Some recent advances in inborn errors of metabolism : proceedings of the fourth symposium of the Society for the Study of Inborn Errors of Metabolism, held in Dublin, July 1966 / edited by K.S. Holt and V.P. Coffey.

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Some Recent Advances in Inborn Errors of Metabolism

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SOME RECENT ADVANCES IN INBORN ERRORS OF METABOLISM

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Some Recent Advances in Inborn Errors of Metabolism

Proceedings of the Fourth Symposium of The Society for the Study of Inborn Errors of Metabolism held in Dublin, July 1966

EDITED BY
K. S. HOLT and V. P. COFFEY

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PREFACE

The Society was more ambitious in 1966 than in previous years. The annual symposium was held across the seas in Dublin and lasted two whole days. It was well attended. The scientific papers were stimulating and original and the discussions were keen and informative. The excellent hospitality of our Irish hosts and friends provided fine support to the meeting and made it a memorable occasion.

It is our pleasure and privilege now to present the proceedings of this symposium to a wider audience. Our task has not been easy as we have had to devote considerable time to the preparation of the papers and discussion to reach what we hope readers will feel is a reasonably clear and uniform presentation. We are grateful for the help of colleagues in this work. Any errors and misrepresentations must be the editors responsibility, but we hope that these are not too numerous and are offset by the final result. Despite this work we can say that we have enjoyed the task if for no other reason than the satisfaction of promoting communication and understanding between professions, which is the primary aim of the Society.

It is a pleasure to acknowledge the support received from Welfare Foods Ltd., Stockport. Many of the tables in this publication were set by Friden Inc. Our publishers have throughout been most helpful and encouraging. To these, and to all the others who made this Symposium and these proceedings possible we tender our grateful thanks.

London, 1968 K.S.H. and V.C.

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THE ROLE OF PTERIDINES IN METABOLIC DISORDERS

W. JACOBSON

Introduction

PTERIDINES are co-factors in many important metabolic processes. In fact, it may not be an exaggeration to say that their role in the synthesis of essential compounds is as vital for the maintenance and growth of cells as the part played by the citric acid cycle and glycolysis in the energy supplying steps, or as indispensable as the cytochromes in cellular respiration.

The question may well be asked: why discuss co-factors of enzymic reactions when we are confronted with inherited defects of the protein moiety—the apo-enzyme—and not with defects of the co-enzyme? However, on closer consideration of the problem a more complex picture emerges.

Many, but by no means all inherited biochemical defects involve the protein moiety of an enzyme. Only rarely do we deal with a *complete* absence of enzyme activity. This may be brought about by two different processes; either the synthesis of a specific protein (or the apo-enzyme) is severely repressed, or a faulty enzyme is made in the cells in either normal or possibly even larger quantities, due to abnormal coding. These possibilities appear to have been studied in detail in only a very few of the inherited defects.

The much more frequently encountered situation involves low enzyme activity, due to either repression of synthesis or faulty amino acid sequence in an 'imperfect' apo-enzyme. The supply of the co-enzyme in surplus may drive the reaction faster by two means; either by completely saturating the enzyme, so that every apo-enzyme molecule is present as a functioning holo-enzyme; or, if the abundance of co-factor affects the repressor system, (which may be geared—though faultily—to the amount of *free* apo-enzyme) by combining every free apo-enzyme with its co-enzyme, the concentration of free apo-enzyme within the cell would be lowered effectively and thus the synthesis of further apo-enzyme would be de-repressed. This would result also in an increase in enzyme activity. Finally, there is a third possibility, that a low enzyme activity may be caused by a fault in the synthesis or supply of the co-factor itself.

As none of these mechanisms have been studied in detail in the relevant inborn errors of metabolism, it is not inappropriate to draw attention to the possibility that an abundant supply of co-factor may be of therapeutic value in a few of the inherited defects. For this reason it is worthwhile to discuss the role of pteridines in metabolic disorders and among these I shall include the acute leukaemia of children, though this is unlikely to be an 'inborn error'.

The pteridine co-factors fall into two classes: unconjugated and conjugated. Of the latter class folic acid and the folinic acid group of compounds are well known. In this context the synthetic folic acid antagonists will have to be discussed as their action in therapy and research has been of great value in unravelling the function of conjugated pteridines.

A. Conjugated pteridines (Fig. 1)

1. *Folic acid.* Soon after its isolation as an essential nutritional factor it was identified as a conjugated pteridine and its synthesis achieved (Subba Row *et al.*, 1946; Hultquist *et al.*, 1946). Its therapeutic effect in the nutritional megaloblastic anaemias was soon established. Very recently it has been pointed out that folic acid deficiencies without pronounced megaloblastic marrow changes are by no means infrequently encountered in old people, in pregnant women, especially multiparae, in women with habitual abortion, and among those who have given birth to a child

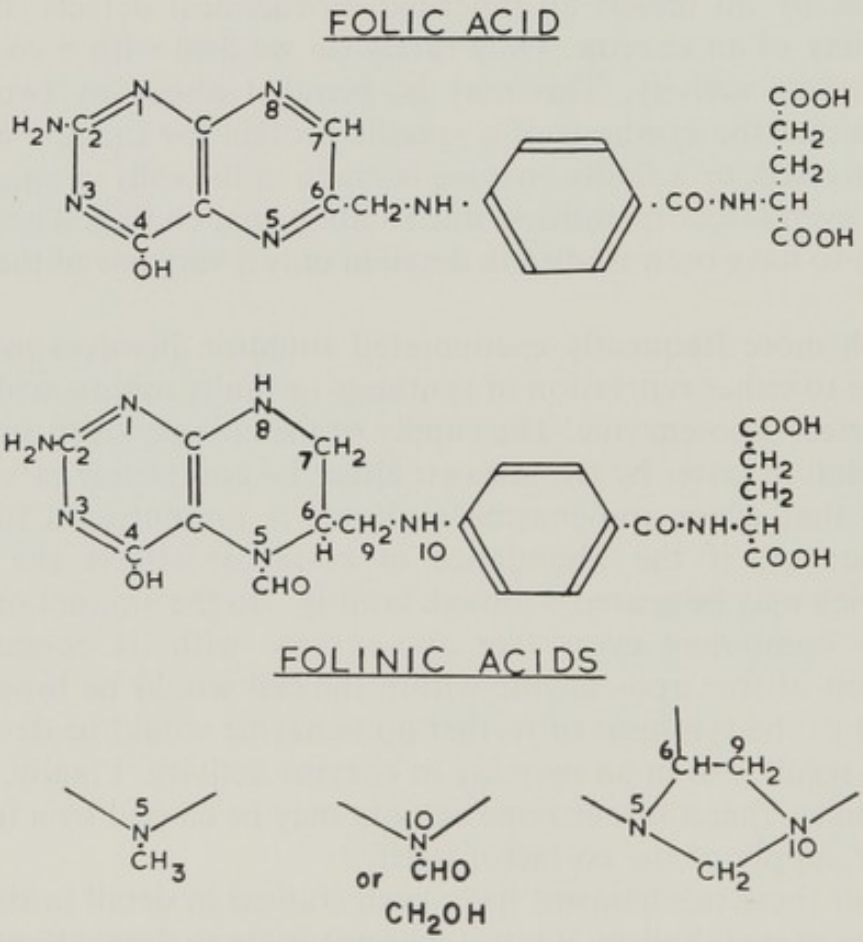

FIG. 1

The formulae of folic acid and the various members of the group of folinic acids are shown. On the left is the pteridine ring. The folinic acid formulae given in full (2nd row) is the 5-formyl-, 5, 6, 7, 8-tetrahydrofolic acid. Of four other members of this group, indicated in the lower part of the figure, only the specific substitutions are shown: 5-methyl-, 10-formyl-, 10-hydroxmethyl and (on the right) 5-10 methylene derivatives of tetrahydrofolic acid.

with overt congenital abnormalities. Folic acid itself is probably biochemically inactive, but it is in this form that it is taken up by the intestine. To obtain its functional form it has to be reduced by an enzyme, folate reductase, via the dihydro to the tetrahydro form.

2. *The folinic acid group.* All members of this group are tetrahydro derivatives of folic acid. The free tetrahydrofolate is oxidized to the dihydro form, in the presence of oxygen, unless special reducing agents are operative. This reduction of folic acid to its tetrahydro form probably occurs in growing or nucleic acid synthesizing tissues.

A discussion of the specific functions of folinic acids as co-factors in cellular processes is closely interwoven with the mode of action of folic acid antagonists, therefore both groups of compounds will be considered together.

3. *Folic acid antagonists.* The usefulness of folic acid antagonists in the treatment of acute leukaemia of children has been known now for almost 20 years. The two most frequently used antagonists are characterized by the substitution of the hydroxyl group at the 4- position of the pteridine ring by an amino group. Aminopterin is a 4-amino folic acid, methotrexate (or by its older name: A-methopterin) has an additional substitution of the nitrogen 10 of the amino group of the p-aminobenzoyl-moiety, and is thus a 4-amino-, 10-methyl-folic acid. On a weight basis methotrexate has approximately one-fifth of the activity of aminopterin. Methotrexate (or aminopterin) is now of considerable value in the treatment of acute leukaemia of children, choriocarcinoma and psoriasis. In these three so very different diseases the drug induces temporary remissions (or even occasional cures in choriocarcinoma). Folic acid antagonists operate by interfering with the physiological function of folinic acid and by blocking the function of the enzyme folate reductase. Much of the function of folinic acid has been established by analyzing the inhibitions caused by folic acid antagonists. This 'inhibition analysis' has proved a fruitful tool in revealing the physiological part played by the conjugated pteridines (Jacobson, 1965).

