	2	0	1	28	5.2	16.2*
Microcephaly	6	5	0	2	2	2	3	20	2.1	14.8*
Cataract	1	1	0	1	0	0	1	4	1.0	1.8
Inguinal hernia	65	8	2	6	2	0	1	84	119.5	7.8*
Pyloric stenosis	2	2	0	0	0	0	0	4	15.7	7.1*
Anencephaly	2	0	0	0	0	0	0	2	10.5	5.8*
Encephalocele	2	1	0	0	1	0	0	4	2.1	0.6
Spina bifida	0	0	0	4	1	3	3	11	14.7	0.6
Cleft lip ± palate	9	17	0	0	0	0	4	30	10.5	9.8*
Limb reduction	3	2	0	0	2	0	1	8	4.2	1.2
Polydactyly	7	12	0	2	1	0	1	23	5.2	11.6*
Omphalocele	0	5	0	1	0	2	1	9	2.1	4.3*
Oesophageal atresia	0	1	0	0	0	0	0	1	2.1	0.4
Anal atresia	2	2	0	0	1	0	1	6	2.1	1.8
An-microphthalmia	1	0	0	2	0	0	1	4	1.0	1.8
Other eye	3	0	0	0	0	0	0	3	1.0	1.0
Heart-great vessels	34	18	1	14	6	2	12	87	104.8	2.1
Hypospadias	7	11	0	3	3	0	2	26	2.1	20.9*
External genitalia	1	1	0	0	0	0	0	2	1.0	0.3
Diaphragmatic hernia	1	5	0	0	0	0	0	6	2.1	1.8
Other digestive	3	1	0	0	0	0	0	4	1.0	1.8
Undescended testis	18	10	0	1	1	0	2	32	141.5	84.5*
Other urogenital	2	2	0	0	0	1	0	5	2.1	1.2
Renal agenesis	0	2	0	0	0	1	0	3	2.1	0.2
Cystic kidney	1	4	0	1	0	0	0	6	1.0	3.6
Branchial	0	0	0	0	0	0	0	0	1.0	—
Respiratory	1	2	0	0	1	0	0	4	1.0	1.8
Other nervous	1	0	0	0	0	0	0	1	1.0	—
Intestinal atresia	2	1	0	0	0	0	0	3	1.0	1.0
Hydrocephalus	7	11	0	7	3	0	3	31	7.3	15.3*
Endocrine + spleen	0	0	0	1	0	0	0	1	1.0	—
Cong. tumours + skin + others	2	1	0	0	0	0	0	3	2.1	0.2
Total	187	155	3	48	31	13	39	476	475.6	

* = $p < 0.05$

congenital abnormalities have a syntropy index significantly greater than 1.0 within the multiple congenital abnormalities [9]. Our O-hypothesis was based on the equality of two conditional probabilities:

$$P(A/B) = P(A/C)$$

where B involves 7 categories of congenital postural deformity studied, C represents all the 33 groups of non-postural type congenital abnormalities, while A is the selected group of just evaluated congenital abnormalities. The O-hypothesis means that the selected group of evaluated con-

genital abnormalities associates on an equal probability with congenital postural deformities and with non-postural type congenital abnormalities. The calculation was based on the following 2×2 contingency tables

1	2	3
4	5	6
7	8	9

where 1 = number of index patients having one or more B abnormalities and A abnormality

2 = number of index patients having exclusively one or more B abnormalities

4 = number of index patients having one or more C abnormalities and A abnormality

5 = number of index patients having exclusively one or more C abnormalities

Thus

$$P(A/B) \sim \frac{1}{3} = P_1;$$

$$P(A/C) \sim \frac{4}{6} = P_2$$

The O-hypothesis was tested by the χ^2 test. First each category of congenital postural deformity was selected and studied as to whether it associated with the other 6 categories of congenital postural deformity with a significantly higher probability than with other groups of non-postural type abnormalities. Furthermore, each non-postural type abnormality group was analysed as to whether it associated more frequently with 7 postural deformity categories than the remaining non-postural type abnormality groups. The value of the χ^2 test may have three meanings (Table VI):

p_1 = The O-hypothesis is refused: there is a positive correlation between the occurrence of A abnormality and of the congenital postural deformities studied;

p = The O-hypothesis is valid; the combination between the A abnormality and the stu-

TABLE V

Values of stochastic syntropology in the combinations of three more common congenital postural deformities without other congenital abnormalities. The "true" and registered birth prevalence of DH are 28.0 and 5.9; of CF they are 1.3 and 1.6; of TC they are unknown and 0.1. The true birth prevalences were determined by the help of specific epidemiological approaches

Combinations	Expected number	Observed number	Syntropy index	χ_1^2
DH + CF	11.2	244	21.7	212.4
DH + TC	0.7	189	269.5	186.9
CF + TC	0.2	19	99.9	18.4
DH + CF + TC	0.001	23	20,499.6	22.9

died congenital postural deformities is due to chance;

p_2 = The O-hypothesis is refused: there is a negative correlation between the occurrence of the A abnormality and the studied congenital postural deformities.

TABLE VI

Rates of combinations among postural and non-postural abnormalities

Congenital abnormality (A)	p_1	p_2	χ_1^2	Positive correlation p_1	Random combination p	Negative correlation p_2
* Postural-type deformity categories						
DH	0.152	0.009	2149.8	< 0.0001	—	—
CF	0.038	0.010	255.2	< 0.0001	—	—
TC	0.023	0.0002	499.5	< 0.0001	—	—
OL	0.0016	0.0009	3.4	—	> 0.05	—
MS	0.002	0.0035	5.3	—	—	< 0.05
FN	0.0005	0.0026	15.6	—	—	< 0.001
AU	0.0010	0.0040	19.7	—	—	< 0.001
** Non-postural type abnormality groups						
Anencephaly	0.0002	0.0063	59.4	—	—	< 0.001
Encephalocele	0.0004	0.0029	20.6	—	—	< 0.001
Spina bifida	0.0013	0.0072	46.0	—	—	< 0.001
Hydrocephalus	0.0033	0.0069	15.5	—	—	< 0.001
Microcephalus	0.0022	0.003	1.6	—	> 0.10	—
Other nervous	too few cases					
Cleft lip ± palate	0.0032	0.0107	46.7	—	—	< 0.001
An-microphthalmia	0.0004	0.0015	78.0	—	—	< 0.01
Cleft palate	0.0023	0.0035	3.4	—	> 0.05	—
Cataract	0.0004	0.001	3.1	—	> 0.05	—
Other eye	0.0004	0.0012	5.0	—	—	< 0.05
Branchial	too few cases					
Respiratory	0.0004	0.003	21.4	—	—	< 0.001
Oesophageal atresia	0.0001	0.0035	32.9	—	—	< 0.001
Pyloric stenosis	0.0004	0.0009	2.6	—	> 0.05	—
Intestinal atresia	0.0003	0.0013	7.4	—	—	< 0.01
Anal atresia	0.0006	0.0051	38.8	—	—	< 0.001
Other digestive	0.0004	0.0036	27.6	—	—	< 0.001
Renal agenesis	0.0003	0.0025	19.2	—	—	< 0.001
Cystic kidney	0.0009	0.0027	11.0	—	—	< 0.01
Other urogenital	0.0006	0.0063	50.7	—	—	< 0.001
External genitalia	0.0002	0.0016	11.7	—	—	< 0.001
Hypospadias	0.0026	0.0082	33.5	—	—	< 0.001
Undescended testis	0.0032	0.0074	20.0	—	—	< 0.001
Heart-great vessels	0.009	0.036	185.4	—	—	< 0.0001
Diaphragmatic hernia	0.0006	0.0044	31.5	—	—	< 0.001
Omphalocele	0.0009	0.0049	30.9	—	—	< 0.001
Inguinal hernia	0.0099	0.0089	0.7	—	> 0.10	—
Limb reduction	0.0008	0.0043	26.8	—	—	< 0.001
Polydactyly	0.0024	0.0061	19.9	—	—	< 0.001
Syndactyly	0.0028	0.0025	0.3	—	> 0.10	—
Endocrine + spleen	0.0001	0.002	18.0	—	—	< 0.001
Cong. tumours + skin + others	0.0003	0.001	4.1	—	—	< 0.05

The rates of combinations of DH, CF and TC with the studied congenital postural deformities are significantly higher than those of their combinations with other non-postural type abnormality groups (Table VI).

OL did not show a significantly higher probability. Furthermore, MS, FN and AU associated significantly less frequently with the studied congenital postural deformities.

All but 6 groups of non-postural type congenital abnormality were significantly less often associated with the studied congenital postural deformities.

The combination of the abovementioned 6 non-postural type abnormality groups with studied congenital postural deformities is due to chance i.e. they are random combinations.

Our results partly confirm previous data about the frequent combinations of postural-type deformities partly disprove them. On the one hand the occurrence of combinations of DH, CF and TC is a manifold of the random combination. These congenital postural deformities involve 92.2% and 79.3% of two and of three or more combinations of congenital postural deformities, respectively. On the other hand the other suggested categories of congenital postural deformities have not a higher predisposition for a combination with each other. Of course, our calculation was based on data registered without any clinical control. The validity of the diagnosis would be important in these types of congenital abnormalities thus we cannot exclude the possibility that the majority of our index patients had in fact no postural-type abnormality.

Dunn [15, 16] found a significant clinical association of DH with CF and of CF with TC but not of DH with TC. He stated that congenital postural deformities are caused by intrauterine moulding and showed that congenital postural deformities not only occur in association with each other but also share a number of pregnancy characteristics including primigravidity, maternal hypertension, oligohydramnios, breech presentation and fetal growth retardation.

The point is that the congenital postural deformity association (CPDA) composed of two or more combinations of DH, CF and TC without other major congenital abnormalities would be treated as a provisional entity. ("Association" is a recognized and characteristic pattern of abnormality combinations.)

475 index patients with CPDA were found in the material of the HCMR, 1970–1976. Their distribution was DH + CF: 244 (0.21 per 1000 births), DH + TC: 189 (0.16 per 1000 births), CF + TC: 19 (0.02 per 1000 births), DH + CF + TC: 23 (0.02 per 1000 births). This means a total birth prevalence of CPDA of 0.4 per 1000 total births. Its participation was 1.25% within all births with congenital abnormality and 9.6% with multiple congenital abnormalities. Thus, after Down syndrome, CPDA is the second common type of multiple congenital abnormality.

Some epidemiological characteristics

Previously, some important epidemiological characteristics connected with the aetiology were observed e.g. a higher rate of first pregnancy, breech presentation [15, 17] and of twinning [23]. They have been confirmed by our material. Here only three further epidemiological variables will be discussed.

Sex ratio. Knowing the sex ratio of isolated congenital postural deformities, the so-called expected sex ratio could be calculated for the different combinations of CPDA. The comparison of expected and observed values did not show a significant deviation (Table VII).

Seasonality of births with CPDA. A significant deviation from the annual average of monthly birth numbers was found by the methods of Edwards [18] and Hewitt et al [22] (Fig. 2). The maximum occurs in October to December, while the minimum was found between April and July. It partly corresponded to the well-known seasonal distribution of births with DH in Hungary [5] having a maximum between November and February and a minimum in May–June. This trend is an important argument for environmental factors.

Birth weight. Dunn [14] demonstrated an aetiological correlation between growth retardation or small-for-dates infants and a higher rate of congenital postural deformities. Our data were remarkable: the different combinations of CPDA were not associated with a lower mean birth weight and a higher rate of newborns with low birth weight than those of

Table VII

Observed and expected sex ratio of different combinations of congenital postural deformities

Type	Sex ratio		χ_1^2
	observed	expected	
DH	0.25* –0.28**	–	–
CF	0.60**–0.68*	–	–
TC	0.63**	–	–
DH + CF	0.37	0.29	3.35
DH + TC	0.33	0.34	0.05
CF + TC	0.50	0.69	1.33
DH + CF + TC	0.39	0.35	0.09
CPDA	0.36	0.33	0.91

* in epidemiological approach.
** in the HCMR, 1970—1976.
The values of CPDA are weighted averages.

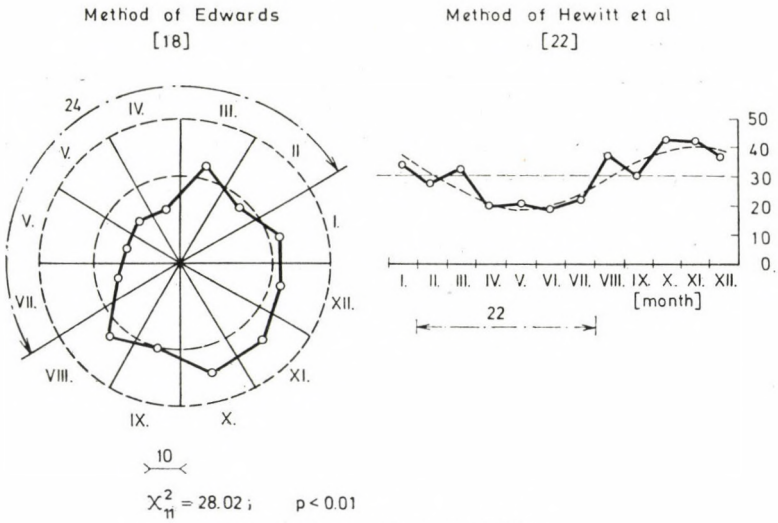

FIG. 2 Seasonality of CPDA

TABLE VIII

Mean birth weight and rate of low birth weight (< 2500 g) newborns in different combinations of congenital postural deformities

Type	Number of index patients	Birth weight		Per cent under 2500 g
		$\bar{X}$	S.D.	
DH	2.684	3142	584	9.8
CF	452	3014	683	19.5
TC	152	3138	635	8.6
DH + CF	165	3214	574	9.1
DH + TC	138	3147	550	11.6
CF + TC	10	3148	481	10.0
DH + CF + TC	24	3136	537	8.3
CPDA	337	3177	557	10.1

single congenital postural deformities or that of the national average (about 3140 g and 11%) (Table VIII).

