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

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

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

Organization of the screening programme

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

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

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

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

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

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

Material

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

Methods

Guthrie-test

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

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

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

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

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

Supplementary tests

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

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

Results

Differential diagnosis of hyperphenylalaninaemia

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

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

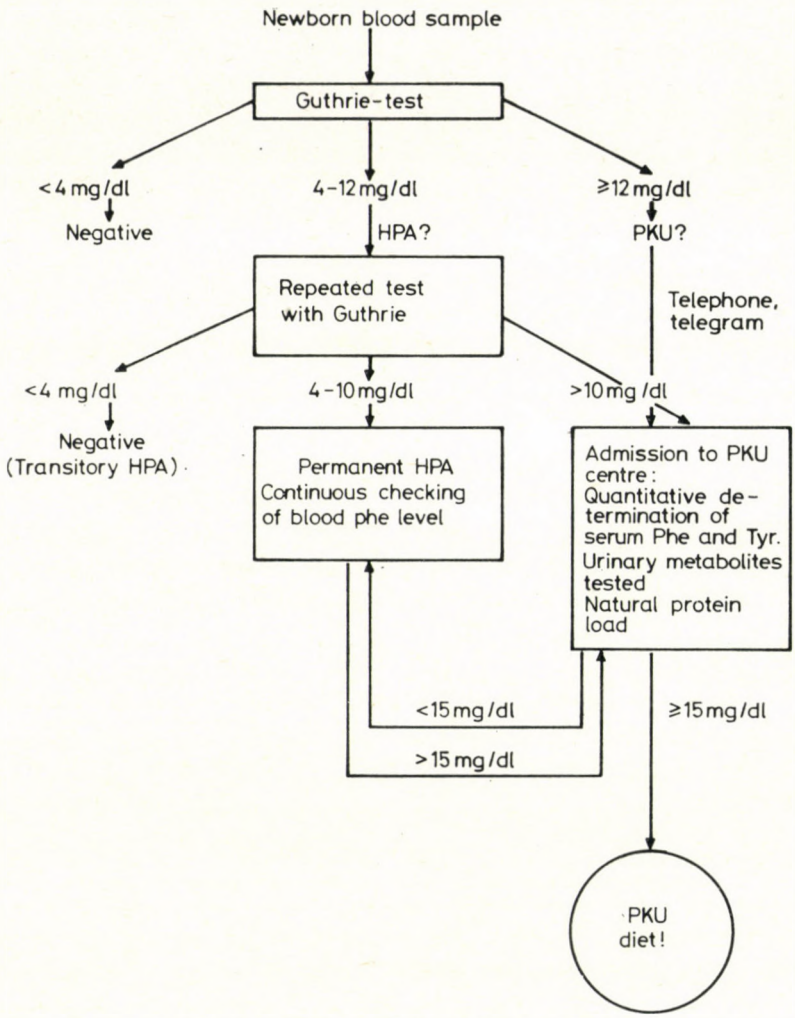

FIG. 1

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

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

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

Classical PKU

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

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

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

Table I

Distribution of various types of hyperphenylalaninaemia among 800 000 screened newborns

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

+: died

Table II

Result of Guthrie test in classical PKU patients at screening

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

TABLE III

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

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

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

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

Atypical PKU

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

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

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

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

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

Permanent benign hyperphenylalaninaemia

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

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

Incidence of hyperphenylalaninaemia

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

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

TABLE IV

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

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

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

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

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

Current practice of care

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

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

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

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

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

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

TABLE V

Preliminary evaluation of therapeutic effect

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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