4. *The functions of folinic acid.* Relatively high doses of folic acid antagonists cause lesions in the mucous membranes of the oral cavity, pharynx, oesophagus, small and large intestine and other epithelia. They also cause leucopenia and anaemia due to depression of cellular activity in bone marrow, lymphnodes and white pulp of the spleen and may lead to severe aplasia. These facts, and the absence of overt toxic lesions in such organs as the central or peripheral nervous systems, the skeletal tissues, liver, kidneys and glands like pancreas and salivary glands, drew attention to the fact that folinic acid must be concerned in processes intimately connected with cellular multiplication, since the common denominator for all those organs which showed toxic lesions in response to folic acid antagonists, was their mitotic activity.

a. *Role in biosynthesis of nucleic acids.* It is now known that folinic acid operates as a co-factor in the *de novo* synthesis of the purine ring, where the labile single carbon residue of folinic acid supplies the carbon atoms 8 and 2 for the future purine ring. In the synthesis of thymidylic acid of DNA, from uridylic deoxyribotide, folinic acid supplies the 5-methyl group of the pyrimidine moiety. In this step folinic acid is also oxidized to dihydrofolate and has to be reduced again to the tetrahydro level by the enzyme folate reductase.

b. *Interconversion of amino acids.* Folinic acid operates as a co-factor in the synthesis (or interconversion) of the following three pairs of amino acids: histidine and glutamic acid; glycine and serine, homocysteine and methionine. This last step requires also the function of vitamin B_{12}.

c. *Function during mitosis.* The synthetic steps involving folinic acid, as listed above, occur in proliferating cells during the interphase, i.e. after completion of cell division, when the two daughter cells emerging from a division have to reduplicate their nuclear and cytoplasmic nucleic acids and proteins. However, folinic acid also plays a vital part in the actual mitotic process itself which takes just over one hour, while cellular synthesis is at a minimum. It can be shown that when normal or leukaemic cells entering into prophase of mitosis are exposed to high concentrations of folic acid antagonists, they will be arrested in the metaphase stage within minutes. At this stage the chromosomes lie free in the cytoplasm and form a single group. This effect can be shown by direct application of folic acid antagonists to cells growing in tissue cultures. Simultaneous and direct exposure of dividing cells both to folic acid antagonists and to folic acid does *not* prevent the action of the antagonist. However, dividing cells can be completely protected from the metaphase arrest if *folinic* acid is supplied simultaneously with the folic acid antagonist. Higher concentrations of the antagonist require much higher concentrations of folinic acid to give complete protection from the inhibitory action of the antagonists. By a series of such experiments in which the amounts of folinic acid that provided complete protection from the antagonist were determined, i.e. an inhibition analysis, the function of folinic acid during mitosis was established. What actually happens when dividing cells perform normally the step from metaphase to the next stage, anaphase? It was found that the chromosomes in prophase (while still within the nuclear membrane) synthesize RNA, so that by the end of prophase, they contain both DNA and demonstrable amounts of RNA. In metaphase when the chromosomes lie free in the cytoplasm they show both types of nucleic acids. When in anaphase each chromosome splits longitudinally to form two chromosomes for the two future daughter cells, RNA is transferred from the chromosomes into the cytoplasm, in other words chromosomal RNA (= 'messenger RNA') passes into the cytoplasm. This step from meta- to anaphase cannot take place when all available folinic acid is blocked by a folic acid antagonist.

5. *Resistance to folic acid antagonists.* The question may be asked: If conjugated pteridines play such important role in the life of proliferating cells, how is it possible that a resistance to folic acid antagonists can, and invariably does develop in, for example, leukaemic children? Can cells 'learn' to dispense with all the important functions of folinic acid? The answer to this question is: no, they cannot. The development of resistance to the blocking action of the antagonist is due to one or both of the following principles: (1) a strictly intracellular process: folic acid antagonists form a relatively stable and inactive complex with the enzyme folate reductase. When the cell is thus deprived of the functioning enzyme, it synthesizes more enzyme. There may well be a limit to this process of sequestering intracellular antagonist. (2) a mainly extracellular mechanism. Many, but not all proliferating cell types (and some non-proliferating cells also) can convert the folic acid antagonists aminopterin and methotrexate into inactive compounds. Thus cells will be able to use again the folinic acid available to them. It may be stated, therefore, that the conjugated pteridines of the folinic acid group are of vital importance to probably all proliferating cells, whether normal or not.

B. Unconjugated pteridines

1. *Hydroxylation reactions.* In 1959 Kaufman established that the hydroxylation of phenylalanine to tyrosine required an unconjugated pteridine compound as a co-factor. Five years later Brenneman & Kaufman (1964), Kaufman (1964) and Nagatsu, Levitt & Undenfried (1964) demonstrated that such unconjugated pteridine was also involved in the second hydroxylation of phenylalanine, i.e. the conversion of tyrosine (4-hydroxyphenylalanine) to 3,4-dihydroxyphenylalanine (DOPA). Already in 1962 Renson, Weissbach & Udenfried had shown that an unconjugated pteridine (probably the same) operated also as co-factor in the enzymatic hydroxylation of tryptophan to 5-hydroxytryptophan. In these three hydroxylation reactions the oxygen of the -OH group is derived from air and the hydrogen comes from the unconjugated pteridine which has to be in the reduced form as a 5, 6, 7, 8-tetrahydro compound (Kaufman, 1964). The tetrahydropteridine acts as a hydrogen donor and is oxidized to a dihydropteridine.

Kaufman had shown that certain synthetic pteridines, in the reduced state, could act as co-factors in the hydroxylation reaction of phenylalanine, among these were the 2-amino-4-hydroxy-6-methyl-pteridine which, on a molar basis, is more effective in vitro than the 6,7-dimenthyl analogue.

Kaufman (1963) reported that tetrahydrobiopterin (Fig. 2) was the naturally occurring co-factor in the enzymatic hydroxylation of phenylalanine to tyrosine.

Patterson *et al.* (1955, 1956 a, b) had isolated biopterin from human urine, established its structure and achieved its synthesis. The configuration

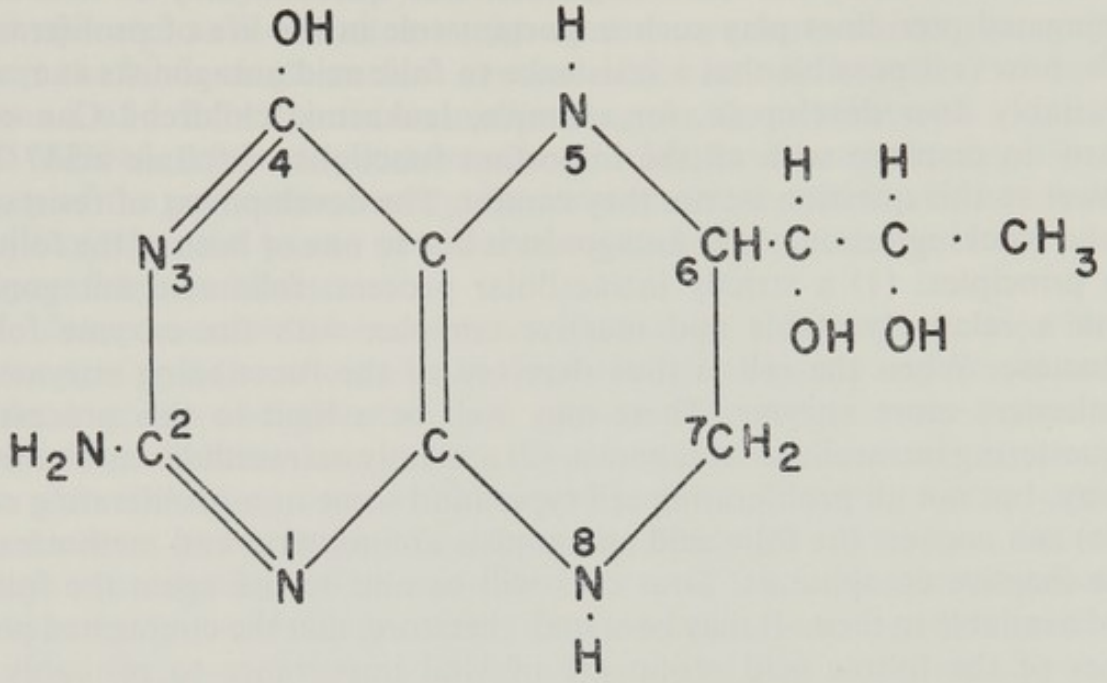

FIG. 2

Formula of tetrahydrobiopterin = 2-amino-, 4-hydroxy-, 6(L-erythro-1·2-dihydroxypropyl)-5,6,7,8-tetrahydropteridine.

of the two hydroxyl groups in the side chain was found to be that of 'L-erythro' (=L-cis). As the naturally occurring compound is still difficult to obtain, a similar synthetic pteridine, neopterin (L-erythro), has been used in experimental investigations; it differs from biopterin only in the side chain where it carries an additional hydroxyl- group at the terminal carbon atom. This synthetic compound is about one-third less active in hydroxylation of phenylalanine than the naturally occurring biopterin (L-erythro). It may be mentioned here that Rembold (see his review, 1965) found biopterin (L-erythro) and neopterin (D-erythro) in the food of the queen bee larva (royal jelly). This latter pteridine (carrying the terminal hydroxyl group in a plane different from the two other hydroxyl groups of the side chain) is considerably less active as hydroxylation co-factor.

The whole system requires at least the following components: the hydroxylase (apo-enzyme), a reduced pteridine (co-enzyme), the presence of a pteridine reductase and $NADPH_2$ (TPNH) to regenerate the functioning tetrahydro form of the co-factor from the oxidized dihydropteridine. In vitro studies indicated that a second reductase was required that may be identical with the enzyme folate (or dihydrofolate) reductase which converts dihydrofolate to tetrohydrofolate, and is inhibited by folic acid antagonists like aminopterin at concentrations of M^{-7}.

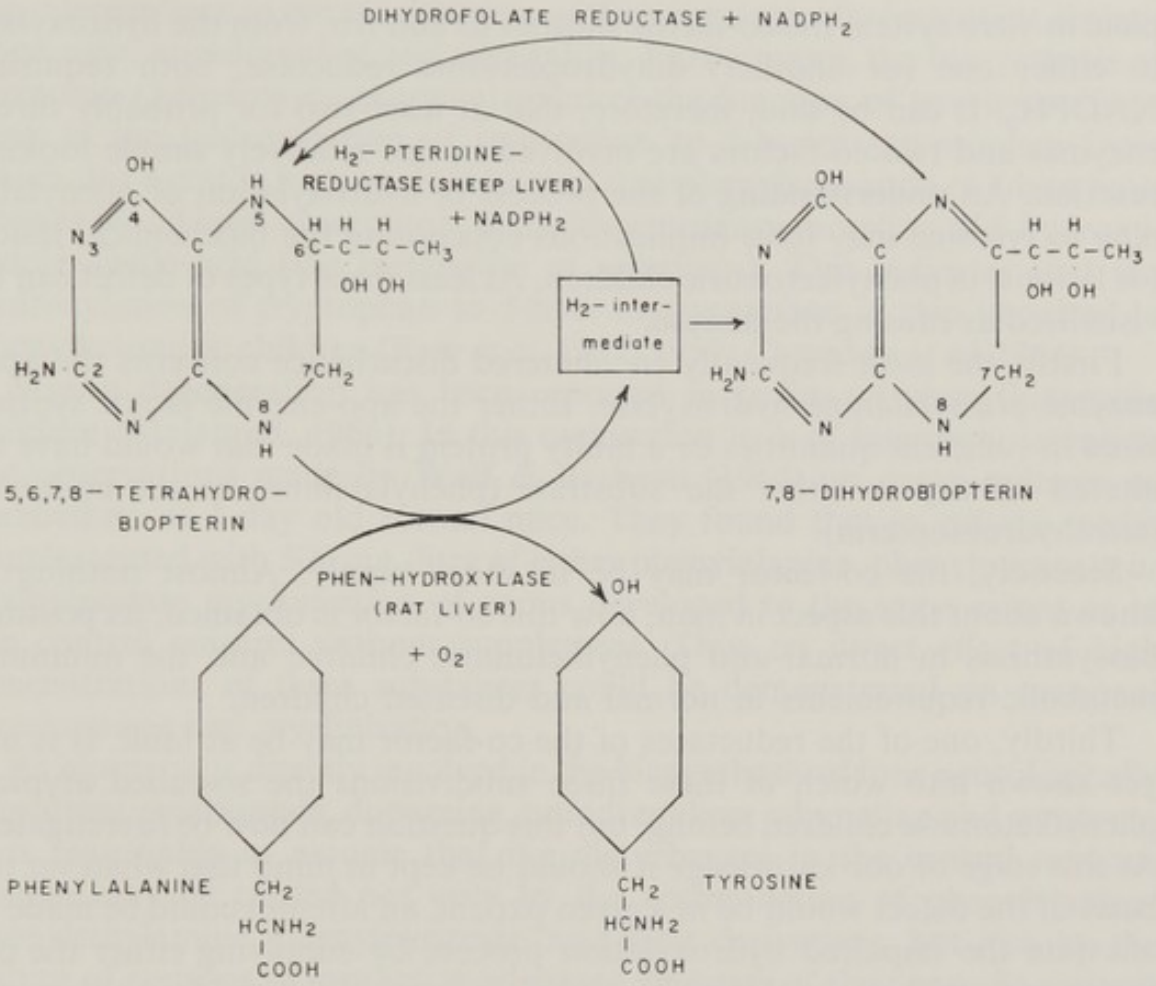

FIG. 3

Hydroxylation of phenylalanine to tyrosine (Kaufman, 1964). Phenylalanine hydroxylase requires a co-factor, tetrahydrobiopterin that donates the hydrogen of the OH- group and is oxidized to an unstable dihydro-intermediate. This can be reduced again by a dihydropteridine reductase + $NADPH_2$ (TPNH) to the functioning tetrahydrobiopterin, or it converts to a stable 7,8-dihydrobiopterin. This requires another enzyme (dihydrofolate reductase) for the reduction to the functioning tetrahydroform.