FAMILY STUDY

A questionnaire was sent to the parents of 475 index patients with CPDA. The purpose was to determine (i) the number of parents and sibs, (ii) the number of relatives affected with different postural-type deformities, and (iii) the number of relatives affected with other non-postural type abnormalities. Owing to the method and the inaccuracy of diagnosis of congenital postural deformities these data have only an informative value. Finally, due to erroneous addresses, no response,

incomplete data of first degre relatives and inaccurate diagnosis of index patients or first degree relatives, only 349 index patients (73.5%) could be evaluated. The affected rates of first degree relatives are shown in Table IX. These figures are completed with the data of family studies of DH [6] and CF [7].

Eight parents (1.2%) and 35 sibs (11.9%) who had DH or CF + TC were mentioned. Six other postural-type deformities occurred in first degree relatives, but all these were different in type. The affected rate of sibs was ten times higher than that of the parents. And it is even more important, that only isolated congenital postural deformities and no combinations were found in the relatives of the index patients. The so-called family cluster, the high K value of Penrose [24], was obvious in all combinations of CPDA except CF+TC. Owing to the isolated manifestation of congenital postural deformities, the use of the additive value of DH and CF seemed to be more advantageous. In this case a familial cluster occurred only in the sibs of index patients. The higher rate of affected siblings than that of parents is a general rule explaining (i) the different standard of medical care in these different generations; (ii) recall bias on account of the mild and perhaps spontaneously recovered or easily treated deformities, thus the parents do not remember their own anomaly they had in childhood; (iii) permanent maternal-factors (which may exist in successive pregnancies), (iv) selection (?) and (v) dominance variance. Still, a ten-fold difference is unusual between parents and sibs. The magnitude of the familial cluster was the same in the sibs of index patients with CPDA and isolated DH and isolated CF. This was an important proof of the common aetiological factors, e.g. mechanical intrauterine influences.

The occurrence of other non-postural type congenital abnormalities was not higher in relatives of the index patients. This refers to specific aetiological factors in CPDAs.

The presence of a familial cluster is a seeming counterargument for the exclusive effect of some extrinsic intrauterine moulding factor. The permanent maternal factors existing in successive pregnancies may, however, explain (i) the higher sib-occurrence, (ii) the considerable difference of affected rates in parents and sibs, (iii) the alternative, (iv) specific and (v) single manifestation of DH and CF in sibs. These general maternal factors are independent of a single maternal effect, e.g. primigravidity.

On the basis of the present data the nosological heterogeneity of DH and CF seems to be obvious. At least four different aetiological categories should be separated: (i) structural (probably of multifactorial origin, i.e. polygenic liability and triggering environmental factors), (ii) syndromes (probably of Mendelian, chromosomal or teratogenic origin), (iii) secondary (probably as consequences of primary nervous or muscular damage, e.g. spina bifida cystica) and (iv) postural

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TABLE IX

Data of family study in index patients with CPDA

Groups of CPDA	N	P_{true}	P_{reg}	Parents					Sibs				
				m	M	q	K	K_{ADD}	m	M	q	K	K_{ADD}
DH	—	28.00	5.9	3534	82	2.3	0.4	—	1070	148	13.8	2.3	—
CF	—	1.30	1.6	348	8	2.3	1.4	—	180	10	5.6	3.5	—
DH + CF	180	0.21	—	360	7*(2)	1.9	9.0	0.3	153	12**(2)	7.8	37	1.0
DH + TC	136	0.16	—	268	1°	0.4	2.5	0.1	110	19°°(1)	17.3	108	2.9
CF + TC	10	0.01	—	20	0	0	0	0	9	0	0	0	0
DH + CF + TC	23	0.02	—	46	0	0	0	0	21	4°°°(1)	19.0	950	2.5
CPDA	349	0.40	—	694	8 (2)	1.2	3.0	0.2	293	35 (4)	11.9	30	2

N = number of index patients
P = birth prevalence per 1000 live births
m = number of given type of relatives
M = number of affected relatives with DH or CF

$$q = \frac{M}{m} \cdot 100$$

$P_{true\,(TC)} = 0.1$

$$K = \frac{q}{P_{reg}}\text{; or if } P_{reg} \text{ is not known } K: = \frac{q}{P_{true}}$$

$$K_{ADD\,(DH+CF)} = \frac{q}{P_{reg\,(DH)} + P_{reg\,(CF)}}$$

P_{true} = epidemiological approach
P_{reg} = data of HCMR

*DH: 1 (father) + 6 (mother)
(+ 2 other postural-type deformities)
**DH: 7; CF: 5
(+ 2 other postural-type deformities)
°DH: 1 (father)
°°DH: 19
(+ 1 other postural-type deformity)
°°°DH: 4
(+ 1 other postural-type deformity)

(probably of maternal origin) ones. This last category has a predisposition to a combination with each other, i.e. the existence of CPDA and a moderate recurrence risk in sibs (on account of the influence of intrauterine maternal moulding factors in successive pregnancies) and a good prognosis (recovery spontaneous or treatable with high efficacy). These points are considered sufficient for a delineation of CPDA.

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HLA antigens in bullous epidermolysis, congenital ichthyosis and ectodermal dysplasia

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HLA–ABC antigen typing was carried out in 4 homozygous patients and 10 family members from three families with bullous epidermolysis, 7 homozygous patients and 19 family members of six families with ichthyosis, and 4 homozygous patients and 8 family members of 4 families with ectodermal dysplasia. The type of heredity was established on the basis of genetical evidence and the clinical picture.

In bullous epidermolysis cases autosomal recessive heredity was detected in two families with congenital bullous epidermolysis, and autosomal heredity in two families with dystrophic bullous epidermolysis. The HLA–Aw 24, B5 combination, which was thought to be significant in epidermolysis bullosa, was found in one, B5 alone in two, out of the four families.

In congenital ichthyosis autosomal recessive heredity was detected in two families out of five. In one, X-linked recessive heredity was found, and in two families X-linked recessive heredity could be supposed. In three families out of the five with congenital ichthyosis, the A2, B18 antigen combination was found.

In a family where two infants died from the most severe form of ichthyosis, epidermolysis and psoriasis also occurred. In this family two grandfathers were brothers, and one of the two was a carrier of the Aw24, B5 combination.

In the case of ectodermal dysplasia, in one out of four families X-linked recessive heredity, in another autosomal recessive heredity was detected. In two families X-linked recessive heredity could be supposed as only the male children were affected and also on the basis of data in the literature. The HLA–A26, B38 antigen combination occurred in three of these four families.

The reports on HLA antigens in skin diseases have revealed an increased frequency of Al, B8, Dw3 and DR3 in dermatitis herpetiformis, B13, B17, B27 and B38 in psoriasis, of A10 in pemphigus [13], and of A32 in porphyria cutanea tarda [7]. In a family suffering from dominantly inherited epidermolysis bullosa dystrophica, identical HLA-haplotypes (Aw24, B5) were found in 71.6% of the patients [10].

In the present paper we report on the results of HLA typing in three skin diseases of genetic origin, bullous epidermolysis, congenital ichthyosis and ectodermal dysplasia, and also on their type of heredity, since we could

TABLE I

HLA pheno- and genotypes in bullous epidermolysis congenital ichthyosis and ectodermal dysplasia

Bullous epidermolysis

	HLA phenotypes	Possible genotypes
P.S. (patient)	A 2, 3; B 5, 18; Cw1, 6	A 2, B 5, Cw1/A 3, B18, Cw6
P.M. (sister)	A 2, 3; B 5, 18; Cw1, 6	A 2, B 5, Cw1/A 3, B18, Cw6
P.S. (father)	A 1, 3; B 8, 18; Cw6	A 1, B 8, Cwx/A 3, B18, Cw6
Mrs P. (mother)	A 1, 2; B 5, Cw1	A 2, B 5, Cw1/A 1, B 5, Cw2
Mrs A. (maternal grandmother)	A 1, 2; B 5, 39; Cw1, 6	A 2, B 5, Cw1/A 1, B39, Cw6
L.Sz. (patient)	A 1, 2; B 8, 17; Cw6	A 2, B17, Cw6/A 1, B 8, Cw6?
L.J. (patient)	A 1, 2; B17, 35; Cw6	A 2, B17, Cw6/A 1, B35, Cw6?
L.J. (father)	A 2, 28; B17, 41; Cw6	A 2, B17, Cw6/A28, B41, Cw6?
Mrs L. (mother)	A 1, ; B 8, 35; Cw6	A 1, B 8, Cw6/A1 , B35, Cw6?
P.J. (patient)	A 3, 24; B 5, 7; Cw6	A 3, B 7, Cw6/Aw24, B5, Cwx?
P.J. (father+patient)	A 2, 3; B 5, 7; Cw6	A 3, B 7, Cw6/A 2, B5, Cwx
P.J. (paternal grandfather, patient)	A 3, 25; B 7, 18; Cw6	A 3, B 7, Cw6/A25, B18, Cwx
P.L. (uncle, patient)	A 2, 3; B 7, 40; Cw2, 6	A 3, B 7, Cw6/A 2, B40, Cw2
Sz.Gy. (patient)	A 1, 3; Bw15, 35; C–	

Congenital ichthyosis

D.J. (patient)	A 1, 3; B 5, 18; C—	
D.F. (patient)	A 2, w23; B18, ; Cw6	A 2, B18, Cw6/Aw23, B18?, Cwx
D.K. (sister)	A 2, w24; B18, 40; Cw3, 6	A 2, B18, Cw6/Aw24, B40, Cw3
Mrs D. (mother)	A 2, 11; B 5, 18; Cw2, 6	A 2, B18, Cw6/A11, B 5, Cw2
Mrs T. (maternal grandmother)	A 2, 11; B 7, 18; Cw6	A 2, B18, Cw6/A11, B 7, Cw6
T.L. (uncle)	A11, ; B 5, 7; Cw2, 6	A11, B 5, Cw2/A11, B 7, Cw6
T.E. (cousin)	A11, 26; B 7, 38; Cw6	A26, B38, Cwx/A11, B 7, Cw6
T.E. (nephew)	A11, 26; B 5, 14; Cw2	A26, B14, Cwx/A11, B 5, Cw2
B.G. (patient)	A 1, w31; Bw15, 35; Cw4, 6	A 1, B35, Cw4/Aw31, Bw15, Cw6
B.M. (sister)	A 1, w31; Bw15, 35; Cw4, 6	A 1, B35, Cw4/Aw31, Bw15, Cw6
B.É. (sister)	A 1, 2; B 8, w50; Cw6	A 1, B 8, Cw6/A 2, Bw50, Cwx
B.M. (father)	A 1, 2; B 35, w50; Cw4	A 1, B35, Cw4/A 2, Bw50, Cwx
Mrs B. (mother)	A 1, w31; B 8, w15; Cw6	A 1, B 8, Cw6/Aw31, Bw15, Cw6

Congenital ichthyosis

	HLA phenotypes	Possible genotypes
D.B. (patient)	A 2, 28; B 5, 15; Cw3	A 2, B 5, Cwx/A28, Bw15, Cw3
Mrs D (mother)	A 2, ; B 5, 12; C–	A 2, B 5, Cwx/A20, B 12, Cwx
V.I. (maternal)	A 2, ; B 5, w50; C–	A 2, B 5, Cwx/A20, Bw50, Cwx
Mrs V. (grandparents)	A 2, 11; B 12, 18; C–	A 2, B12, Cwx/A11, B 18, Cwx
D.I. (paternal)	A 11, 28; B 38, 40; Cw2, 3	
Mrs. D (grandparents)	A 2, 28; Bw15, 27; Cw2, 3	A 2, B27, Cw2/A28, Bw15, Cw3

Congenital ichthyosis

	HLA phenotypes	Possible genotypes
M.S. (father)	A 2, 28; B 7, 35; Cw4	
Mrs S. (mother)	Aw24, 32; B 5, 27; Cw2	Aw24, B 5, Cwx/A32, B 27, Cw2
L.P. (grand-	A 2, w24; B 5, 17; C–	Aw24, B 5, Cwx/A 2, B 17, Cwx
Mrs L. parents)	Aw23, 32; B 13, 27; Cw2	Aw23, B13, Cwx/A32, B 27, Cw2
L.S. (aunt)	A 2, w23; B 13, 17; C–	Aw24, B13, Cwx/A 2, B 17, Cwx
D.P. (patient)	A 2, 3; B 18, 35; C–	A 2, B35/A 3, B18
D.J. (patient)	A 1, 2; B 8, 35; C–	A 2, B35/A 1, B 8
Mrs D. (mother)	A 2, 28; B 27, 35; C–	A 2, B35/A28, B27
	Ectodermal dysplasia	
O.Gy. (patient)	A 1, 2; B 35, w50; Cw4	A 1, B35, Cw4/A 2, Bw50, Cxw
Mrs O. (mother)	A 1, 26; B 35, 38; Cw4	A 1, B35, Cw4/A26, B 38, Cwx
Mrs K. (maternal grandmother)	A 1, 2; B 12, 35; Cw4	A 1, B35, Cw4/A 2, B 12, Cwx
D.Zs. (patient)	A 2, w33; V 13, 39; C–	A 2, B39/Aw33, B13
D.L. (father)	A 2, w33; B 13, 18; C–	A 2, B18/Aw33, B13
Mrs D. (mother)	A 2, ; Bw15, 39; C–	A 2, B39/A20, Bw15
Mrs I. (aunt)	A 1, 32; B 8, 39; C–	
B.L. (patient)	A 25, 26; B 38, 40; Cw2	
Mrs B. (mother)	Aw24, 25; B 38, 40; Cw2	
N.E. (patient)	A 2, 26; B 7, 38; C–	A 2, B 7/A26, B38
Mrs N. (mother)	A 2, 3; B 7 C–	A 2, B 7/A 3, B7
N.L. (father)	A 11, 26; B 38, 37; C–	A 26, B38/A11, 37

find no literary data concerning the question or else they awaited confirmation [10].

Material and Methods

Four families with bullous epidermolysis, 7 homozygous patients in 6 families with congenital ichthyosis as well as 4 homozygous children from 4 families with ectodermal dysplasia were typed for HLA–ABC antigens, using the NIH lymphocytotoxicity test [15]. The pedigrees were recorded on the basis of the clinical picture and the genetic history.

Results

The HLA pheno- and genotypes are shown in Table I.

Bullous epidermolysis

The pathological condition of baby P.S. was diagnosed in newborn age; his mother had had a similar dermatological abnormality in her babyhood and infancy. The patient is a male child (Fig. 1. III/1) who had the haplotype HLA–A2, B5, Cw1 in common with the gene-carrier mother

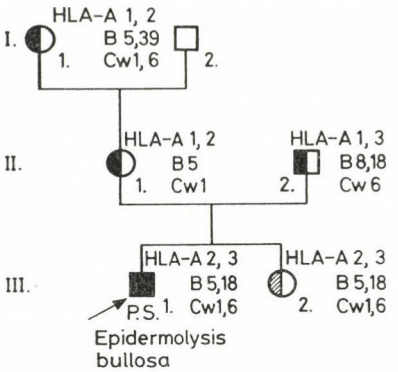

FIG. 1

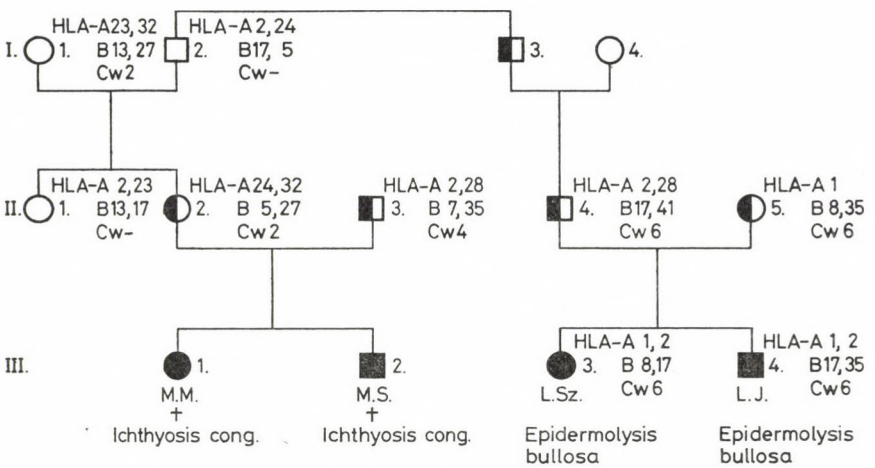

FIG. 2

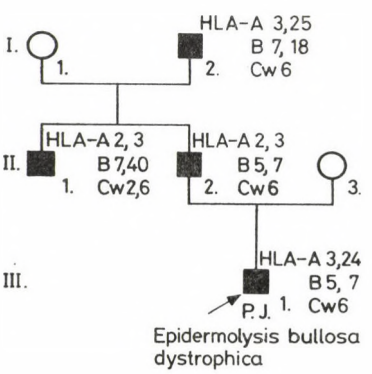

FIG. 3

and maternal grandmother. The genotype of the gene-carrier father is A1, B8, Cwx/A3, B18, Cw6, of which the combination A3, B18, Cw6 was represented in both of his children. On the basis of these common haplo-types and of the HLA-identity of the children, the sister III/2 may also be considered a gene carrier. The disease gene transfer in the family was of the autosomal recessive type.

In family L. heredity was also of the autosomal recessive type (Fig. 2). The common HLA haplotype of the two homozygous children (III/3, III/4) was A2, B17, Cw6. The single common antigen of the obligate gene-carrier parents is Cw6.

In the case of family P. (Fig. 3) epidermolysis bullosa dystrophica of dominant heredity was observed. The common haplotype of the homozygous family members was A3, B7, Cw6. In patient P.J. also Aw24, B5 haplotype occurred which has been observed to be characteristic of bullous epidermolysis [10]. B5 alone occurred in the father, too. In the adult patient Sz. G., B35 was found as a shared antigen in the bullous epidermolysis family L.

Congenital ichthyosis

In family D. (Fig. 4) where four male individuals were affected, the heredity was X-linked recessive. In the two homozygous patients in the family who were HLA-typed, no common antigen was found. One common haplotype of the obligate heterozygous mother and grandmother, A2, B18, Cw6, could be found in one of the homozygous patients (IV/3) and in the supposed gene-carrier sister (IV/4).

Baby B. G. was born of the third pregnancy. Three of the four children of the maternal grandmother's brother died (Fig. 5) in early infancy, two of them of congenital hydrocephalus, and one of spina bifida. The common haplotype of the propositus, his sister, and of the mother was A31, B15, Cw6. The heredity is, according to literary evidence, X-linked recessive. We suppose that the sister (III/3) is a possible gene-carrier, on the basis of the HLA-haplotype shared with the gene-carrier mother.

In the family of the female infant D. B. (Fig. 6), no similar disease occurred excluding thus an autosomal dominant heredity. The sex of the child excluded an X-linked recessive heredity, therefore transmission of the gene may be qualified as autosomal recessive. The common haplotype of the mother, the maternal grandfather and of the female child is A2, B5.

In family D. (Fig. 7) the two male twins suffer from ichthyosiform congenital erythroderma. We must consider the mother to be an obligate gene-carrier. The heredity is, on the basis of the family data and the literature, X-linked recessive. The common haplotype of the two homozygous male children and the possible gene-carrier mother is HLA–A2, B35.

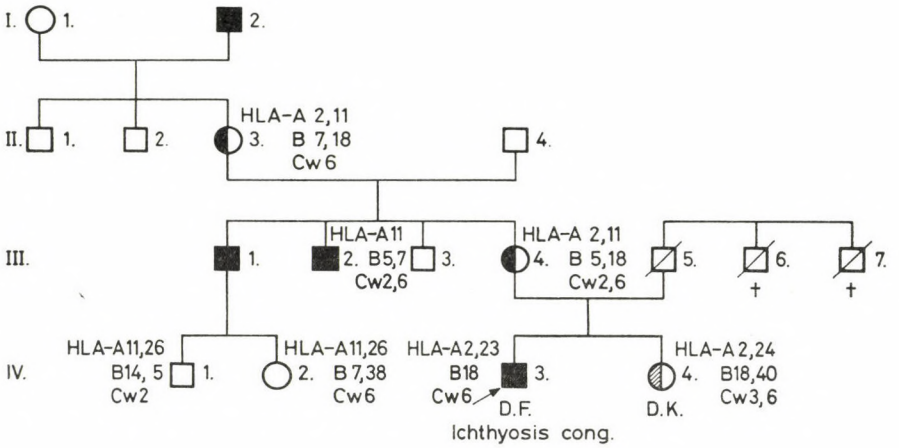

FIG. 4

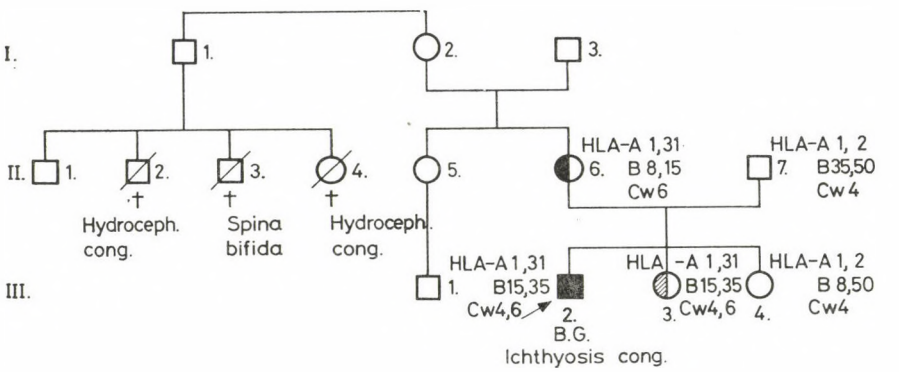

FIG. 5

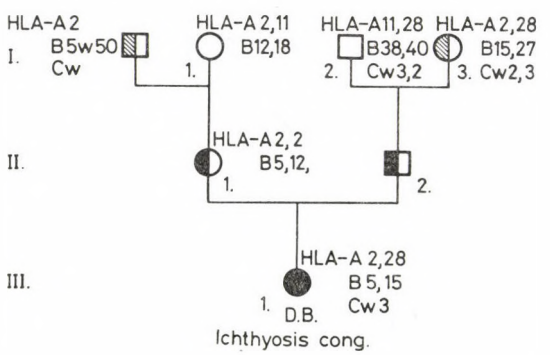

FIG. 6

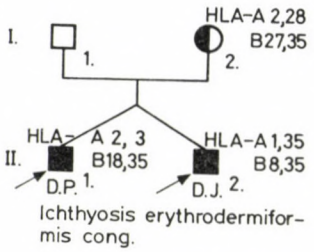

FIG. 7

In family M. (Fig. 2), on the basis of the affected infants of different sexes, gene transfer must have been of the recessive type. The two collodium babies (III/1, III/2) had died so that HLA-typing could not be done, therefore in Table I. only the HLA phenotypes of the obligate heterozygous parents are given.

Ectodermal dysplasia

The male infant O. G. was admitted as a newborn with anhydrotic ectodermal dysplasia (Fig. 8). The mother and the maternal grandmother have slight symptoms. The type of heredity is, according to the literature [13], X-linked recessive. The patient, the mother and the maternal grandmother share the HLA-A1, B35, Cw4 haplotype.

In the case of D. Zs. (Fig. 9) who is a male individual, X-linked recessive heredity may be suspected. Of the HLA-antigens of D.Zs., A2, B39 are shared with the gene-carrier mother. The mother's sister does not have these antigens.

The anhydrotic ectodermal dysplasia of the male child B.L. proved to be of X-linked recessive heredity, as there were three homozygous male children in the family (Fig. 10). Three sons of the mother's brother died in infancy; according to the history they have probably been also affected. The mother was found to

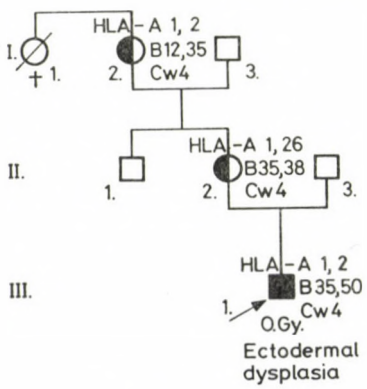

FIG. 8

have cystinuria. The affected male child and the gene-carrier probably had the common haplotype A25, B40.

The type of heredity of the female infant N.E. was, on the basis of her sex, autosomal recessive (Fig. 11). Her HLA antigens inherited from the

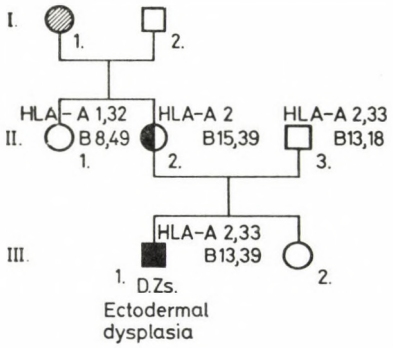

FIG. 9

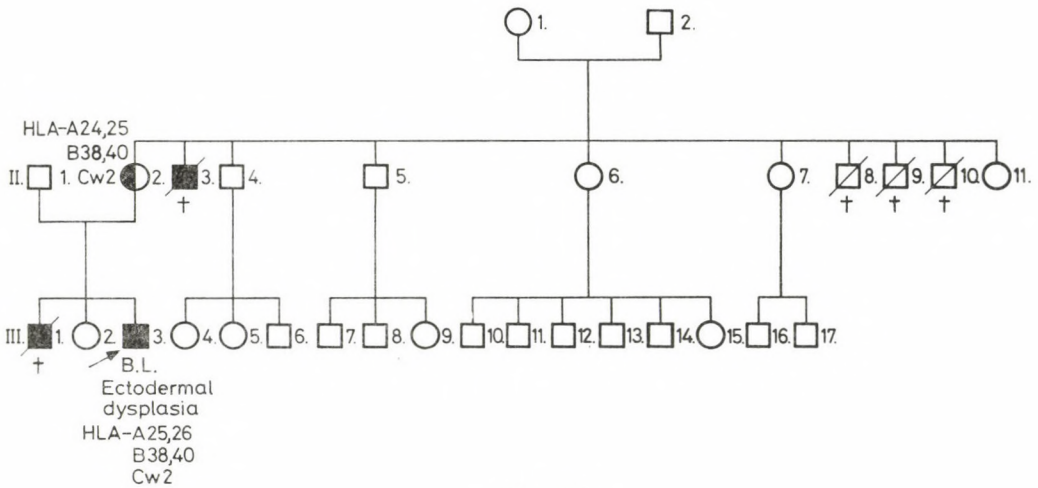

FIG. 10

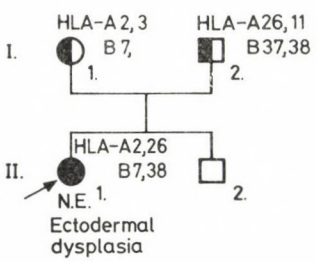

FIG. 11

mother were HLA–A2, B7, those inherited from her father, A26, B38.

The haplotype A26, B38 probably occurred in three of the four cases shown in the ectodermal dysplasia group.