In figure 3 is shown in more detail the system involved in hydroxylation on phenylalanine. It can be seen that 5, 6, 7, 8-tetrahydrobiopterin is not directly oxidized to 7,8-dihydrobiopterin which is fairly stable. First an unstable intermediary form appears (Kaufman suggested the quinonoid form of dihydrobiopterin) which changes to the stable 7,8-dihydrobiopterin. In vitro experiments indicated that when the unstable form of dihydrobiopterin is immediately reduced again by dihydropteridine reductase, prepared from sheep liver, + $NADPH_2$ (TPNH), the reaction will continue, i.e. the phenylalanine hydroxylase (prepared from rat liver) can operate. When the intermediary, unstable form of dihydrobiopterin is allowed to convert to the stable 7,8-dihydrobiopterin, another pteridine reductase (dihydrofolate reductase) + $NADPH_2$ will be required to reduce the 7,8-dihydrobiopterin to the functioning tetrahydro form. In the com-

plete in vitro system the co-factor shuttles to and fro, from the hydroxylase to either one (or another) dihydropteridine reductase, both requiring $NADPH_2$. It can be said, therefore, that at least two (or probably three) enzymes and two co-factors are involved in this relatively simple looking reaction. An understanding of the process of hydroxylation of phenylalanine to tyrosine may have implications concerning the biochemical lesion (or lesions) in phenylketonuric children. At least three types of defect can be visualized as causing the disease.

Firstly, the most frequently encountered disturbance concerns the apo-enzyme phenylalanine hydroxylase. Either the apo-enzyme is not synthesized in sufficient quantities or a faulty protein is made that would have an altered affinity to either the substrate (phenylalanine) or the co-factor (tetrahydrobiopterin).

Secondly, the co-factor may be in short supply. Almost nothing is known about this aspect in man, how this co-factor is obtained, its possible biosynthesis in normal and phenylketonuric children and the minimum metabolic requirements in normal and diseased children.

Thirdly, one of the reductases of the co-factor may be at fault. It is not yet known into which of these three subdivisions the so-called atypical phenylketonuric children belong, but this question can now be investigated. At this stage of our knowledge it should be kept in mind that whatever the basis of the defect would be in a given patient, an attempt could be made to alleviate the impaired hydroxylation process by supplying either the co-factor or a synthetic analogue in surplus: in some of the children this might lead to an improvement in coping with phenylalanine and thus may allow them a less restricted diet.

2. *Neurological implications*. Tetrahydrobiopterin is also the co-factor in the hydroxylation of tyrosine to form DOPA (3,4-dihydroxyphenylalanine (Fig. 4). That this reaction, involving another apo-enzyme (tyrosine hydroxylase), is impaired in phenylketonuric children is manifested by their usually light complexion, as the cells of the epidermis, hair follicles and iris do not form much melanine from DOPA. This compound is also

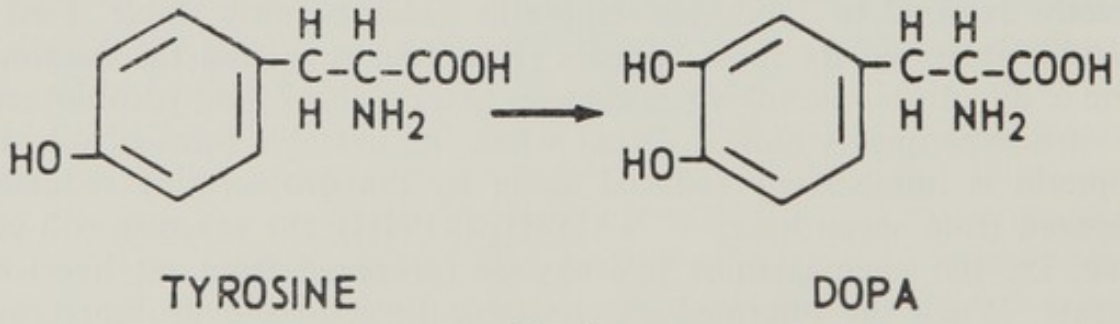

FIG. 4

Hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) requires tetrahydrobiopterin as co-factor. From DOPA is derived dopamine which gives rise to noradrenaline and adrenaline.

the intermediary in the biosynthesis of the neurologically important amines dopamine, noradrenaline and adrenaline. Furthermore, the biosynthesis of serotonin (5-hydroxytryptamine) requires the function of tetrahydrobiopterin in the hydroxylation of tryptophan to 5-hydroxytryptophan from which this agent is formed. It is known that phenylketonuric children have a lower blood level of serotonin and a lower urinary excretion of 5-hydroxyindole acetic acid (the metabolite of serotonin). This indicates that the hydroxylation of tryptophan to 5-hydroxytryptophan is also impaired in phenylketonuric children (Pare *et al.* 1957, 1959; Carreddu *et al.* 1964).

Myelin degeneration has been reported in brains of phenylketonuric children (Malamud, 1966). In this connection it is of interest to mention the observations made by Wolf & Barbeau (1964) on organ cultures of cerebellum of 1-day old *normal* mice. They found that in culture media supplemented with 500 mg./litre of either phenylalanine, phenylpyruvate or phenylacetate myelinisation of axons developed to the same extent as in the control cultures without supplement. Thus no direct effect of high concentrations of these substances could be demonstrated on neuronal development and myelinisation.

As biopterin is directly involved in the biosynthesis of four neurologically important compounds, dopamine, noradrenaline, adrenaline and serotonin it is reasonable to assume that the disturbances in the central nervous system may be caused not only by the accumulation of phenylalanine, phenylethylamine, phenylpyruvate, and their derivatives, but also by the failure of neurons to synthesize adequate amounts of their specific amines.

Phenylketonuria occurs as a genetic defect in mice where it manifests itself in the homozygous recessive animal. The diseased mice have a gray fur (in contrast to the balck coat of the heterozygous, normal parents) and they die around the 21st day. During the last ten days of their lives they show steadily increasing ataxia, clonic epileptiform fits and opisthotonus. The seizures occur finally with very high frequency. The liver phenylalanine hydroxylase activity is low (Rauch *et al.*, 1963). Their central nervous system shows myelin degeneration in some areas and tracts, among these the spino-cerebellar, tecto- and vestibulo-spinal tracts are much affected (Kelton *et al.*, 1962). Thus, some general points of similarity exist between the inherited disease in mice and man. High levels of phenylalanine, phenylpyruvate or phenylacetate do not appear to affect the normal process of myelin formation and maintenance, as Wolf & Barbeau found in cerebellar cultures of normal mice up to the 54th day, i.e. more than twice the life span of phenylketonuric mice. Thus the failure to synthesize adequate quantities of amines may play a greater part in the development of disturbances in the central nervous system than has lately been considered.

Finally another functional connection of nerve tissue and an unconjugated reduced pteridine should be mentioned: Batyl alcohol has been found to occur in the white matter of the brain (and also in the bone marrow). It is

a stearyl-glycerol ether (CH_3-$(CH_2)_{17}$-0-CH_2-CHOH-CH_2OH); to the glycerol moiety may also be linked phosphatidyl-ethanolamine (as in cerebrosides). Tietz *et al.*, (1964) found that the enzymatic cleavage of the ether link (-0-) requires as co-factor a tetrahydropteridine, possibly biopterin. The reaction results in setting free glycerol and stearylaldehyde, the latter being oxidized subsequently to stearic acid. The functional role of batyl alcohol and its enzymatic cleavage remains to be elucidated.

C. Conclusion

In view of the considerations discussed in the previous section it may be worth the effort to ascertain in a group of phenylketonuric children the pattern of their metabolic defects. In the first instance this would entail determining the effect of a single loading dose of L-phenylalanine (100 mg./kg. body weight) on the serum level of this amino acid for a period of 24 hours. A peak concentration will be reached between the 6th and 12th hour, not after 4 hours which is the conventional length of the test. Three days later a repeat test is undertaken with the same amount of phenyla-

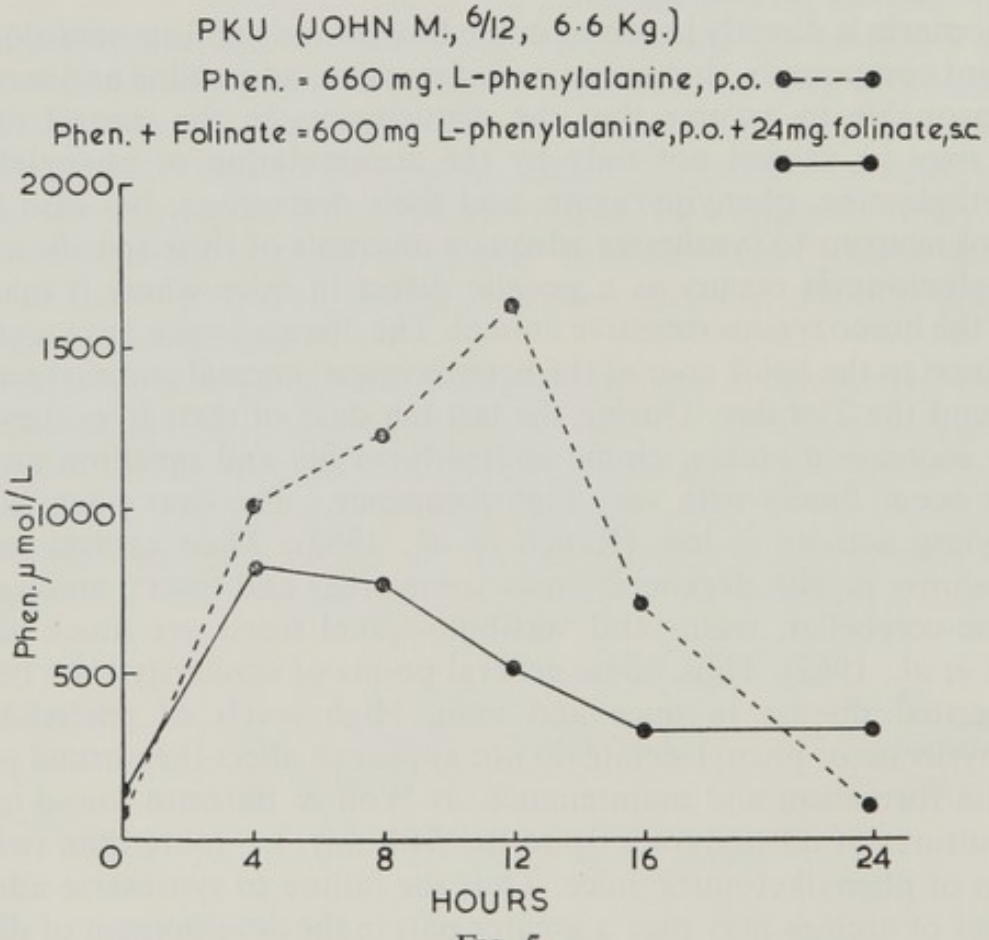

FIG. 5

A loading dose of L-phenylalanine given to a phenylketonuric child causes a characteristic rise in serum phenylalanine (· — — — ·). A second loading dose, given a few days later together with 24 mg. folinate (s.c.), results in a smaller rise in serum phenylalanine (·——·). This effect has been obtained in two of four phenyl ketonuric children.