Discussion

Bullous epidermolysis

On the basis of the clinical picture, the pathological findings and the prognosis, 8 types of bullous epidermolysis may be distinguished. I. Epidermolysis bullosa simplex (type Koebner) with autosomal dominant heredity; II. Weber–Cockayne syndrome with autosomal dominant heredity; III. epidermolysis bullosa lethalis (type Herlitz) of autosomal recessive heredity; IV. locally missing skin with blisters and dystrophy of the nails (Barty syndrome), with autosomal dominant gene transfer; V. epidermolysis bullosa dystrophica (autosomal dominant); VI. epidermolysis bullosa dystrophica (autosomal recessive); VII. Macular dystrophic epidermolysis bullosa (Mendes Da Costa syndrome) with X-linked recessive heredity; and VIII. epidermolysis bullosa dystrophica neuropathica which may be associated with deafness of the perceptive type [4].

In the two families L. and P. (Figs 1–2) the heredity of congenital epidermolysis was autosomal recessive and in a third family (P. J., Fig. 3), autosomal dominant in type. In the last case the clinical picture was somewhat different as it was a dystrophic bullous epidermolysis with the haplotype Aw24, B5. This might be characteristic of bullous epidermolysis [10] as we found it in a homozygous patient (P. J. Fig. 3) and B5 alone in his father. In the case of P.J. as well as in the one published by Ozawa et al [10], the gene transfer was of the autosomal dominant type. B5 alone was observed in addition in four members of Family P.S. (Fig. 1) with bullous epidermolysis of the autosomal recessive type.

In family P.S. A2, B5, Cw1 were demonstrated in the homozygous male child patient (III/1), in her sister, a suspected gene carrier (III/2), as well as in the gene-carrier mother and maternal grandmother. From the gene-carrier father both children received the haplotype A3, B18, Cw6.

In family L. (Fig. 2) the common haplotype of two homozygous children (III/3, III/4) was A2, B17, Cw6, and they were different in the antigens inherited from their mother.

In the case of the male patient Sz.Gy. who only shared a B35 with some members of the family L., the mother was affected, thus the heredity was autosomal dominant.

Congenital ichthyosis

Heredity is different in the different ichthyosiform dermatoses [4]. The heredity of ichthyosis vulgaris is autosomal dominant, the X-linked recessive ichthyosis also belongs to the group of normokinetic ichthyoses. In the group of hyperkinetic ichthyoses, epidermolytic hyperkeratosis has an

autosomal dominant, while lamellar ichthyosis an autosomal recessive heredity. To this last group belong the ichthyosiform erythrodermia, the Harlequin fetus, lamellar ichthyosis of infants, and the collodium baby.

Voigtländer et al [16] classified the hereditary ichthyoses as follows.

1. The group of ichthyosis vulgaris includes (i) autosomal dominant ichthyosis, (ii) X-linked recessive ichthyosis (iii), ichthyosis in Refsum syndrome.

2. The congenital ichthyosis group consists of (i) ichthyosis congenita gravis (Riecke I, Harlequin fetus), (ii) ichthyosis congenita mitis (Riecke II) and tarda (Riecke III), (iii) ichthyosis in the Sjögren–Larsson-syndrome, (iv) ichthyosis in the Rud-syndrome.

3. In the ichthyosis hystrix group (i) erythrodermia congenitalis ichthyosiformis bullosa and (ii) the four subtypes of ichthyosis hystrix gravior are to be found.

Palásty [11] observed in a gipsy family five collodium babies and supposed an autosomal recessive heredity, in agreement with Burgoon [3] and Lencz and Altman [6].

Mevorah et al [8] observed autosomal dominant and X-linked recessive variants of ichthyosis vulgaris within one family. Phenotypically the ichthyosis was of the dominant type in the mother, while it was dominant and X-linked recessive in her sons. Ultrastructural examination of the keratinization disorders in the ichthyoses of different hereditary types may provide important clues regarding the type of heredity [1].

Koppe et al [5] reported on cases of ichthyosis congenita vulgaris with X-linked heredity. They considered the decrease in arylsulphatase-C activity of the skin and the placenta to be the causative factor.

In two of our families with congenital ichthyosis we demonstrated an autosomal recessive heredity(M. and D.B., Fig. 2 and 6) and X-linked recessive heredity in one family (D.F., Fig. 4). In one case (family B., Fig. 5) we supposed an X-linked recessive gene-transfer on the basis of literary data.

In the case of family D.F. (Fig. 4) with X-linked recessive heredity the combination of HLA–A2, B18, Cw6 antigens could be demonstrated in four out of the eight family members, viz. in a homozygous male child and three obligate gene carriers. The similarly affected maternal uncle (III/1) totally differed in his HLA-antigens from the genetically affected nephew. Among his antigens A11, B5, and Cw2 agreed with those of some other family members, while in A11, B7, Cw6 he was identical with his daughter and the obligate heterozygous maternal grandmother (IV/2, II/3). The antigen combination A26, B38 in the daughter is apparently very frequent in our families with ectodermal dysplasia.

In the family of the collodium babies (Fig. 2) the HLA types of the two obligate heterozygous parents may be important. The phenotype of the father was A2.28; B7.35; Cw4, and that of the mother Aw24, B5, Cwx/A32, B27, Cw2. It is interesting

that she carried Aw24, B5 in one haplotype, an antigen combination which was thought to be significant in bullous epidermolysis [10]. As regards our own cases with bullous epidermolysis, we found this combination in one out of three families. It is assumed that Aw24 and B5 are markers of some sort of general sensitivity to certain dermatological disorders. It was remarkable that this antigen combination seemingly important in bullous epidermolysis should occur in a family where the grandfathers were brothers and in which one psoriasis and two collodium baby cases occurred. Only one of the two grandfathers was HLA-typed; he was healthy and a carrier of the Aw24, B5 combination. Another frequent antigen combination in this family, A2, B17, Cw6, was found in five out of ten persons and in a sixth case it was very probably present. B17, however, did not occur in some obligate ichthyosis gene-carriers (II/2, II/3).

Ectodermal dysplasia

Concerning the inheritance of ectodermal dysplasia, the anhydrotic form was reported to be an X-linked condition [13] while Bernard et al [2a] demonstrated an autosomal dominant heredity through three generations.

In one (family B.) out of four families with ectodermal dysplasia, the heredity was clearly X-linked recessive (Fig. 10). In two other families (D. and O.) (Figs 8, 9). owing to the genetic affection of the male persons and on the basis of literary data, heredity was considered to be X-linked. Finally, in the fourth family (Family N., Fig. 11) gene transfer was found to be autosomal recessive.

In three of these four families (see Table I) the combination of A26 and B38 was demonstrated and in the fourth family B39 occurred in another combination. Thus, the former Bw16 antigen (B37 and B38 together), which has a 9 % frequency in European populations, was present in four out of the four families with ectodermal dysplasia.

As regards prevention, odontological screening, detection of agenesis or hypoplasia of the teeth may also throw light upon gene transfer in ectodermal dysplasia [11].

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Neonatal oxygen toxicity and its prevention: D-penicillamine offers benefits without harmful side-effects

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Oxygen toxicity in the newborn and the possibilities of its prevention are discussed. The role of active radicals developing from molecular oxygen, lipid peroxidation and its prevention by antioxidants, oxygen toxicity and its importance in neonatology, and facilities for overcoming hyperoxia of the newborn are considered. The antioxidant effects based on experimental and clinical observations of vitamin E and D-penicillamine are described. Since the introduction of D-penicillamine therapy the incidence of retrolental fibroplasia has steeply decreased. It is emphasized that in the neonatal period the drug has no adverse effects.

Oxygen (O_2) is indispensable for the highly organized functions of life. Oxygen therapy may be life-saving in hypoxia, but hyperoxia may lead to severe damage to cells and tissues.

The role of active radicals arising from molecular oxygen

During inspiration, O_2 dissolved in the lipids and the water of lung tissue diffuses through the alveolar membranes into the capillaries where it is bound to the haemoglobin of the erythrocytes; oxygen is then carried by the blood to the various tissues. It is well known that O_2 is indispensable for a large number of biochemical processes working at cellular and tissue levels. Most oxygen undergoes the four-electron reduction carried out by mitochondrial cytochrome oxidase; the end product of this process is water [12]:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Reduction by two electrons performed by other enzymes results in hydrogen peroxide

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$$

while a reaction using one electron leads to the superoxide radical

$$O_2 + e^- \rightarrow O_2^-.$$

In addition, O_2^- is produced by auto-oxidation of normal cellular components like thiol derivatives, haemoglobin, or epinephrine [67, 69, 70]. The bactericidal effect of O_2^- developing during phagocytosis by polymorphonuclear leucocytes is a characteristic property [3, 85]. H_2O_2 is comparatively well tolerated by most aerobic organisms in spite of its much higher reactivity to biological molec-

ules. The compound may be harmful if it is transformed to hydroxyl radical (·OH) in the presence of certain metal ions [33]. This radical is extremely aggressive. It may also be formed during the reaction of O_2^- and H_2O_2 catalyzed by iron chelates [24, 34]:

$$O_2^- + H_2O_2 \xrightarrow{Fe} 2\cdot OH + O_2$$

The interaction between various oxygen and peroxide radicals may result in a very reactive oxygen derivative toxic to most biological systems, in singlet oxygen ($^1O_2$). In this radical, one electron of the oxygen is pushed to a high energy orbit [48, 91].

H_2O_2, O_2^-, $^{\cdot}OH$ and $^1O_2$ are the main factors responsible for the tissue damage provoked by hyperoxia; "oxygen radical" is an oversimplified common term for them.

Lipid peroxidation and its biological consequences

According to the most accepted hypothesis, O_2 toxicity is provoked by free radicals arising from O_2 and by other active oxygen metabolites; their effect is mediated by increased lipid peroxidation (LP) of biological membranes, thus leading to specific cell damage [25, 67]. Oxidability of fatty acids depends on the number of double bonds, i.e. only unsaturated fatty acids (linolic acid, linolenic acid, arachidonic acid, etc.) are prone to peroxidation. Cellular membranes, containing a considerable quantity of unsaturated fatty acids, are especially vulnerable to peroxidative damage [16, 81, 99]. ·OH and probably also O_2^-, react with the lipid components of biological membranes; this results in peroxide (ROO.) and hydroperoxide (ROOH) radicals of lipids and in other compounds like malonyldialdehyde [5, 17, 47]. Malonyldialdehyde forms covalent bonds with the free amino-groups of proteins, lipids and nucleic acids, and on this reaction is based the chemical detection of LP precesses [9]. This chain reaction, initiated by the free oxygen radicals is catalysed by heavy metals, especially iron, and copper at several levels [51, 53, 99] leading to a loss of the integrity of membranes of erythrocytes, mitochondria, microsomes and lysosomes, to inactivation of enzymes in these structures and to damage to nucleic acids [50, 65, 71].

LP may accompany a number of physiological and pathophysiological events. Such normal physiological processes are prostaglandin synthesis, phagocytosis and aging [3, 37, 40, 65, 85]. Certain experimental and clinical observations have indicated the damaging effect of LP in haemolytic anaemia, dysfunctions of reproduction, certain pulmonary disorders, muscular atrophies, liver necrosis, encephalomalacia, atherosclerosis, testicular atrophy, diabetes mellitus, irradiation disease, postresuscitation syndrome, mutagenesis, carcinogenesis [16, 18] and other conditions including certain neonatal disorders to be described in this paper.

Antioxidant prevention

The antioxidant defence mechanisms are indispensable in maintaining life since the potentially destructive free radicals are continuously produced in the organism and they keep combining with each other [28]. The first line of defence consists of various physiological barriers hindering the oxygen to react with the tissues while entering from the alveolar space into the pulmonary capillaries and carried by haemoglobin to the cells [36]. The second type of defence is bound to specific enzymes: superoxide dismutase, catalase, peroxidases, glutathione and its enzyme system, and the NADPH producing system closely coupled to the hexose-monophosphate shunt [87, 106]. Since these enzymes exhibit activity induction during adaptation to hyperoxia, their function will be described in some detail.

Superoxide dismutase (SOD)

This metalloprotein enzyme has been found in three main forms. In eukaryotic cells the metal is copper and zinc in the cytoplasm, and manganese in the mitochondria. A third type of SOD contains iron and occurs in a number of bacteria. Their main task is to enhance the dismutative inactivation of O_2^- radicals produced during auto-oxidation of various biological components or arising during enzymatic processes. The dismutation can be described by the reaction,

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

As a result of this reaction, O_2^- is rapidly removed before it could induce direct cellular damage or lead to production of aggressive $^{\cdot}OH$ radicals by combination with H_2O_2 [25, 66, 100].

Catalase and other peroxidases

These are enzymes of haem-protein nature. Their main biological role consists in the elimination of H_2O_2:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Although H_2O_2 itself participates in LP at a low degree and slow reaction rate, its elimination is imperative because it reacts with O_2^- in the presence of metal ions, leading thus to $^{\cdot}OH$, the most dangerous radical from the aspect of oxygen toxicity [27, 33].

Glutathione and NADPH producing systems

Glutathione is a thio-tripeptide. A high concentration of its reduced form (GSH) and a low concentration of its oxidized form (GSSG) are essential in the maintenance of animal life. The antioxidant effect of GSH is exerted by two routes: it prevents the oxidation of sulfhydryl groups of the proteins by serving as a substrate for the oxidants; in addition, GSH is a substrate of the enzyme glutathione peroxidase which inactivates both H_2O_2 and lipid peroxides, stopping thus the chain reaction initiated by the oxygen radicals [35, 68]. The optimum GSH–GSSG ratio is maintained by the enzymes glutathione reductase and glucose-6-phosphate

dehydrogenase. The latter forms an essential step of the hexose-monophosphate shunt. Its production is greatly enhanced by the NADPH (reduced nicotine amide adenine dinucleotide phosphate) system, supporting in this way the important biosynthetic reactions necessary for the reparation of tissues damaged by lipid peroxidation [11].