lanine, given again as a single dose, and a suitable pteridine, given in three divided doses (a few hours before the phenylalanine, together with the phenylalanine, and a few hours later). If after the second loading dose of phenylalanine (while on a pteridine) the plasma concentration of the amino acid does not rise to a peak of similar height as during the first test (without pteridine), the patient might respond favourably to a supplement with a suitable (reduced) pteridine. In Fig. 5 the result of one such test is shown, but it must be pointed out that these observations are of a quite preliminary nature. A defective hydroxylation of phenylalanine, tyrosine and tryptophan may be alleviated by giving a suitable co-factor in surplus.

The strict dietary regime of a low phenylalanine intake, as generally used nowadays, is both expensive and beset with practical difficulties. A less restricted intake of phenylalanine may be permissible in this type of patient when supplied with an appropriate pteridine.

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DISCUSSION OF PRESENTATION BY DR. JACOBSON

Raine (Birmingham). I would like to refer to the very interesting experiment in which Dr. Jacobson gave a phenylketonuric subject a load of phenylalanine with, and without, folinic acid. I noticed that the dose of phenylalanine was relatively small compared with some loading doses

(I think about 0·5 gm.). Would he comment on this and also tell us whether the plasma levels of phenylalanine were determined by a microbiological assay.

Jacobson. The loading dose was 100 mg. of phenylalanine per kg. body weight, which, I believe, is about the standard for a loading dose. The phenylalanine level was determined by amino acid chromatographic analysis.

Ireland (Liverpool). We have carried out a number of tests as suggested by Dr. Jacobson on three phenylketonuric children. Phenylalanine tolerance tests were carried out under normal conditions and again after loading with folic acid and vitamin C for one week and giving a large dose of folic acid immediately prior to the second tolerance test. We found that there was hardly any alteration in the levels of phenylalanine reached and in the time taken for them to return to their ordinary level. But three other children—the three heterozgote siblings of Dr. Allan's case—showed typical heterozygote curves on the first loading dose when this dual tolerance test was performed and in all three the peak reached was lower and the time to return to their normal level less, after the folic acid loading. It would appear that, in the heterozgote where there is some enzyme present, the enzyme was stimulated by the folic acid, but that in the homozygote with no enzyme present the folic acid wouldn't have any effect.

Jacobson. When folic acid is given to a patient it is converted to tetrahydrofolic acid, by the enzyme folate reductase. This enzyme is activated by ascorbic acid. I wouldn't be surprised if some PKU children fail to respond to tetrahydrofolate (derived from folic acid in the presence of ascorbic acid), as, on a molecular basis, it shows less than one fifth the activity of tetrahydrobiopterin in vitro providing any hydroxylase is present.

Bickel (Marburg). A recent paper by La Du given in Washington reported that the reduced pteridines have a strong influence on the action of the phenylalanine hydroxylases, particularly on the in vitro estimation of the hydroxylase. The lower the concentration of hydroxylase in the biopsy specimen, the greater seemed to be the influence of the pteridine. He has not yet given his views about the clinical importance of this finding for phenylketonuria, but his data suggests that the enzymatic system catalyzing the oxidation of phenylalanine to tyrosine is very complex. Further information will be required before the assay of this system can be considered satisfactory. It is possible that the different forms of phenylalaninaemia are distinguished by different disturbances of this system.

Woolf (Oxford). Large doses of folic acid continued for a long time are not by any means safe. Next to the fat soluble vitamins, I think folic acid is perhaps the most toxic of all vitamins. I am thinking of a boy

given 20 mg. folic acid daily for quite a long time—several months—who developed severe epilepsy and mental retardation, exactly in fact what we are trying to prevent (he didn't have phenylketonuria) and he had macrocytic anaemia. This may be related to B_{12} depletion of the C.N.S.

Jacobson. We should be careful in labelling a substance as 'toxic'. If you have a child who shows neurological symptoms while on high doses of folic acid, we have to assume that this may be a child low on Vitamin B_{12}. Only under these conditions could folic acid lead to neurological symptoms of Vit. B_{12} deficiency. This is unlikely to occur in children. Folic acid is **completely** harmless in doses of 20-50 mg. per day provided there is no kidney disease or error in B_{12} metabolism. Incidentally, I should like to know why folic acid was chosen in this case.

Woolf (Oxford). First of all, Dr. Jacobson is quite right. The child had an oral intake of Vit. B_{12} which was regarded as adequate unless he had fits. It was when we gave intramuscular B_{12} that fits started. This was almost certainly a case of depletion of C.N.S. What I meant to say was that giving high doses of folic acid without ensuring adequacy of Vitamin B_{12} by plasma assay is dangerous. In this case the boy probably had an inborn error in the transport of folic acid across the gut wall (not certain, but quite possible). He developed a macrocytic anaemia which did not respond to Vit. B_{12} orally or intramuscularly, but responded to folic acid 20 mg./day, not less.

Poley (Zurich). It is interesting to note that when methotrexate is given to normal subjects and a phenylalanine tolerance test is performed afterwards, their response is akin to individuals heterozygous for the PKU gene. A change in the pteridine metabolism in bringing about this response may have to be considered.

MATERNAL PHENYLKETONURIA AND FOETAL BRAIN DAMAGE
AN ATTEMPT AT PREVENTION BY DIETARY CONTROL

J. D. ALLAN and J. K. BROWN

Introduction

IN recent years there have been a number of reports suggesting that damage to the foetal brain may occur in pregnancy when the mother herself is a phenylketonuric. The subject is an important one for several reasons. Firstly, survey of these reports gives the impression that damage occurring in this way is probable rather than possible. Secondly, mental deficiency arising in this way may be more common than has been realised. We do not know with any accuracy the number of adult females with undiagnosed phenylketonuria in the general population. Surveys of distribution of intelligence (Partington, 1962) in untreated phenylketonuria show that from 2·5 to 6·6 per cent have I.Q.'s of more than 60. This percentage of the total would therefore be at risk of pregnancy in that they are unlikely to be in institutions. The figure, however, may be slightly higher as most of these surveys are based on series collected in hospitals for retarded children and thus reflect the worst aspect of the disease. Furthermore the incidence of phenylketonuria may be higher than the generally quoted figure of 1-20,000 to 1-25,000. Recent surveys suggest an incidence in the range of 1:8,000 to 1:10,000 (Guthrie & Susi, 1963; Guthrie & Whitney, 1963) (Massachusetts screening programme, 1965). Also, even though modern screening techniques are used, as intelligence tends to have an inverse relationship to that of the serum phenylalanine (Low *et al.*, 1956; Cowie & Brandon, 1958; Tischler *et al.*, 1961), it is likely that it will be the high I.Q. phenylketonuric who will be missed in screening programmes. Thirdly, and most important, is the fact that we are currently rearing a generation of phenylketonuric females, many of whom are now at school. Experience so far seems to indicate that the inevitable relaxation of strict diet which accompanies this stage in life, does not result in significant lowering of the intellect or in emotional disturbance. Thus, in a generation or so we may reap from this source a harvest of brain damaged children.

It is in the light of the above background that we record in this paper our experience and observations in a family in which the mother is a phenylketonuric of near normal intelligence who has had 4 children. Three were born before the diagnosis of phenylketonuria was established and all are of sub-normal intelligence. The fourth child was born one year after the diagnosis of phenylketonuria had been established in the mother. We thus had the opportunity of studying the dietetic control of phenylketonuria in the non-pregnant and in the pregnant state. During the last 5 months of

pregnancy the mother was maintained on a controlled diet which was continued for 14 days after parturition to cover the establishment of lactation.

The family

The pedigree is shown in Figure 1.

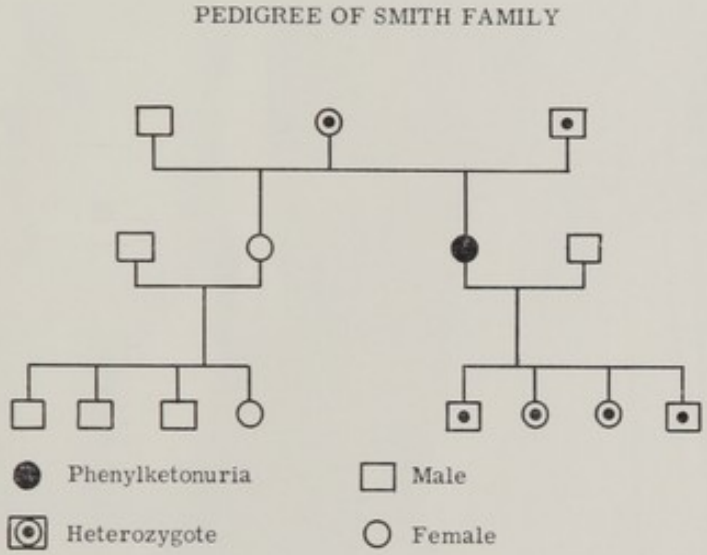


FIG. 1

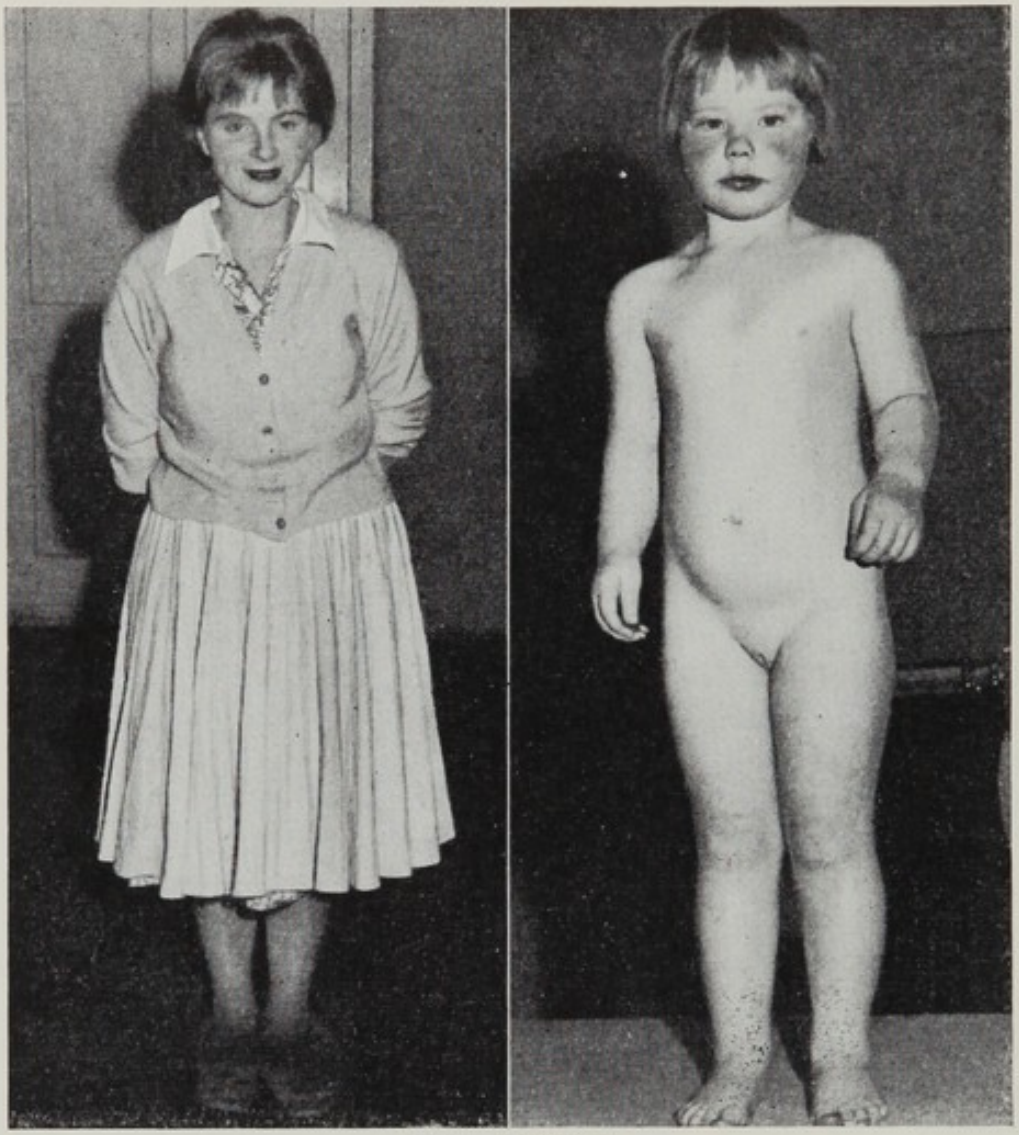
The family first came to light when a school medical officer drew attention to the occurrence of three mentally retarded children in one family. The urine of the mother was strongly positive for phenylketones and that of each of the children was negative. All four were admitted to hospital for further study. The father, unfortunately, refused to co-operate, but he is normal in intelligence and there is no history of mental defect in his pedigree. The maternal mother and father are mentally normal, as is the

Table I

PHENYLALINE - LOAD TEST ON FAMILY

PLASMA VALUES IN mg%

	I.Q.	AGE YRS.	FASTING PH.AL.	FASTING TYROSINE	ONE HOUR AFTER PH.AL. 0.1g./kg PH.AL.	ONE HOUR AFTER PH.AL. 0.1g./kg TYROSINE	PH.AL/TYROSINE RATIO
MATERNAL GRANDFATHER	N	60	3.0	0.6	23.2	2.0	12.76
MATERNAL GRANDMOTHER	N	67	4.4	1.0	22.6	2.2	11.30
MOTHER	72	27	27.0	-	-	-	-
STEPHEN	54	6	1.5	1.9	18.4	2.2	10.00
LINDA	88	4	3.7	1.6	15.1	1.4	11.90
PAMELA	46	1½	3.0	1.6	11.5	2.0	6.32
NORMAL	-	-	1.3	1.5	12.1	2.8	5.00
HETEROZYGOTES	-	-	1.4	1.3	14.8	1.8	8.90



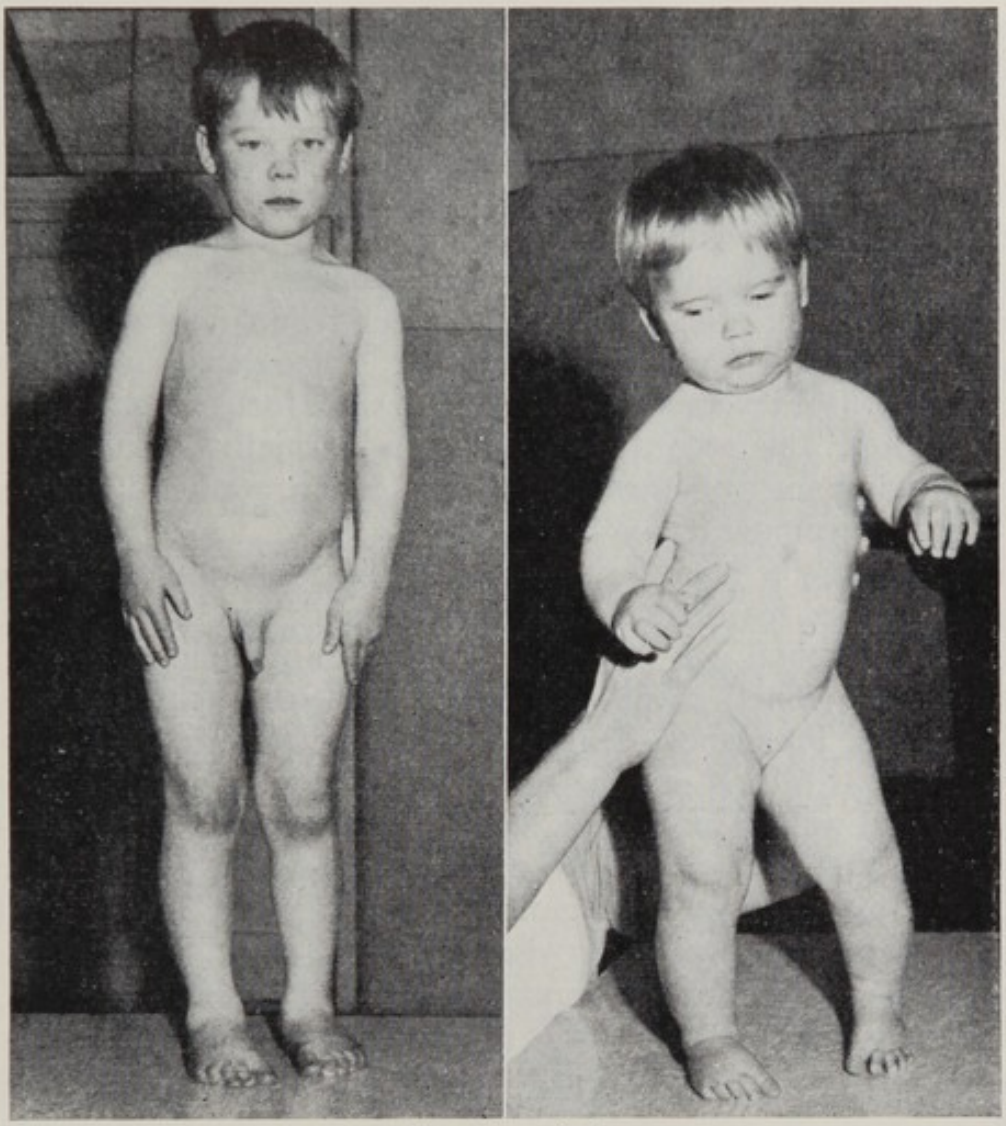
FIGS. 2 and 3.

maternal step-sister and her 4 children and there is no family history of mental defect in the maternal pedigree. The results of the serum phenylalanine levels and of phenalalanine blood tests on members of the family and on normal individuals and heterozygotes for phenylketonuria are shown in table I.

Case reports

The appearance of the mother and her three elder children is as shown in Figures 2 to 5. *The Mother* (aged 27 years) was a fair haired blue eyed woman of small proportionate build. Height 4 ft. 9 in., weight 51 Kg. Her I.Q. (Weschler) was: Full scale 66, Verbal 77, Performance 55. Her reading age was $9\frac{3}{4}$ years and in arithmetic she could add and subtract, but could not do multiplication or division.

She had a pleasant extrovert personality, was co-operative and anxious to please, was a good mother and could carry on a reasonable conversation. She had been born at full term after a normal pregnancy. There was no



FIGS. 4 and 5.

neo-natal asphyxia or jaundice. Development seemed normal in all respects except that she was slow in her milestones—not speaking until about 3 years of age. She went to normal school until she was 11 years old, but as she could neither read nor write she was transferred to a special school. She left school at 16, and had two jobs—making towels and stringing tennis racquets. She married at 18 and had her first child at 22 years of age. There was no significant past medical history, in particular no convulsions. Examination revealed no abnormality except slightly scaly skin and a head circumference of 20½ in. Her E.E.G. was reported as normal. She had three children, as follows.

S. aged 6 4/12 years, was a pleasant, but obviously backward little boy. He had fair hair and blue eyes, but the hair was darker than his mother's. His mental age was 3 years 10 months; I.Q. (Binet) 56. His social scale was higher at 4 years 5 months. He attended a Junior Training Centre. He was born at full term, normal delivery, birth weight 6 lb. 8 oz. Neo-natal period was normal, he sat unsupported at 2 years was

walking and saying a few words at 3 years. There was no history of convulsions or of any illness or episode of note. He was below the 3rd percentile for height, but not for weight. His head circumference was 18 in. which was below the average for his age. He had rather highly coloured cheeks and the skin on his face was dry and a little scaly. Physical examination was otherwise negative except for a precordial systolic bruit considered to be physiological, and, unexpectedly, he had already had successful removal of congenital cataracts. The retinal field and discs were normal. His E.E.G. was reported as follows: "some excess theta in the left parietal and a phase reversing slow and sharp wave focus in the left-parieto-occipital region. This record is not what is normally found in phenylketonuria and seems to indicate some focal lesion, probably atrophic, in the left parieto-occipital region".

L. aged 4 years 6 months, appeared to be more intelligent than S. She carried on a reasonable conversation, and was friendly and co-operative. Her mental age was 4 years against an actual age of 4 years 6 months; I.Q. (Binet) of 88. Her social age was slightly higher at 4 years 4 months. Pregnancy, delivery and neo-natal period were normal. Birth weight was 6 lb. 4 oz. She sat up at 10 months, said single words at $1\frac{1}{2}$ years and walked at 2 years. She had blue eyes and fair hair. There was no history of convulsions, or other illnesses. She too had a rather high colour over the malar regions. She had small epicanthic folds. Physical examination was otherwise negative except for an unexpected ophthalmological finding of increased density of the nucleus of each lens of a non-specific nature. This is referred to later. Her head circumference was $18\frac{1}{2}$ in. which is below the average for her age. Her E.E.G. was normal.