The third line of antioxidant defence comprises endogenous antioxidants, the adequate tissue level of which offers satisfactory protection against oxygen toxicity. These compounds are vitamin E, selenium, sulfur containing amino acids, vitamin C [27, 97], and transferrin and coeruloplasmin, proteins performing the task of extracellular transport of iron or copper [19].

The special situation of neonates and low-birth-weight newborns

As shown above, oxygen toxicity is the resultant of two biological processes acting in two opposite directions. On the one hand, production of free radicals and the chain reaction initiated by them would tend to lead to disintegration of the cell and the cellular organelles. On the other hand, there exists a sophisticated defence system acting at several levels that prevents or even repairs the tissue damage. Under physiological conditions there is an equilibrium between the two systems; the identical rate of production and elimination secures only the "normal" function of lipid peroxidation. Pathological newborns and preterm infants are, however, in a peculiar situation. In these infants and to some extent in normal term babies as well, a disequilibrium resulting in increased vulnerability to oxygen toxicity may develop during adaptation to extrauterine life. In addition, defence mechanisms against the oxygen radicals are also immature in them. There are two main reasons for this increased vulnerability, (i) an increased exposure to situations of prolonged oxidative stress; (ii) deficient defence at all levels.

Ad (i)

1. Before and during birth the newborn may be exposed to maternal and fetal injuries resulting in an increased rate of LP: EHP-gestosis, intrauterine infections, various maternal diseases, placenta praevia, premature rupture of the membranes, exposure of the mother to drugs and radiation, especially tocolytics, procedures during labour like oxygen inhalation, anaesthesia, analgesia, oxytocin etc. [89].

2. The greatest risk is oxygen therapy some time after birth, frequently applied as a life-saving measure necessary for the prevention of sequelae of hypoxia [42]. The most vulnerable individuals in this respect are babies of very low birth weight and newborns in whom the partial tension of arterial oxygen continuously exceeds the tolerable level. For this reason, in newborn units all over the world attempts at measuring arterial oxygen tension are made to avoid prolonged hyperoxaemic

periods [49, 96]. Unfortunately, only partial success has been achieved as even in intensive care units not all the grave sequelae of oxygen toxicity can be avoided [83].

3. A frequent damaging factor is hyperbilirubinaemia. Apart of its unfavourable effect on the central nervous system [63], bilirubin has been shown to be a photodynamic agent i.e. it produces $^1O_2$ in the presence of oxygen and light, thus enhancing the peroxidation of unsaturated fatty acids [15, 29, 32].

4. Certain therapeutic measures destined to surmount neonatal hyperbilirubinaemia like phototherapy and exchange transfusion may also be potentially harmful in this respect. Phototherapy enhances the isomerisation of bilirubin into rapidly eliminated and less toxic compounds. This process is accompanied by an increased production of $^1O_2$. This radical has a minor role in bilirubin oxidation but it may be responsible for the potential hazards of phototherapy: damage to DNA, mutagenesis, carcinogenesis, sexual dysfunction, etc. [14, 93]. Similarly, exchange transfusions and transfusion of adult blood may increase the oxygen toxicity by the lower oxygen affinity of adult haemoglobin compared to that of fetal haemoglobin [13].

5. Septic and hypotensive conditions, including the idiopathic respiratory distress syndrome (IRDS), resuscitation and reparation after resuscitation increase the risk of oxygen damage. In chronic and acute inflammations the polymorphonuclear leucocytes, macrophages and monocytes produce a considerable amount of O_2^-; this radical has a direct bactericidal effect and promotes the formation of the superoxide dependent chemotactic factor and the production of $^{\cdot}OH$ radicals. Both compounds have a favourable effect in the killing process directed against the bacteria and other invasive agents [3, 85]. In the immature organism, however, with its deficient antioxidant defence, the aggressive radicals may damage the cellular integrity.

6. Certain drugs may increase the proneness to oxygen toxicity. Some have a direct sensitizing effect in favour of oxygen toxicity, e.g. riboflavin, methylene blue, salicylates, etc. [92]. Others enhance the albumin-bilirubin dissociation or weaken the activity of glucuronyl transferase thus promoting bilirubin and oxygen toxicity at the same time [63]; sulfonamides and chloramphenicol are in this group. Finally, artificial ventilation and X-ray exposure, not infrequent in modern intensive care, directly lead to overproduction of harmful oxygen radicals [4].

Ad (ii)

1. In newborn babies the physiological barriers lining the internal route of oxygen transport are immature. Immaturity and gestational age show a direct correlation. Size, weight, alveolarity, collagen content, concentration of the pulmonary surfactant, the proportion of interstitial elements, capillary microcirculation, the number of alveolar epithelial

cells, respiratory capacity and compliance of the lung all differ from the situation prevailing in the baby born at term [41].

2. The activity of specific antioxidant enzymes gradually increases during gestation and in the early postnatal period. The most potent inductor of this activity is the oxygen itself, and hyperoxia speeds up the enzyme adaptation process [1, 6, 107]. This may explain the finding that under prolonged oxygen exposure newborn animals survive much longer than adult animals; this does not imply that they do not suffer permanent tissue damage. Similarly, pretreatment with antioxidants abolishes the usual biochemical reaction to hyperoxia [103]. Our own experiments with SOD on the effects of D-penicillamine resulted in similar findings [46].

The neonatal insufficiency of the enzymatic defence is markedly promoted by heavy metals, especially by iron and copper ions. These latter accumulate in a considerable degree in the fetal tissues during the last weeks of gestation [88]. After birth an additional unfavourable factor is the increased haemolysis of fetal erythrocytes. The metal ions directly influence the oxidative processes and, in addition, they inhibit the activity of delta-aminolaevulinic acid synthetase, an enzyme governing the production of haeme-containing proteins like catalase, peroxidase and cytochrome P-450. They also stimulate haemoxygenase, an enzyme initiating haeme catabolism [62].

3. Newborn babies, especially very low birth weight preterm babies, are insufficiently supplied by endogenous antioxidants like vitamin E, selenium, transferrin and coeruloplasmin [23, 38].

Neonatal disorders related to oxygen toxicity

These disorders are specific to the newborn period. They have a multicausal aetiology, their common feature being the chain reaction leading to tissue damage, initiated by free oxygen radicals.

Haemolytic diseases

Auto-oxidation of the newborn's erythrocytes proceeds at a rate three to five times higher than in adults [94]. An especially unfavourable situation is encountered in babies affected by congenital deficiency of glucose-6-phosphate dehydrogenase, pyruvate kinase or glutathione reductase [95].

Retrolental fibroplasia (RLF)

Terry [98] gave the first description of RLF of premature babies in 1942. The pathology of the condition was clarified 10 years later [26] when it was also recognized that the principal cause was an uncontrolled high oxygen supply [10, 80]. Although this view has repeatedly been confirmed, no efficacious prophylactic or therapeutic measure has been available and RLF has become the heaviest burden of modern intensive care treatment [90]. The increasing

survival rate of very low-birth weight infants has led to an increase in the number of blind people [42, 89, 96].

Bronchopulmonary dysplasia (BPD)

A better understanding of the pathomechanism of IRDS has led to the introduction of continuous positive airway pressure (CPAP) ventilation, bringing about a dramatic improvement in the prognosis of the disease [31].

However, BPD, a new clinical entity has appeared on the scene [75]. The condition is a progressive pulmonary fibrosis leading to severe respiratory failure; it is caused, in addition to oxygen therapy, by prolonged mechanical ventilation and patent ductus arteriosus [104].

Hyaline membrane disease or IRDS

This condition is specific to preterm babies and pathological newborns and leads to alveolar hyaline membrane production. The process is related to oxygen toxicity in several respects. Increased catabolism due to the severe condition of the newborn may increase the production of free radicals, and the therapeutic efforts may directly enhance LP. Recently it has been shown that the peroxidative chain reaction triggered by oxygen radicals may play a primary role, since the hyaline membrane is basically a LP product [8]; it can be induced experimentally by ionizing radiation and type II pneumocytes producing the surfactant are damaged by oxygen radicals [2].

Possible methods for surmounting the adverse effects of hyperoxia

Theoretically, there are two possibilities to increase the defence against oxygen toxicity. Antioxidant agents are substances directly neutralizing the free radicals. Other compounds enhance the activity of enzymes counteracting the effect of oxygen radicals and repair the damage caused by lipid peroxidation. Vitamin E is a good example of antioxidants while D-penicillamine plays an active role in both respects.

Vitamin E

Vitamin E has been known for more than fourty years [22] but the exact mechanism of its antioxidant effect has not been clarified [82]. Vitamin E deficiency was observed to lead in certain animals to disorders probably due to increased auto-oxidation, i.e. infertility, encephalomalacia, muscle dystrophy or demyelinisation. Human hypovitaminosis E occurs only in preterm infants or in chronic fat malabsorption [30, 105]. Several authors attribute a primary aetiological role in neonatal oxygen toxicity to the low vitamin E concentration in the preterm babies' tissues [78, 79]. Vitamin E has been applied for its antioxidant effect by several authors; some reported on success in the prevention of BPD [20] or of RLF [45, 39], others have not been able to observe any convincing effect [42, 44, 84, 89, 102]. We feel that Dormandy's remark still holds [18]: "Forty years after its discovery,

vitamin E remains a biochemical Don Basilio, not to be trusted or believed but impossible to dismiss".

D-penicillamine (DPA)

In previous studies we have shown that intravenous DPA is a potent drug in the treatment and prevention of neonatal hyperbilirubinaemia [56, 58]; it was especially effective in haemolytic disease due to incompatibility in the Rh and ABO systems [57]. Its use combined with phototherapy allowed to avoid exchange transfusions in preterm babies affected by hyperbilirubinaemia [7]. A new impetus to our research was the observation of a reduced incidence of RLF among very low-birth-weight infants treated with DPA during the newborn period. This retrospective observation was later confirmed by a comparative study of preterm babies weighing less than 1500 g at birth, 109 of whom were treated with DPA and 86 without the drug [54, 55]. The two groups were shown to be comparable in a number of perinatal factors including those known to increase oxygen toxicity. The incidence of cicatricial RLF was 0.9 % in the treated group while it was as high as 11.6 % among the babies not treated with DPA. Since our first observation in 1979, all preterm babies weighing less than 1500 g at birth and undergoing oxygen therapy have been given DPA treatment. Table I shows our recent results. It is seen that while intensive perinatal care has brought about a marked increase in the survival rate of very low-birth-weight infants, this was accompanied by a sharp increase in the incidence of loss of sight due to RLF. On the other hand, ever since the introduction of DPA treatment, only one case of RLF has been observed and none during the last two years. Mortality, a sensitive indicator of adequate oxygen therapy, has been unchanged during the same period.

I. DPA directly neutralizes free oxygen radicals. DPA is dimethylcysteine and like all compounds contain-

TABLE I

Number, mortality rate and incidence of retrolental fibroplasia in premature infants weighing less than 1500 g at birth, 1977–1981

Year	Intensive neonatal care				
	Without DPA		With DPA		
	1977	1978	1979	1980	1981
All cases	90	142	140	138	134
Deaths, No.	36	56	64	54	51
per cent	40.0	39.4	45.7	39.1	38.1
No. of survivors	54	86	76	84	83
RLF, No.	6	4	1	0	0

DPA was administered to all preterm babies from May, 1979.

ing SH-groups it is a hydrogen and electron donor [21, 60, 74]. It has been shown that the red-violet complex of copper and DPA exerts SOD activity [61]; other authors, however, assumed that not the complex itself but other copper complexes of low molecular weight liberated by gradual decomposition of the whole complex are responsible for the dismutation of O_2^- [86].

It is highly probable that the antioxidant property of DPA is largely due to its chelating capacity, binding heavy metal ions known to catalyse LP [43].

As every thiol-derivative, DPA forms mixed disulfide bonds with serum proteins containing sulfhydryl groups; during this process it undergoes oxidation and in this way prevents the development of free radicals [73].

It has been shown [59] that the radioprotective property of DPA is more expressed in suckling mice than in adult animals.

The fact that the drug markedly decreases the serum bilirubin level, seems to point to its potent antioxidant activity [13].

DPA treatment leads to an increased intracellular concentration of free glutathione [72].

II. DPA increases the activity of enzymes participating in the antioxidant defence of newborn babies. This may explain the age dependent differences in the action of DPA. It was shown earlier [64] that in newborn animals DPA enhances the activity of hepatic peroxidase and catalase while such an effect could not be observed in adult animals. Similar results were obtained concerning lipid peroxidation in erythrocyte membranes, induced by hydrogen peroxide or phenylhydrazine [76].

The age dependence of the DPA effect was shown in rat experiments by measuring hexobarbital sleeping time. A marked reduction of the parameter by DPA treatment could be achieved in newborn animals. In addition, DPA induced an increase in microsomal cytochrome P-450 and led to multiplication of mitochondria in the liver cells only in newborns. In newborn animals, DPA inhibits haemoxygenase, the enzyme initiating haeme catabolism and so it not only inhibited bilirubin production but also ensured the persistence of high levels of the antioxidant enzymes of haemeprotein structure [77].

Recently, direct evidence has been produced of the sight protective effect of DPA, demonstrating that the drug significantly inhibited the proliferative processes in the corpus vitreum [101].

DPA treatment in the newborn may deeply affect some complex biochemical events and as a result it may shorten the postnatal adaptation period and reduce in that way the incidence and severity of some pathological phenomena appearing characteristically in the course of adaptation. In spite of this, many paediatricians are reluctant to apply DPA in newborn babies. This may be attributed to a fear of the unpleasant and dangerous side-effects of pro-

longed DPA therapy observed in some patients affected by rheumatoid arthritis [52]. It must, however, be emphasized that preterm infants are not small adults with rheumatoid arthritis. Even a high dose of DPA administered for some days has no acute side-effect in babies nor could any chronic side-effect be detected by prolonged observations. The drug had no effect on the albumin-bilirubin complex [7], and none of the outward effects described in adults have ever been encountered in our material. To date a total of 2500 newborn babies has been treated with DPA during the last 9 years, without any complication whatever.