P., aged 2 years 8 months. When first seen at 1 year 7 months she could sit up, but could not walk and said no words. At 2 years 8 months she had been walking for one month and had a vocabulary of approximately 20 words. On the Cattell Infant Intelligence Scale she functioned at a 10 month level against her actual age of 19 months (D.Q. 52). Her social age equated to this level. She was a 4 lb. 2 oz. premature baby, born at 36 weeks gestation. Neonatal period was uneventful, with no history of jaundice, convulsions or other illnesses. She, like S., was below the 3rd percentile for height. She had marked epicanthic folds and her head circumference was only $16\frac{1}{8}$ in., again below average for age. Physical examination otherwise was negative except for an opthalmological finding identical to that of her sister, namely increased density of the nucleus of each lens of a non-specific type. Her E.E.G. was normal.

Comment

At this stage extensive investigations were carried out on both the mother and the three siblings to exclude any other coincident cause of brain

damage. These investigations included the following: chromatography of the urine for sugars, aminoacids, indoles, immidazols, keto-acids, hydroxy-acids, organic acids, phenolic acids, purine, hydroxy proline and proline, homocystine and cystothionine. The mother's urine revealed the metabolites usually found in phenylketonuria, but no evidence of any other abnormal substances, and all the children's urines were completely negative. The urines were also examined for the presence of metachromatic granules, fatty particles, cytomegalic inclusion bodies, and porphyrins. These examinations were completely negative in all four individuals. The blood was examined for the amino acid pattern, CO_2 combining power, phosphorous, calcium levels, the protein bound iodine, the toxoplasma dye test, and liver function tests. The only exceptions to these tests were that the blood sugar was not estimated on the mother, and the P.B.I. on one of the siblings. The results of these examinations confirmed that the mother had a phenylketonuric amino acid pattern. All the other estimations were completely normal. The following enzymes were also estimated; L.D.H. S.G.O.T., and S.G.P.T., on all the individuals with the exception of L.D.H. on the mother's blood. All these tests gave normal results. The cerebral spinal fluid of the three siblings was examined for cellular content, protein, and Lange and Wasserman reactions. Normal responses were obtained in all three. Other investigations included x-ray of skull, skeletal survey for abnormality, mineralisation and bone age, E.C.G.'s, full haematological survey, electrolytes, urea, and all were within normal limits.

From the above survey it was concluded that we were dealing with a high grade phenylketonuric mother, and as there was no evidence of other cause of the mental deficiency in the children, that this was most likely due to intra-uterine brain damage from the unusual metabolites of the phenylketonuric state of the mother. On this premise it was decided to attempt dietetic control of the phenylketonuria in the mother. As she subsequently became pregnant it was possible to study administration of the diet in both the pregnant and non-pregnant states.

The diet

1. *Phenylalanine requirements.* In order to establish the phenylalanine requirements a diet was used based on Albumaid—a synthetic food containing 40 per cent L-aminoacids and virtually totally free of phenylalanine. Phenylalanine was added as necessary in the form of milk. Our findings are recorded in Figures 6 and 7. It will be noted that the daily requirements of phenylalanine in the non-pregnant state were in the region of 5 mg. per kilo, whereas during pregnancy from the 5th month onwards the requirements were virtually twice as much at approximately 10 mg. per kilo. The spikes in the serum phenylalanine level in Figure 6 before stabilisation was achieved were 'accidental' in origin. They were due to a night orderly taking pity and giving her a glass or two of milk. Though disturbing at the time,

retrospectively they are revealing and rewarding, indicating the overflow of urinary metabolites in relationship to the serum phenylalanine level.

2. *The Constitution of the Diet.* In planning the diet we had to be as certain as possible of its nutritional adequacy for pregnancy as we were using a largely synthetic diet. The basic quantity of Albumaid (Table II)

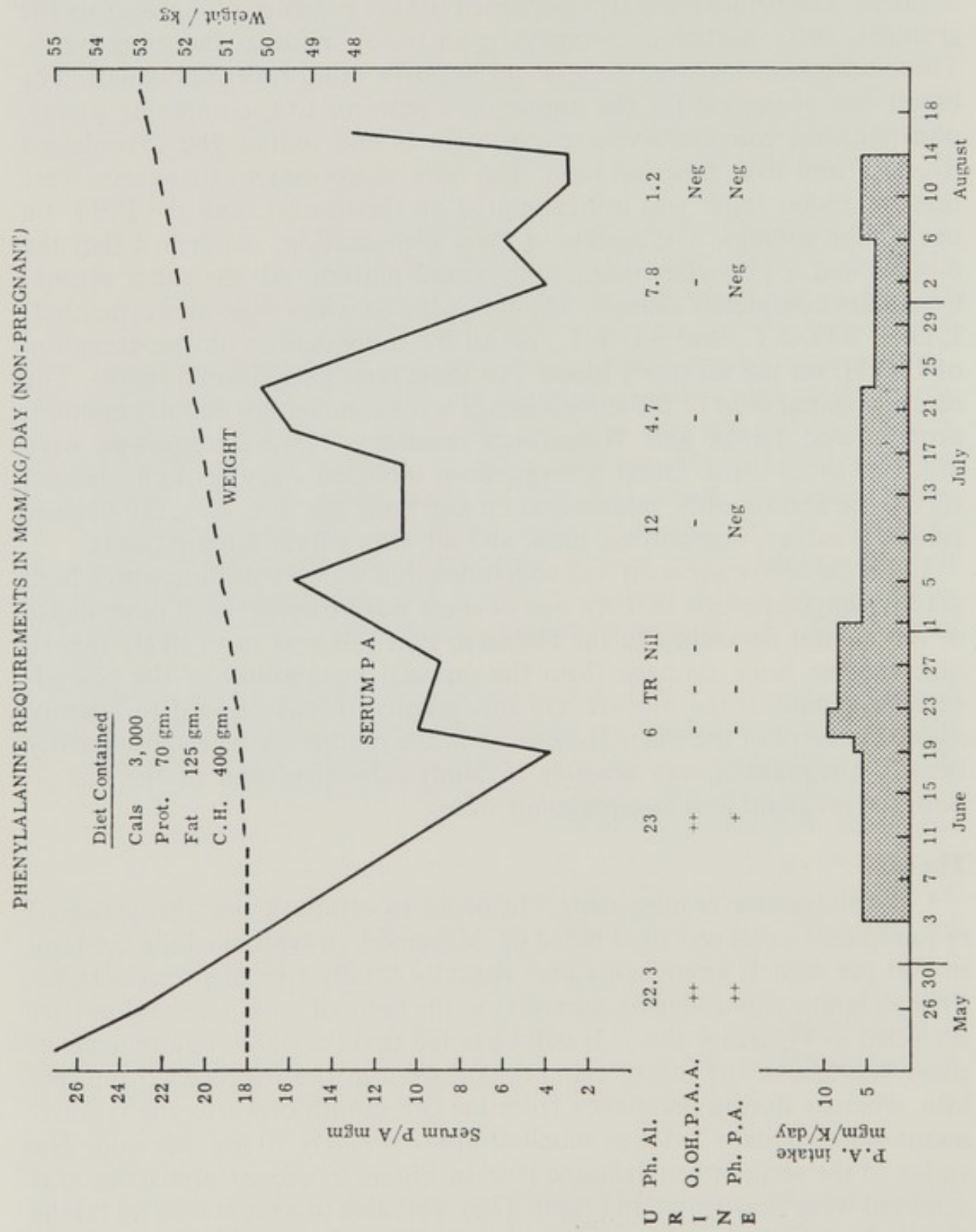


FIG. 6.

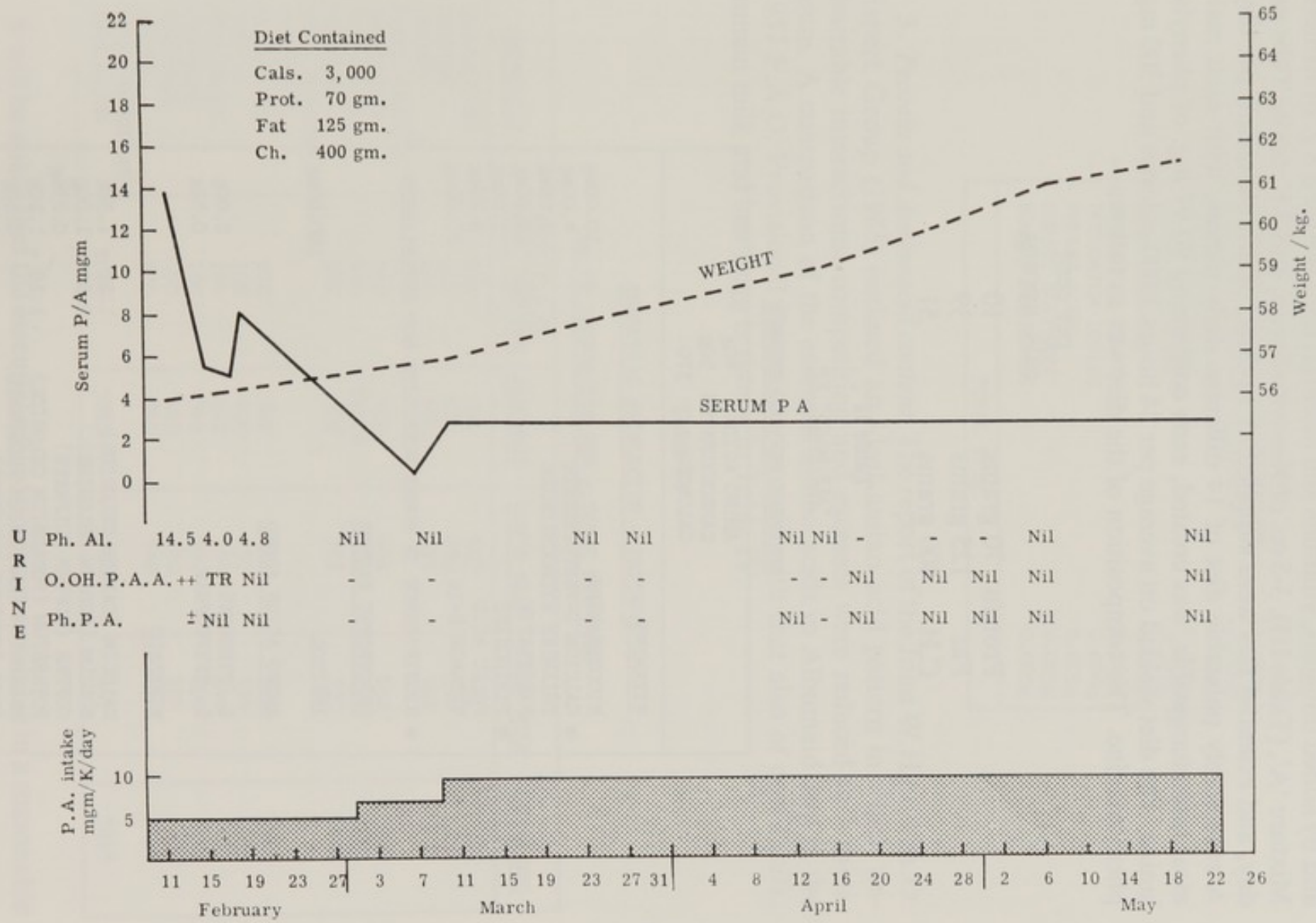

PHENYLALANINE REQUIREMENTS IN MGM/KG/DAY (IN PREGNANCY)
Diet Contained
Cals. 3,000
Prot. 70 gm.
Fat 125 gm.
Ch. 400 gm.
Serum P/A mgm
Weight / kg.
WEIGHT
SERUM P A
URINE
Ph. Al. 14.5 4.0 4.8 Nil Nil Nil Nil Nil Nil - - - Nil Nil
O.OH.P.A.A. ++ TR Nil - - - - - - Nil Nil Nil Nil Nil
Ph.P.A. ± Nil Nil - - - - Nil - Nil Nil Nil Nil Nil
P.A. intake mgm/K/day
11 15 19 23 27 3 7 11 15 19 23 27 31 4 8 12 16 20 24 28 2 6 10 14 18 22 26
February
March
April
May

FIG. 7.

was fixed at 200 grams (80 grams amino acid = 60 grams of Protein). This was supplemented by fruit and vegetables, P.K.N. Bread and Flour, and by added minerals, vitamins and essential fats. We used 8 g. of Mineral Mixture 'A', (Table III), 3-5 cc. of Ketovite Syrup, and 3 Ketovite Tabs per day, and essential fats were supplied as $1\frac{1}{2}$-2 oz. of double cream per day. A reasonably palatable diet of 14 different daily menus, with each main meal interchangeable, was created, each containing 90-97 mg. of phenylalanine. The diet yielded on average per 24 hours 3,000 calories and 300 mg. phenylalanine. The composition of the diet was as follows—

		per cent cals. approx.
Protein	70 grams	10
Fat	125 grams	39
C.H.	400 grams	51

Table II

"ALBUMAID" XP

ANALYSIS PER 100 GRAMMES

AMINO ACIDS	40g
CARBOHYDRATES	50g
CALORIES	370

VITAMINS	
NICOTINAMIDE	10.00mg
* CALCIUM D-PANTOTHENATE	4.00mg
THIAMINE HYDROCHLORIDE	2.00mg
RIBOFLAVIN	2.00mg
* PYRIDOXINE	1.00mg
FOLIC ACID	0.20mg
VITAMIN B-12	20.00ug
* SIGNIFICANCE IN HUMAN NUTRITION NOT ESTABLISHED	
LIPOTROPIC FACTOR	
INSITOL	100.00mg
AMINO ACIDS ADDED	
L. TYROSINE	2.00g
L. TRYPTOPHAN	0.32g
MINERALS	
CALCUIM (CALUIM CHLORIDE)	1.20 - 1.30g
SODIUM (SODIUM CHLORIDE)	0.90 - 1.00g
COPPER (COPPER SULPHATE)	0.23mg
POTASSIUM (POTASSIUM CHLORIDE)	1.10 - 1.20g
MAGNESIUM (MAGNESIUM PHOSPHATE)	0.10g
IRON (FERROUS SULPHATE)	1.90mg

Table III

MINERAL MIXTURE A

CALCIUM LACTATE	450.000g
CALCIUM CHLORIDE DIHYDRATE	30.000g
DIPOTASSIUM HYDROGEN PHOSPHATE	15.000g
DISODIUM HYDROGEN PHOSPHATE	10.000g
MAGNESIUM SULPHATE	8.000g
FEROUS SULPAHTE	2.000g
COPPER SULPHATE	0.200g
ZINC CHLORIDE	0.200g
MANGANESE SULPHATE	0.200g
POTASSIUM IODIDE	0.008g
POTASH ALUM	0.003g
COBALT SULPHATE	0.003g
SODIUM MOLYBDATE	0.003g
TOTAL 515g.	

3. *Protein and Aminoacid content*. The report of the Joint W.H.O./F.A.O. Expert Group (1965) outlined an ideal amino acid pattern in which undesirable interactions, competition and excesses were reduced to a minimum. A comparison of the essential amino acids in Albumaid and in the 1957 F.A.O. Provisional Pattern for protein with that also of cow's milk, human milk and hen's egg is shown in Table IV.

Table IV

ESSENTIAL AMINOACIDS - PATTERN

mg OF AMINOACIDS PER g.TOTAL NITROGEN

AMINOACID	1957 F.A.O. PROV. PATTERN	ALBUMAID	COW'S MILK	HUMAN MILK	HEN'S EGG
ISO-LEUCINE	270	215	407	411	415
LEUCINE	306	642	630	572	553
LYSINE	270	680	496	402	403
TOTAL AROMATIC AMINOACIDS	360	400	634	652	627
PHENYLALINE	180	TRACE	311	297	365
TYROSINE	180	400	323	355	262
TOTAL SULPHUR CONTAINING AMINOACIDS	270	622	211	274	346
CYSTINE	126	400	57	134	149
METHIONINE	144	222	154	140	197
THREONINE	180	542	292	290	317
TRYPOTHAN	90	120	90	106	100
VALINE	270	542	440	420	454
TOTAL ESSENTIAL AMINOACIDS	2016	3763	3200	3127	3215

It will be noted that the essential amino acids are available in a reasonable proportion.

Table V

COMPARATIVE TABLE SHOWING METABOLIC REQUIREMENTS OF AN ASTRONAUT AND A PREGNANT WOMAN, AND THE INTAKE OF THE PATIENT

	PREGNANCY REQ. 60kg WOMAN *	PATIENT'S INTAKE	ASTRONAUT'S INTAKE
NITROGEN 95mg/kg	5.70g	9.00g	
PROTEIN N.P.U. 100			
0.7g/kg	42.60g		
plus 6g	48.60g		
N.P.U. 70			
1.0g/kg	60.00g		
plus 6g	66.00g	70.00g	
ESSENTIAL AMINOACIDS			
LEUCINE	2.33g	5.70g	3.83g
ISO-LEUCINE	2.04g	1.94g	2.42g
VALINE	2.04g	4.88g	2.87g
LYSINE	2.04g	6.12g	2.60g
TRYPTOPHAN	0.68g	1.08g	0.75g
THREONINE	1.36g	4.88g	2.42g
METHIONINE	1.06g	2.00g	1.75g
ARGININE		1.84g	

* AS PER F.A.O./W.H.O. EXP. GROUP

Table V gives the requirements in pregnancy for nitrogen, protein and essential amino acids, based on the W.H.O./F.A.O. recommendations and findings, and compares this intake with that of the 'man-in-space' diet

Table VI

ANALYSIS OF THE MINERAL CONTENT OF THE DIETS

MINERAL	DAILY REQUIREMENTS IN PREGNANCY	ANALYSIS OF 8 (RANDOM) DAYS INTAKE RANGE	ANALYSIS OF 8 (RANDOM) DAYS INTAKE AVERAGE	ASTRONAUT DIET
SODIUM	3.000 - 7.00g	3.00 - 7.00g	4.80g	3.100g
POTASSIUM	1.950 - 5.85g	2.00 - 4.50g	3.90g	2.800g
CALCIUM	1.50g	3.00 - 3.60g	3.30g	0.600g
PHOSPHOROUS	2.25g	3.00 - 3.90g	3.70g	0.700g
IRON	15.000 - 22.00mg	17.00 - 21.00mg	17.50mg	104.000mg
MAGNESIUM	200.000 - 400.00mg	300.00 - 500.00mg	412.00mg	240.000mg
COPPER	2.50mg	1.30 - 2.60mg	2.30mg	0.800mg
IODINE	0.050 - 0.30mg	0.18mg	0.18mg	0.190mg
ZINC	10.000 - 15.00mg	3.00mg	3.00mg	0.600mg
MANGANESE	5.000 - 10.00mg	1.00 - 2.00mg	1.30mg	4.100mg
SULPHUR	0.125 - 1.06g		1.40g	0.055g

(Winitz *et al.* 1965). It may be accepted that the diet was adequate in respect of total nitrogen, total protein and essential amino acids. As, however, Arginine may be an essential amino acid for foetal nutrition it is included in the above table, and as can be seen, it too was adequate. With respect to the proportion of essential to non-essential nitrogen, it is worth noting that for W.H.O./F.A.O. 'reference' protein the proportion is 31 per cent, that for the 'man-in-space' diet is 39·4 per cent, and that of the patients diet is 45·3 per cent.

Table VII

ANALYSIS OF THE DIETS WITH REGARD TO THE VITAMINS AND ESSENTIAL FATS

	DAILY REQUIREMENTS IN PREGNANCY	ANALYSIS OF 8 (RANDOM) DAYS INTAKE		ASTRONAUT DIET
		RANGE	AVERAGE	
WATER SOLUBLE				
THIAMINE	1.100 - 1.40mg	7.20 - 8.00mg	7.60mg	0.89mg
RIBOFLAVINE	1.500 - 2.00mg	3.80 - 7.30mg	7.40mg	1.50mg
NICOTINAMIDE	11.00mg	30.00 - 35.00mg	33.70mg	10.00mg
PYRIDOXINE	1.500 - 3.00mg	3.00 - 4.00mg	3.80mg	1.30mg
BIOTIN	0.15mg	0.61mg	0.61mg	0.80mg
CYANCOBALAMIN	1.00ug	1.4ug	1.4ug	1.67ug
FOLIC ACID	100-200µg	1.00 - 1.20mg	1.20mg	1.60mg
PANTOTHENIC ACID	10.000 - 15.00mg	9.00 - 13.20mg	11.30mg	3.80mg
PARA-AMINOBENZOIC ACID	?	?	?	416.50mg
CHOLINE	250.000 - 600.00mg	450.00mg	450.00mg	104.00mg
INOSITOL	?	350.00mg	350.00mg	0.83mg
ASCORBIC ACID	40.00mg	90.00 - 268.00mg	175.00mg	62.50mg
FAT SOLUBLE				
VITAMIN A I.U.	6,000	9,000 - 11,000	10,500	10,600
VITAMIN D I.U.	600	1,300 - 1,500	1,400	570
VITAMIN E	15.000 - 20.00mg	15.50 - 22.40mg	19.40mg	52.30mg
VITAMIN K	1.000 - 2.00mg	3.70 - 5.00mg	4.20mg	4.58mg
ESSENTIAL FATS				
ARACHIDONIC LINOLENIC ALPHA-LIPOIC	$1\frac{1}{2}$ - 2 OZS. OF CREAM			ETHYL-LINOLEATE 2g

4. *Minerals Vitamins and Essential Fats.* Tables VI and VII give, in so far as can be ascertained, the 24 hour requirements of minerals, vitamins and essential fats in pregnancy, and show the intake on the diet, and that on the synthetic diet of the 'man in space' (Winitz, 1965) per 24 hours. The intake of the patient was in the main, satisfactory except that it was low in zinc and manganese. The importance of trace elements in animal nutrition is exceedingly well realised and documented (*Lancet*, 1966) and we are consequently determining the rarer trace elements in our diet.