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The prognosis of severe bronchial asthma in childhood on the basis of late reexaminations

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Fourhundred and forty one patients treated for asthma between 1954 and 1978 were reexamined. Their age ranged from 15 to 36 years at the time of reexamination. To regard a case as severe, at least four factors out of the following six had to be present:

1. Attacks more frequent than one every week;
2. attacks lasting for more than one day;
3. six or more hospitalizations;
4. absence from school exceeding one month per school-year;
5. drop-out from school for at least one school-year;
6. steroid treatment for more than one week.

59 patients (13.3%) fulfilled the criteria. The sex ratio within the severe group was 1:1 although there was a male preponderance of all asthmatics. The recovery rate for the severe group was lower (40.6%) than for all patients (75.0%). Prognosis was less favourable in female than in male patients.

In the severe group the disease appeared earlier, eczema during infancy occurred more frequently and allergic disorders had a higher incidence in the family than in the group as a whole. Chest deformities were observed in 48.7% of severe patients while its incidence was only 18.6% for all patients with asthma. Acetylcholine-induced bronchial hyperreactivity was more frequent in the severe patients than in all patients irrespective of the degree of severity while the prevalence of exercise induced asthma showed no relationship to the degree of severity. Among the severe cases 31% failed to cope with a standardized physical load.

Childhood asthma varies greatly in severity. The life-style of slightly ill patients hardly differs from that of healthy age-mates, and to these children only slight changes in the way of life, reduction of exposure to environmental allergens and occasional treatment with symptomatic drugs have to be recommended. Severe cases need concentrated medical and social help, have a high drop-out rate at school, and a reduced working capacity narrowing the quality of their life. In this study we have attempted to define severe asthma and to examine the prognostic value of certain factors occurring during childhood.

Judgment of the severity of asthma is not uniform in the literature. The term severe asthma is used for different clinical conditions. The most important factor is the frequency of attacks. Table I shows the classifications used by many authors; it is seen that the criterion of severe asthma varies from three attacks per year up to one or even more per week.

TABLE I

Criteria of severe asthma

Reference	Criteria
Blair 1977	More than 3 attacks/year
Ryssing 1963	More than 10 attacks/year
Dawson 1969	More than 10 attacks/year
Leveque 1972	More than 12 attacks/year
Kraepelien 1963	More than 25 attacks/year
Osváth 1976	More than 25 attacks/year
Vialatte 1972	More than 50 attacks/year
Charpin 1972	More than 60 attacks/year

TABLE II

Incidence of severe asthma in all cases of asthma

	Proportion of severe asthma per cent	Total No. of asthmatic	Note
Leveque 1972	16.0	206	Children
Antonsen 1974	20.0	331	Children
Editorial 1978	20.0	?	Children
Charpin 1972	25.9	589	Children
Pearson 1958	28.0	625	Adults
Vialatte 1972	33.3	741	Children
Mukoyama 1975	35.4	1000	Children
Ryssing 1963	42.0	442	Children

The criteria of Kraepelien [10] are widely accepted; in his classification mild means less than five attacks per year, moderate is characterized by six to ten, severe by eleven to twenty-four and very severe by twentyfive or more attacks per year. In the score system of Osváth [16] not only the frequency of attacks is taken into consideration but also their duration, the severity of wheezing and the number of days with dyspnoea per year; on this basis patients with scores between sixteen and twenty-one are regarded as very severe cases. The annual frequency of attacks has a primary role in this system too, as 12 of the possible 21 points reflect this factor.

It is thus not surprising that the proportion of severe cases shows wide variations. Table II illustrates the discrepancies in the literature: the lowest proportion is 16% and the

highest one 42%. Other factors too influence the proportion of severe cases, thus the ratio of children and adults in the material, whether the patients are collected from a geographic area or from a special hospital department [2], and whether they are hospitalized or out-patients [11].

The clinical characteristics, the long-term course and the late prognosis of severe asthmatics were investigated by several authors. Severe asthma usually has an early onset [4, 5, 18], simultaneous or previous eczema foretells a bad prognosis [5], chest deformities, especially barrel chest and pigeon chest, develop more frequently in severe patients [18].

Growth and weight development may be impaired either by the disease itself or by the prolonged steroid treatment [7, 17, 18]. Asthmatics with severe complaints, are practically never free of symptoms [8] and they are usually on long-term steroid treatment [13, 17]. In this group of asthmatics the positive skin test, especially that for house-dust, frequently persists into adulthood [3, 5].

The relationship between the severity of asthma and the gender of patients is not clear. Some authors [1] believe that the overwhelming majority of severe cases are males while others [2] found a better recovery trend in males. Pearson [14] observed no difference in severity between the two sexes.

Similar differences prevail in the recovery outlook of severe asthmatics. In a study [3] checking the childhood cases twenty years later, the recovery rate was 69% in mild cases and 33% in the severe group of patients. Cases classified as severe during childhood exhibit a reduced tendency to recovery during puberty, and so the relative proportion of severe cases increases with age. A high frequency of attacks predicts a bad prognosis in adulthood [11, 14].

Death from asthma occurs nearly exclusively among severe patients. In a series of 11,646 mild and moderate cases collected in Japan [12] there was a single fatal case while eleven of 354 severe cases in the same series died of the disease.

Among severe asthmatics exercise induced asthma has a higher incidence but the severity of the latter and that of the disease itself shows no close relationship [7, 9].

In this work we have studied the severe cases of our material by comparing their anamnestic, clinical and prognostic findings with an unselected group of asthmatic children treated over the same period of time.

Material

During the twenty-five years 1954–1978, more than 1200 asthmatic children were treated in our Department. Of these, we selected those who had completed 14 years of age in 1979; they were adolescents or young adults with their age ranging from 15 to 36 years. The home address of 525 such patients was available and 441 of them (84%) answered our letter; 381 reported for examination an ients or their parents completed a questionnaire. In the latter case eventual dubious answers were clarified by correspondence. The rea-

sons for their absence were as follows: 5 patients had died, the others were lactating or doing military service or were abroad. In the patients reporting at our clinic the tests described in detail below were carried out. In some exceptional cases not all examinations were performed, physical loading and the acetylcholine provocation test had to be omitted in some pregnant mothers, physical loading was omitted in two men who had been injured or had an ankylosed knee. Six patients refused to have skin tests.

Methods

Every patient had to complete a questionnaire. The answers given were thoroughly examined in the presence of the patient, anamnestic data were checked by comparison with the original history and eventual corrections were made. We had the opportunity to talk to one or both parents of 40% of the patients.

Physical examination was performed and the eventual chest deformity noted. Prick tests were carried out with Bencard allergens, in the evaluation of results the prescriptions of the makers were observed. The following allergens were used: A_1 and A_2 mixed inhalants, house-dust, house-dust mite, feathers, horse, cat and dog hairs, hay-dust, straw-dust, mixed threshing powder, cotton flakes, grass pollen B_2, tree pollen B_3, flower and shrub pollen B_5, nettle, plantain, mixed moulds A_{13}, M_{10}, and M_{11}. The acetylcholine provocation test was performed by three minute inhalation of 1% nebulized acetylcholine. Vital capacity (VC) and forced expiration volume during 1 sec (FEV_1) were registered with a Spiroscreen 11 device before and three, five and 10 minutes after provocation.

Physical loading was performed by the help of an ergometer; 1 Watt/kg was applied over 6 minutes. VC and FEV_1 values were registered before and three, five and ten minutes after loading. Some patients did not tolerate the load and gave

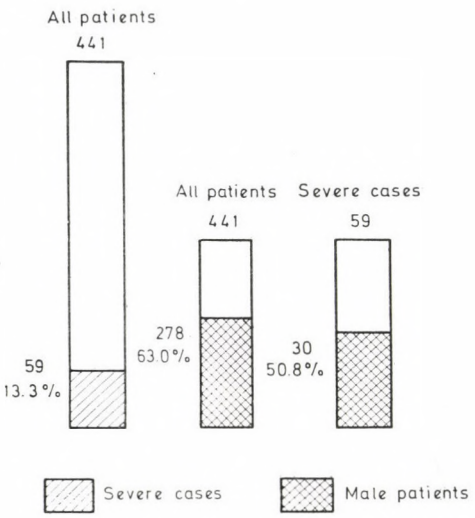

Fig. 1. Prevalence of severe asthma among all patients with asthma. Criteria of severity: 1. Attacks once per week or more frequent. 2. Attacks persisting for more than 24 hours. 3. Six or more hospital admissions. 4. Absence from school for more than one month. 5. Drop-out from school for at least one school-year. 6. Steroid treatment for a period exceeding one week. At least four criteria were present

up before completion of the six minutes period; the fact and time were then noted. In both loading tests a decrease of FEV_1 exceeding 10% of the initial value was regarded as a sign of bronchospasm.

The severe cases were selected by the following method. From the data registered the criteria listed in Figure 1 were examined, taking into consideration the duration of attacks and the frequency of complaints. The following events were also considered to indicate a high degree of severity. Six or more hospital admissions, long absence or drop-out from school for a whole year or more. Since we apply steroids according to very strict indications, steroid treatment over a period exceeding one week could also be regarded as a sign of severity. The presence of at least four of the six criteria led us to classify the patient as severe.

Results

Of the 441 patients available for follow-up 59 (13,3%) were classified as severe (Fig .1). As mentioned above, five patients had died (2 from a cause other than asthma, 2 others from asthma and in the fifth fatal case asthma was complicated by chronic myocarditis). The asthmatic deaths were classified into the severe group.

The unselected asthmatics showed a 2:1 male-female ratio (278 men and 163 women), in accordance with the literature. On the other hand, the severe group contained a comparable number of men and women, 30 and 29 respectively.

The criterion of recovery was a complete freedom from asthmatic symptoms without any treatment over a period exceeding one year. Fig. 2 shows the data. The overall recovery rate was 75% while in the severe group only 40.6% recovered; the difference was significant statistically. Separate analysis for the two sexes showed that the unfavourable outcome of severe asthma was due largely to the low recovery rate in

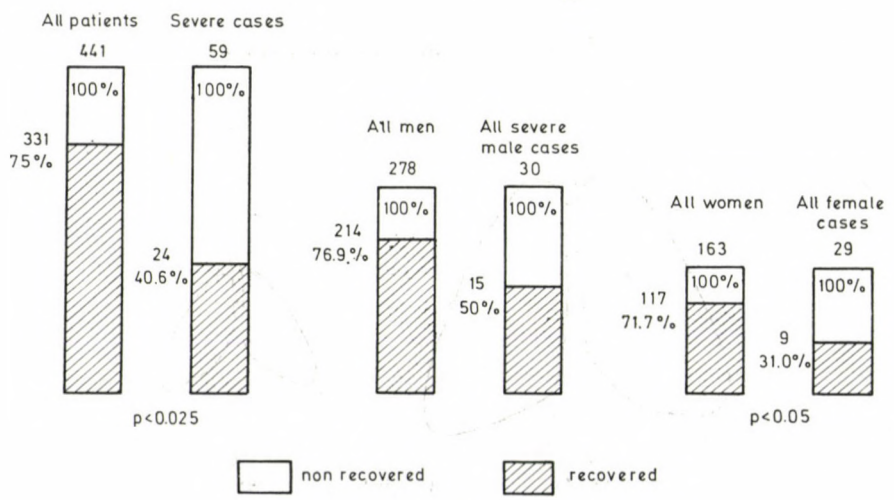

Fig. 2. Recovery rates in asthmatic patients

TABLE III

Data of the history, per cents

	Overall	Severe	
Onset before two years of age	37.9	49.2	NS
Infantile eczema	22.2	28.8	NS
Positive skin test at follow-up	38.9	48.7	NS

NS (Non significant)

women. The proportion of recovery was 50% in severe asthma while the overall recovery rate for men was 76.9%. The corresponding figures for women were 31 and 71.7%, respectively.

Table III presents the data in the history. A higher proportion of severe asthma patients had experienced the onset of the disease before completion of the second year of life and had had eczema during infancy. Positive skin

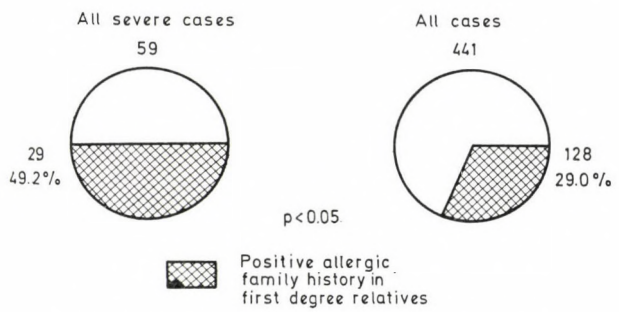

FIG. 3. Family history of asthmatic patients

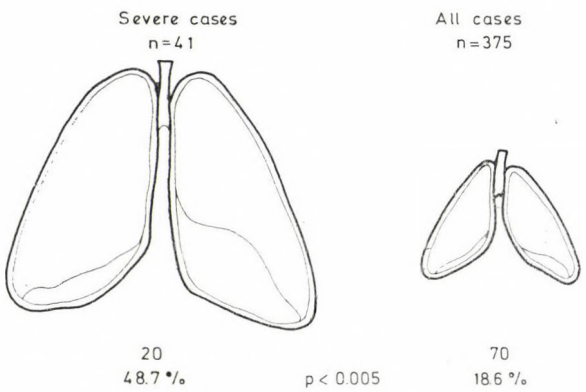

FIG. 4. Incidence of chest deformities

tests at follow-up in adult or adolescent age were more frequently encountered in the severe group. These differences were, however, not significant statistically.

The incidence of allergic diseases in close relatives (Fig. 3) was higher in the severe than in the moderate group. No distinction was made between the atopic (asthma, pollinosis, eczema, etc.) and non atopic (urticaria, drug hypersensitivity, etc.) forms of allergy.

Within the severe group a much higher proportion of the patients exhibited chest deformity than in the whole material (Fig. 4). Among the 375 patients appearing at follow-up, 55 had a slight and 15 a grave chest deformity while among the 41 severe

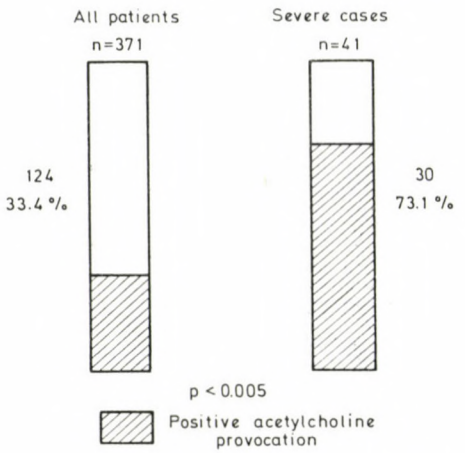

FIG. 5. Acetylcholine provocation

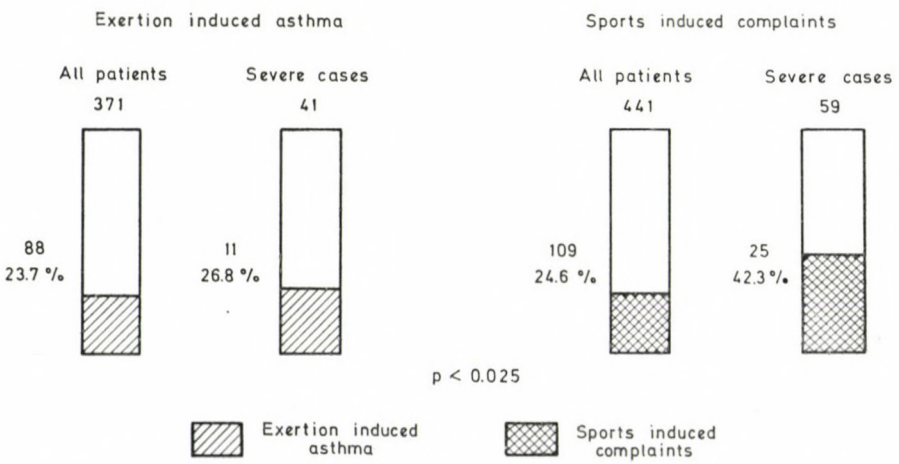

FIG. 6. Physical exertion. 13 severe cases could not complete the test

cases there were 15 with slight and 5 with severe chest deformity. The difference was significant statistically.

Fig. 5 shows the results of acetylcholine provocation at follow-up: again, a larger number of patients had a positive reaction in the severe than in the moderate group.

Physical exercise including sports caused complaints in a higher number of severe patients than in the whole material (Fig. 6). Out of the 441 patients 93 complained of dyspnoea and 16 of asthmatic attacks elicited by exercise while of the 59 severe patients 18 respectively 7 displayed such complaints. The bicycle ergometer test revealed that exercise induced asthma was hardly more frequent among the patients labelled as severe. It is noteworthy, however, that of the severe patients 13 (31.0%) were unable to complete the 6 minute load; they soon became tired and they also lacked the self-confidence needed for the task. For the overall data this proportion was 10% (38 cases).

Discussion

The main purpose of this study was to select the really severe cases of childhood asthma. Our principle of selection, the occurrence of at least four of the six criteria, can only be applied in a given material and for a given period of time. The frequency and severity of attacks has always been a good indicator of grave asthma. The other criteria such as the duration of absence or the drop-out from school and frequent hospitalisations prolonged steroid treatment have become comparatively unfrequent. Still, we feel that by our principle of selection all grave asthmatics in the material could be classified into the severe group, including all the children who had died of asthma and all the adults who were incapable to work. By the score system of Osváth [13], all our cases considered severe by our own criteria had a score exceeding 15, i.e. they would have been classified as very severe.

It may be argued that the patients' memory could fail in answering questions pertaining to events 10 or even 25 years back in the past. We are however certain that they did correctly remember their drop-out from school, their treatment in sanatoria and the long-term steroid treatment since these events must have left deep traces in their or their parents' memory.

Comparing the various parameters in the severe group with those observed in the whole asthmatic material, it turned out that the severe patients form a distinct group in several respects.

The proportion of severe cases amounted to 13.3%, with an equal representation of the two sexes. This markedly differed from the 2:1 male-female ratio found in our unselected asthmatics and in reports in the literature.

The long-term prognosis of severe asthma especially in female patients

is much worse than the average. An earlier onset, a higher incidence of infantile eczema in the patients, of allergic diseases in the close relatives, and of positive skin tests at follow-up are all characteristics of the severe group.

The consequences of asthma notably the chest deformity, especially its severe forms such as a barrel chest or a pigeon chest occur with a higher than normal frequency in patients having had severe asthma during childhood. In addition, bronchial hyperreactivity, as revealed by the acetylcholine provocation test, persists in a higher proportion of severe than of moderate asthmatics. Exercise induced asthma does not occur with increased frequency in the severe group but these persons cannot endure physical exercise and so they rarely participate in sports or other physical activities.

All this forces us to concentrate medical help and social assistance to the severe group. The criteria described in this study may help in selecting severely ill children from the patients affected by asthma in general. They must be advised concerning their way of life and the choice of profession [6]. The family members must be instructed about the importance of rational body exercise, holidays under a favourable climate, the avoidance of smoking by the patient and his environment, etc. Environmental antigens must be kept within reasonable borders, and pets like dogs, cats or guinea-pigs should not be kept by the child.

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Book reviews

Pancreatic Disease in Clinical Practice. Edited by C. J. MITCHELL, J. KELLEHER. XV + 432 pages. Pitman, London 1981. Price £ 20

This book contains the papers given by invited speakers, most of them English, at a symposium held at Leeds in 1980. It is an up-to-date piece of work containing most of the recent advances in the field, concentrating especially on the diagnostic possibilities. At the same time the contents mirrors clearly that in spite of the progress in diagnostics, the treatment of the diseases of our most hidden organ is still in its infancy and has made practically no advance in the last 50 years.

It will be interesting to mention some remarks of the authors on the most recent attempts. Thus tomographic images of function with ^{11}C-agents (instead of ^{75}Se-selenomethionine) are a promising possibility if a cyclotron is near. There is an extensive chapter on ultrasonography with instructive figures but false positive results in normal cases range from 0 to 28%, and false negatives in cancer of the pancreas from 0 to 33%. The great possibilities of CT achieved with current apparatus having scan times between 1 and 5 seconds are enumerated; quite small tumours can be detected but distinction between chronic pancreatitis and cancer is not always possible. Angiography has lost much of its importance but may be useful if the other tests are not in accordance with clinical evidence. Laparoscopy: its only real advantage is said to be its information concerning hepatic and peritoneal metastases, sparing the patient the discomfort of an unnecessary laparotomy. Cytological examination of duodenal juice and even more of pure pancreatic juice obtained by endoscopic retrograde cholangiopancreatography is deemed important but percutaneous fine needle aspiration should only be considered when an enlarged pancreas or mass has been identified radiologically of by sonography. In this case, however, advantage is given to biopsy (with the Trucut needle instead of the Vim-Silverman one) at laparotomy.

Among the pancreatic function tests, the oral ones deserve attention. Of these, the synthetic tripeptide (BTP) administered with a Lundh test meal and releasing PABA is treated in some detail together with Kaffernik's fluorescein dilaurate test the use of which is still limited to some German centres. These tubeless tests might prove useful but for the time being such optimism must be guarded. The same seems to be the conclusion voiced at the symposium by a team from Göttingen University.

As to children, some interesting points have been mentioned. A few years ago it has been reported that a reduction of gastric acid output by the use of cimetidine improves lipid solubilization and the performance of administered pancreatic enzymes. Now we read that in an unpublished study by Matthew and Cameron the increase in steatorrhoea in two children did not occur at least six months after stopping the cimetidine. As an explanation it is

assumed that the reconstitution of the bile salt pool might be an important factor. Little progress has been made in the therapy of acute pancreatitis since the introduction of peritoneal lavage more than 15 years ago and on the whole the same should be said of the treatment of chronic pancreatitis, be it conservative or surgical. And as regards the cases of pancreatic cancer the incidence of which now has reached 10% of all cancers with less than 2% of the patients surviving five years, the best advice is still the old one that efforts should be directed to early detection.

P. V. VÉGHELYI

D. MÜCKE, J. BARTEL: *Pädiatrische Elektrokardiographie.* Band 2., Teil III: *Diagnostisches Vademekum und Atlas der klinischen Elektrokardiographie im Kindesalter.* 351 Seiten mit 275 Abbildungen und 11 Tabellen. Georg Thieme Verlag, Leipzig 1981. Preis M 49,—

Im ersten Band wurden die elektrophysiologischen Grundlagen der Entstehung der Herzstromkurve, deren Eigenheiten im Säuglings- und Kindesalter, die Bewertungsmethoden in 5 Altersgruppen gegliedert (Frühgeborene, Neugeborene, Säugling, Kleinkind, Schulkind) erörtert und die Störungen der Reizbildung und Reizleitung geschildert. Jene Zielsetzung wurde also nicht überschritten, dem Leser gerade soviel Wissensmaterial zu übermitteln, daß er in der Lage sei, ein physiologisches von einem pathologischen EKG zu unterscheiden und letzteres in großen Zügen zu bewerten.

Dem Untertitel entsprechend, bietet der vorliegende Band 2 umfassendere Kenntnisse, die den Kinderarzt zur kompletten Interpretation einer Kurve befähigen sollen. Zu diesem Zweck werden einleitend die Vektortheorie, die verschiedenen Ableitungssysteme, auch die plenothorakalen Ableitungen nach Nehb oder die korrigierten orthogonalen Ableitungen nach Frank behandelt.

Auf etwa 250 Seiten werden in Atlasform 206 komplette Kurven ausführlich besprochen. Jeder EKG-Kurve schließt sich eine kurze Bewertung des klinischen Status und des Elektrokardiogramms an, letztere aufgrund der im ersten Band zusammengefaßten Gesichtspunkte. In diesem Teil sind etwa 85 Kurven den angeborenen Herzfehler gewidmet, die übrigen demonstrieren die Veränderungen bei Lageanomalien, Elektrolytstoffwechselstörungen, Digitalismedikation- und intoxikation, Kardiomyopathien, entzündlichen Krankheiten des Herzens, Herztumoren. Ausführlich werden auch die Herzrhythmusstörungen, faszikuläre Hemiblöcke und Blockkombinationen im Kindesalter behandelt. Abschließend wird auf das EKG unter Provokationsbedingungen und sportlicher Belastung hingewiesen.

Eine nützliche Zusammenstellung von Dia- und Nomogrammen und ein Literaturnachweis schließen das Buch, das sich für den in der Kinderkardiologie tätigen Arzt als ein wertvolles Vademekum erweisen wird.

J. KAMARÁS

BIER O. G, DIAS DA SILVA W, GÖTZE D, MOTA I: *Fundamentals of Immunology.* Springer-Verlag, Berlin–Heidelberg–New York 1981. 442 pages with 164 figures. Price DM 48,—

As stated in the Preface, this textbook of basic and clinical immunology has been written for medical students. The authors seem to have underestimated their production: the book with its clear and concise style, a detailed glossary, a list of the immunological discoveries by year and authors, is an outstanding source of data for everybody interested in the subject.

The first five chapters discuss the tissues of the immune system, the activity of immune cells, the antigens, the antibodies

and the complement. Then follows a chapter on the major histocompatibility complex embracing almost all of the recent knowledge. The next chapter deals with the antigen-antibody interaction; it gives an informative survey of the reactions for qualitative and quantitative assay, among others. The subjects discussed in the remaining chapters are the blood groups, transplantation and hypersensitivity. This last one discusses the four types of allergic reaction according to the Coombs and Gell classification. The demonstratively clever figures and drawings illustrating the book deserve to be mentioned separately.

E. Cserháti

Pädiatrische Intensivmedizin. II. Herausgegeben von P. Lemburg. (Schriftenreihe INA Band 27) XVI + 208 Seiten mit 124 Abbildungen und 100 Tabellen. Georg Thieme Verlag, Stuttgart 1981. Preis DM 90,—

Der Band faßt das Material des 1978 in Düsseldorf veranstalteten Symposiums zusammen. Die Hauptthemen: Vergiftungen, Respiratorbehandlung, Krankenbeobachtung, kardiologische Probleme, Schock, Intensivtherapie in der Kinderchirurgie, epidemiologische organisatorische und gesundheitspolitische Fragen.

Seit 1970 war das wohl das fünfte derartige Treffen der deutschen Fachärzte; die Behandlung der obigen vielversprechenden Themen scheint aber dieses Mal etwas blasser zu sein, und man hat den Eindruck, daß nach einem Jahrzehnt der initiale Schwung auf diesen Gebieten von einem Innehalten abgelöst wurde. Statt einer ausführlichen Besprechung soll hier lediglich auch einige zukunftverheißende Gedanken hingewiesen werden.

In der Technologie der Respiratorbehandlung muß das Erscheinen der Mikroprozessoren als Neuheit hervorgehoben (Heinrich) und auch eine neue Betrachtungsweise des Verhältnisses Maschine-Patient (Frankenberger) erwähnt werden. Die sich aus diesen ergebenden Möglichkeiten dürften zur Zeit eher eine theoretische Note haben, solange das Tempo der Erneuerung des Maschinenparkes jenes des Veralterns desselben nicht übertrifft.

Die vielen Variationen der heutigen Beatmungstechnik dienen dem Zweck, die nachteiligen Folgen — z.B. Barotrauma — abzuschaffen. Müller und Schober berichten über die Anwendung von Niederfrequenz bei den schwersten RDS Fällen, womit die Mortalität wesentlich herabgesetzt werden konnte. (Das Wuchern der bezüglichen Terminologie ist geradezu störend geworden; mit einer ähnlichen Benennung wurde 1980 von Gattinoni eine Methode beschrieben, die nur in ihrer Zielsetung der eben erwähnten ähnlich ist.) Hervorzuheben seien ferner die Untersuchungen von Linderkamp: bei wegen IRDS beatmeten 16 Neugeborenen konnte eine mit der Erhöhung des Ausatmungsenddruckes einhergehende ansteigende Depression des peripheren Kreislaufes beobachtet werden, die besonders bei gleichzeitiger Hypovolämie bedeutend war. Auf diesen, im Erwachsenenalter wohlbekannten Umstand soll unbedingt gedacht werden, da ein herabgesetztes Minutenvolumen nicht nur den entsprechenden Gasaustausch der Gewebe — das Ziel der Behandlung — gefährdet, sondern durch die erhöhte venöse Stauung mit einer Häufung intrakranialer Blutungen einhergehen kann. Die Autoren betonen, daß ein Ausatmungsdruck über 4 H_2O nur dann angewandt werden darf, wenn der Verdacht der Hypovolämie ausgeschlossen werden kann.

Die Ergebnisse der Computer-Trendanalyse bei Neugeborenen in schwerem Zustand wurde von Frank und Pachal besprochen. Dieser Beitrag zeigt die zukünftigen Wege der Diagnostik, wobei die in der Industrie eingebürgerte Computertechnik zur Bewertung des Zustandes und der Veränderungen des Neugeborenen verwertet wird; dieses komplexe Autore-

gulationssystem führt wohl zu einer gewissen Automatisierung der ganzen Intensivtherapie.

I. SZÁNTÓ

BLAUTH, W. SCHNEIDER–SICKERT: F. *Congenital Deformities of the Hand. An Atlas of Their Surgical Treatment.* XV + 387 pages with 426 figures (most n colour). Springer-Verlag, Berlin–Heidelberg–New York 1981. Price DM 440,—

In the past 15 years great advances have been achieved in the surgical correction of congenital malformations of the hand. The progress was partly due to the thalidomide catastrophe as the many children with phocomelia and other malformations were probably responsible for it and also for this book which originally was published in 1976 in German under the title Handfehlbildungen. As Professor Buck–Gramcko, the well-known Austrian hand surgeon then wrote, up to that time there existed no such book in the German literature and a slightly revised text of this atlas-like work has now been published in English.

The work has been written for the practising surgeon. It is based on the own vast experience of its two authors and it only describes operations and techniques that have presented satisfactory long-term results. The many figures serve to explain every move to be done from the incision to the last suture and the most important steps are marked in red. In addition, a written text explains in detail the technique to be followed and, what is more, it lists the possible errors and their consequences. The 16 chapters of the book discuss after an introduction dealing with general principles and technical details, the syndactylies, hypoplasias, variations in number and size of the fingers, cleft hand, trigger thumb, hereditary exostoses and the enchondromas.

All in all, the authors have succeeded in filling an important gap with their atlas. It will be very useful and offer excellent guidelines in the surgical treatment of the deformities of the hand.

J. MANNINGER

H. HARBAUER, R. LEMPP, G. NISSEN, P. STRUNK: *Lehrbuch der speziellen Kinder- und Jugendpsychiatrie.* 4., neubearbeitete und erweiterte Auflage. XVI + 535 Seiten mit 54 Abbildungen und 12 Tabellen. Springer-Verlag, Berlin–Heidelberg–New York 1980. Preis DM 124,—

Das vorbildlich verfaßte, einheitlich und verständlich dargestellte Lehrbuch hat in zehn Jahren seine vierte Auflage erlebt. Die Fülle von neuen Erkenntnissen und Betrachtungsweisen auf dem auch wegen sozialen Interessen so wichtig gewordenen Gebiet der Kinder- und Jugendpsychiatrie haben die überarbeitete vorliegende Auflage notwendig gemacht. Der Inhalt einiger früher nur kurz berührten Teilen, wie z.B. Verwahrlosung, Schulfähigkeit oder Suizide ist beträchtlich angeschwollen, und viele neue Momente findet man hinsichtlich der kindlichen Entwicklungspsychologie und den psychischen Störungen. Neue Gesichtspunkte melden sich in bezug auf die psychischen Erscheinungen und Störungen bei chronischen und malignen Krankheiten.

Ein ausführliches Kapitel befaßt sich mit Elternberatung, wobei betont wird, daß die psychisch gestörten Eltern der geschädigten Kinder auch behandelt werden müssen.

Das Kapitel über die Störungen der Intelligenz ist mit Anspruch auf Vollendung zusammengestellt. Dieser Teil des Buches überschreitet den Rahmen eines Lehrbuches und kann als Handbuch betrachtet werden. Das bezieht sich auch auf das Kapitel der so aktuell gewordenen organischen Psychosyndrome.

Ein Kapitel befaßt sich ausführlich und mit Hilfe erschreckender Statistiken mit der jugendlichen Drogen- und Alkoholabhängigkeit, ein anderes ist der jugendlichen Kriminalität und den diesbezüglichen präventiven- und Strafmaßnahmen gewidmet.

Jedem Kapitel ist ein weiterführendes Literaturverzeichnis beigefügt.

Das in seiner Auffassung moderne, klar redigierte Werk können alle zu Rate ziehen, die sich mit dem Kind als Kinderarzt, Psychiater oder Neurologe, oder aber als Sozialbetreuer befassen.

L. CSÉPÁNYI

H. HAUPT, *Das Neugeborene. Untersuchung. Diagnose, Therapie. Ein Leitfaden für die Praxis.* 3. überarbeitete Auflage. XII + 188 Seiten mit 89 Abbildungen und 19 Tabellen. Georg Thieme Verlag, Stuttgart–New York 1981. Price DM 68,—

The third edition of this booklet represents, in fact, a practical guide in the examination, diagnosis and therapy in the newborn. Particularly interesting and useful is the chapter dealing with the diagnostic work-up based on the presenting symptoms. This is followed by a section dealing with the clinical manifestations of the various adaptation disturbances. The author also focusses on the examination techniques of the various organ systems, and a number of photos and drawings make it easy to understand the descriptions of the techniques. Well-constructed tables give a clear survey of the differential diagnosis of the most important pathological conditions.

This little book will serve well the needs of the students and paediatricians interested in practical perinatal medicine. I also recommend it to those who are in training to acquire skill in neonatal intensive care.

J. MESTYÁN

L. WILLE, M. OBLADEN: *Neonatal Intensive Care.* XX + 238 pages with 49 figures and 76 tables. Springer-Verlag, Berlin–Heidelberg–New York 1981. Price DM 49,50

The original edition of this book appeared in 1977 in German under the title Neugeborenen-Intensivpflege. It was a manual mostly containing practical hints and technical solutions as practised in the neonatal intensive unit of the Paediatric Department of Heidelberg University. The book was a success and its revised edition published in 1979 has now been translated into English, with some recent revisions and useful additions.

The book is somewhat of an exception among the many works on intensive treatment published in almost every country year for year, in that it wastes little or no time on explaining the underlying causes and the pathomechanism of some clinical pattern. It only describes the symptom, its diagnosis and features, tabulating sometimes the latter, and proceeds to detailing the therapy. In this way the chapters are extremely short, for instance that on the disturbances of electrolyte balance takes together with a one-page list of up-to-date references not more than nine pages and the chapter describing the infections takes even less space. Besides, as far as the reviewer was able to see, nothing important is missing and there are very short but always correct answers to every problem arising in the course of intensive care. In addition we find an Appendix containing very detailed and precise technical instructions, even on catheterization of the superior vena cava and the technique of drainage of a pneumopericardium, further a list of the necessary instrumentarium and of the drugs and their doses to be applied, statistical data of the results of neonatal intensive care including its economic aspects, and on the parents' reaction to the birth of a sick infant and his death.

To end with, it is perhaps worthwhile and by all means illuminating to quote one sentence from this appendix. The cost

of neonatal intensive care amounts to DM 600 in Germany and that of a baby of less than 1000 g from admission to discharge to $ 40,000 in the USA. Still, if only a single serious brain injury is prevented each year by a single six-bed unit, all its expenses are paid for in the economic sense.

Elisabeth VÁRADY

Pädiatrie: Weiter- und Fortbildung. Herausgegeben von H. EWERBECK. *Neuropädiatrie* (Redaktion: F. HANEFELD). XII + 102 Seiten mit 5 Abbildungen. Springer-Verlag, Berlin–Heidelberg–New York 1981. Preis DM 19,80

In der Reihe »Pädiatrie« finden wir zur Weiterbildung dienende Arbeiten, die den in der Praxis tätigen Kinderarzt über die zeitgemäße Diagnostik und Therapie auf einem Gebiet der Kinderheilkunde informieren; und tatsächlich sichert jede dieser Publikationen die entsprechenden Kenntnisse, die der Arzt ohne Zeitverlust und ohne unwesentliche Einzelheiten erwerben möchte.

Das vorliegende Büchlein befaßt sich mit den drei aktuellen Themen der Kinderneurologie: die Neurolipidosen, die kindliche muskuläre Hypotonie und die Enzym- und Proteindiagnostik des Liquors.

Unter Neurolipidosen versteht man jene angeborene Defekte des Lipidstoffwechsels, die zu Schädigungen des zentralen oder peripheren Nervensystems führen. Diese Erkrankungen sind zur Zeit unheilbar, können jedoch mittels pränataler Diagnostik erkannt werden, so daß sich die Möglichkeit zur genetischen Beratung bzw Prävention bietet.

Die Ursache der muskulären Hypotonie kann eine geerbte oder erworbene Schädigung sein. Das Kapitel bespricht die Pathophysiologie und Klinik der Erkrankung, auf die zerebral verursachten Formen wird nicht eingegangen.

Aus dem Liquor werden meistens die bei Serumuntersuchungen üblichen Enzyme — GOT, LDH, CPK — geprüft. Neben der radialen Immunodiffusion (RID) ist heute die Elektroimmunodiffusion (EID) die präziseste Methode zur Bestimmung der Einzelproteine im Liquor. Leider ist der Nachweis der Enzyme oder spezifischen Proteine der Nervengewebe aus dem Liquor nicht geeignet, entsprechende eindeutige diagnostische Beweise zu sichern (z.B. frühe Erkennung eines Hirntumors), dennoch bietet ihr Kenntnis ergänzende Stützpunkte zur Beurteilung des klinischen Bildes.

Es wird betont, daß sich eine detaillierte neurologische Diagnostik nur auf die allgemeine pädiatrische Untersuchung stützen darf. Das Buch ist in diesem Sinne an den mit neurologischen Problemen konfrontierten Kinderarzt gerichtet.

J. SZÉNÁSY

R. SACHSENWEGER, U. SACHSENWEGER: *Sehübungen. Ein Bilderbuch.* 6., stark veränderte Auflage. 100 Seiten mit 96 Abbildungen. Georg Thieme Verlag, Leipzig 1981. Preis M 9,60 und Gustav Fischer Verlag, Stuttgart 1981. Preis DM 16,80

Nichts deutet den Erfolg und Wert des vorliegenden kleinen Büchleins besser an, als daß es sich hier schon um die 6. Auflage handelt. Es ist für 4–10jährige Kinder bestimmt, mit dem Ziel, diese in einer spielerischen Form zum bewußten Erkennen, Deuten und zur Zusammenarbeit von Auge und Hand zu erziehen. Mit Aufgaben verschiedener Schwierigkeitsgrade soll die Sehleistung, Intelligenz, Konzentrationsfähigkeit und das logische Denken des Kindes gefördert werden. Die Bilder sind nicht farbig, doch interessant und entsprechen hinsichtlich Thema und Zeichentechnik der kindlichen Gemütsart. Bei einigen Aufgaben muß das Kind zwischen zwei nahezu identischen Bildern die Abweichungen, bei anderen unter verschiedenen Bildern die identischen Elemente finden, oder aber sich in einem

Labyrinth orientieren. Auch die optisch täuschenden Abbildungen können das Interesse der Kinder erwecken und ihnen Spaß bereiten. Am Ende des Buches kann mit Hilfe von Bildreihen die Entwicklung des Sehvermögens kontrolliert werden.

Das Bilderbuch kann normal- und schwachsichtigen Kindern gleichfalls zum häusliche Üben empfohlen werden.

Edith SZALAY

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CONTENTS

Assessing obesity with body weight and height. *M Amador, C Rodriguez, ME González, J Bacallao* 381

Diagnosing and misdiagnosing malnutrition. *M Amador, ME González, L Córdova, N Pérez* 391

Effect of exercise on the lipid parameters in the fasting state and under glucose load. *A Czinner, L Barta, M Tichy* 403

Ring chromosome 15. *P Kiss, M Osztovics* 409

Abnormal dermatoglyphics in absence of thumb. *L Borbolla* 417

Antenatally diagnosed thanatophoric dysplasia. *Z Tóth, J Vachter, G Szeifert, Z Nemes, K Csécsei, O Török, A Harsányi, Z Papp* 423

Congenital postural deformity association. *I Pazonyi, A Kun, A Czeizel* 431

HLA antigens in bullous epidermolysis, congenital ichthyosis and ectodermal dysplasia. *A László, G Kaiser, M Simon* 447

Neonatal oxygen toxicity and its prevention: D-penicillamine offers benefits without harmful side-effects. *G Oroszlán, L Lakatos, L Karmazsin* 459

The prognosis of severe bronchial asthma in childhood on the basis of late reexaminations. *E Cserháti, G Mezei, J Kelemen, G Póder, A Gegesi* 473

Book reviews 483

Index: 26.021

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