5. *Adequacy of the diet.* The mother was maintained on this diet for three months while non-pregnant and for five months while pregnant (the last

five months). During the periods on the diet she remained well and, in fact, improved in health and gained weight. Her weight after delivery was nine kilograms more than her weight on admission when four months pregnant. There was no upset in her behavioural or emotional pattern while on diet. The baby was born seven days post-maturely, weighed

Table VIII (1)

ANALYSIS OF LABORATORY STUDIES ON MOTHER AND BABY. PART 1

SUBSTANCE	NORMAL RANGE	BEFORE PREGNANCY	DURING PREGNANCY	BABY
MINERALS				
SODIUM	135.00 - 145.000mEq	148.0mEq	137.000mEq	137.0mEq
POTASSIUM	3.50 - 5.000mEq	4.4mEq	4.100mEq	5.3mEq
IRON	80.00 - 180.000µg	170.0µg	110.000µg	130.0µg
COPPER	0.08 - 0.235mg	-	0.250mg	-
CALCIUM	9.00 - 11.000mg	9.3mg	9.000mg	10.0mg
CHLORIDE	95.00 - 105.000mEg	102.0mEq	105.000mEq	101.0mEq
BICARBONATE	24.00 - 32.000mEq	-	27.000mEq	26.0mEq
PHOSPHOROUS	3.00 - 4.500mg	5.0mg	3.700mg	4.0mg
VITAMINS				
FOLIC ACID	> 2.5mµg	> 6mµg	> 6mµg	> 6mµg
B-12	>200.0µµg	>600µµg	>600µµg	>600µµg
C (saturation)	100%	100%	100%	100%
PROTHROMBIN	100%	100%	100%	100%

Table VIII (2)

ANALYSIS OF LABORATORY STUDIES ON MOTHER AND BABY. PART 2

SUBSTANCE	NORMAL RANGE		BEFORE PREGNANCY	DURING PREGNANCY	BABY
PROTEIN	ADULT g%	BABY g%	g%	g%	g%
TOTAL	6.90	6.40	6.80	6.70	6.00
ALBUMIN	4.14	3.95	3.16	3.10	2.93
GLOBULIN	2.80	2.45	3.64	3.40	3.07
ALPHA 1 GLOBULIN	0.40	0.41	0.46	1.07	0.44
ALPHA 2 GLOBULIN	0.57	0.52	0.82	0.80	0.80
BETA 1 GLOBULIN	0.55	0.39	0.79	0.78	0.52
BETA 2 GLOBULIN	0.28	0.15	0.40	0.46	0.29
GAMMA GLOBULIN	0.99	0.98	1.17	1.02	1.02
	mg%		mg%	mg%	mg%
UREA	20.0 - 40.0		16.0	22.0	18.0
URIC ACID	2.5 - 6.0		-	2.3	-
NITROGEN BALANCE			POSITIVE	POSITIVE	-
AMINOGRAM			NORMAL	NORMAL	NORMAL
CREATININE	0.8 - 1.5		-	0.8	-
SUGAR	60.0 -100.0		-	64.0	64.0

Table VIII (3)

ANALYSIS OF LABORATORY STUDIES ON MOTHER AND BABY. PART 3

SUBSTANCE	NORMAL RANGE	BEFORE PREGNANCY	DURING PREGNANCY	BABY
FATS				
CHOLESTEROL	150.0 - 260mg%	-	177.0mg	185.0mg%
TOTAL ESTERIFIED FATTY ACIDS	10.0 - 19mEq/l	-	18.7mEq/l	-
PHOSPHOLIPIDS	6.1 - 9.9 mg/PH.L.PH per 100ml serum		6.7 mg/PH.L.PH per 100ml serum	
LIPOPROTEINS				
ALPHA		-	NORMAL	-
BETA		-	NORMAL	-

Table VIII (4)

ANALYSIS OF LABORATORY STUDIES ON MOTHER AND BABY. PART 4

	BEFORE PREGNANCY	DURING PREGNANCY	BABY
LIVER FUNCTION TESTS	NORMAL	NORMAL	NORMAL
BLOOD			
HAEMOGLOBIN	88%	88%	130%
WHITE CELL COUNT	7,500	5,900	14,000
POLYMORPHS	62%	59%	44%
LYMPHOCYTES	32%	32%	53%
MONOCYTES	2%	7%	2%
EOSINOPHILS	4%	2%	1%
PLATELETS	NORMAL	NORMAL	NORMAL
E.S.R.	11mm	80mm	18mm
FILM	NORMAL	NORMAL	NORMAL
X-RAYS			
MINERALISATION	NORMAL	NORMAL	NORMAL
OSTEOPOROSIS	NIL	NIL	NIL

6 lb. 12 oz., and was the heaviest at birth of the four siblings. He was apparently adequately nourished. The analytical data in respect of nutritional status of mother and baby are shown in Table VIII (Parts 1-4) and in each case are satisfactory. Lactation was fully established on the 4th day. The quantity of breast milk was less than normal and presumably would have been insufficient for breast feeding had this been carried out. This was similar to the state of affairs in the other pregnancies. Analysis of the colostrum and two specimens of breast milk for the major constituents showed no deviation from normal.

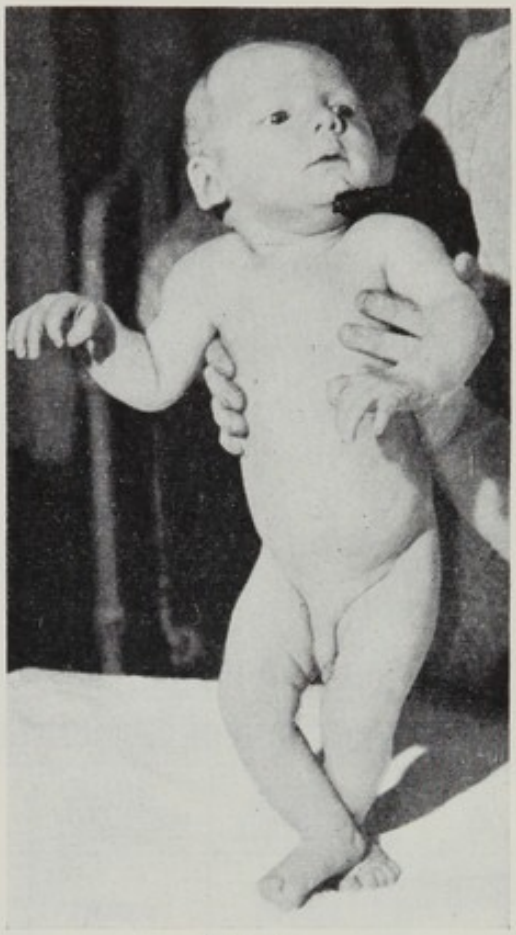


FIG. 8.

The 4th sibling—the baby. The appearance of the baby is shown on the first day of life (Fig. 8). He was born 7 days post-maturely by normal delivery. He cried lustily immediately after birth. There was no neo-natal abnormal episode, except for a little difficulty with feeding in the first day or two, but by day 10 he had regained his birth weight and on discharge at 3 weeks of age he weighed 7 lb. 8 oz. Physical examination was negative. Head circumference at birth was 13 in. so that he fell below the average normal of 13·8 in. This, however, has to be considered in the light of his length which was 19 in. at birth against the normal average of 20 in., i.e., the slight reduction in head circumference may be simply proportionate and genetic in his case. (See discussion re 3 sibs later). It is as yet too soon to assess his intelligence. At 9 months the mother thought he was much more advanced than any of his 3 sibs at a comparable age. He could stand upright holding a chair and drank from a cup. The report on his psychological test (Miss Withers) was, Cattell Infant Scale, D.Q. 108, Vineland Social Maturity Scale, SQ 110.

Investigations and comment

Electro-encephalogram. Two electroencephalographic recordings were taken. The first was an attempt at an intra-uterine (foetal) E.E.G. at 32

weeks of pregnancy. This was reported by Mr E. Manley, Parkside Hospital, as follows:

'I have no previous experience of foetal E.E.G's and the literature is sparse and inconclusive. However delta and theta activity of low voltage is seen, appearing rather erratically. This is an accepted normal finding. Low voltage fast activity is also seen. This has been reported, but whether the source is foetal or not is undecided. Movement and uterine contraction are clearly distinguishable. The conclusion is that this is a normal foetal E.E.G.'

The second tracing was recorded on Day 6 of life and the report is as follows:

'The record shows a low voltage symmetical activity. Theta appears at 4-5 c.p.s. with occasional Delta at 5-6 c.p.s. No spike or spike and wave activity is seen in this recording. The recording appears within normal limits for an infant of this age.'

On this rather poor premise then it may be considered that we have no evidence of intra-uterine brain damage.

The Liquor. A specimen of liquor was obtained at 34 weeks pregnancy and examined for phenylalanine and metabolites. Full screening for these substances was negative. We were prevented from repeating this examination at delivery as three earlier false labours contributed to a final dry delivery.

Phenylalanine. The phenylalanine and tyrosine serum levels of the baby are shown in Table IX. The level in the baby's blood at birth was comparable to that of the mother's at this time. The mother had been moved for

Table IX

SERUM PHENYLALANINE (mg%) - BABY			
TIME	PHENYLALANINE	TYROSINE	MOTHER
CORD BLOOD	7.6	3.0	8.0
6 HOURS	5.2	3.0	-
12 HOURS	2.6	3.1	-
18 HOURS	2.2	2.2	-
24 HOURS	2.2	2.6	-
36 HOURS	1.6	2.4	-
48 HOURS	1.4	2.4	-
4 DAYS	2.4	-	-
8 DAYS	2.0	-	-

delivery to the Maternity Unit and strict attention to diet had lapsed, accounting for this rise in phenylalanine. It is to be noted that a normal level had been reached at 48 hours. The baby has since been proved to be a heterozygote.

Discussion

Review of Literature

Jervis (1937, 1939, 1954) was the first to report on the mating of phenylketonuric women. He recorded four such cases resulting in eight children of which five were 'normal', in one the evidence regarding normality was fairly reliable, and two were phenylketonuric. Dent (1956) aware of a phenylketonuric mother who had three retarded non-phenylketonuric children by three separate illegitimate unions, first suggested the concept of brain damage *in utero*. He believed that the mother's high blood phenylalanine level might have damaged the children's brains and considered that the occurrence of three such retarded children in these circumstances was more than coincidental.

Woolf *et al.* (1961) described two atypical phenylketonuric women who had between them six normal children (heterozygotes). They considered this to suggest that there was no biochemical reason why any phenylketonuric woman should not have normal children.

Partington (1962) referred to a phenylketonuric woman who had three children. Two were phenylketonuric with low intelligence, but one (heterozygote) had normal intelligence. Mabry *et al.* (1963) described three phenylketonuric women who between them had 14 children. Seven of these (Mabry family 2) died in infancy or early childhood. The remaining seven, however, though they did not have phenylketonuria were mentally retarded. Mabry considered this to suggest that a high serum phenylalanine level in the pregnant women may cause permanent damage to the foetal brain. Of these seven, five were proved to be heterozygotes and the other two were assumed to be heterozygotes.

Coffelt (1964) drew attention to a phenylketonuric mother of two children, each non-phenylketonuric and each with mild brain damage which he says may have occurred in utero.

Perry & Tischler (1966) reported the case of a phenylketonuric mother whose child was also phenylketonuric, but these authors raise the question as to whether intra-uterine brain damage could have been a co-incidental factor in the production of the child's mental defect.

Finally Forbes *et al.* (1966) report a phenylketonuric mother of three children all with impaired intellect and all non-phenylketonuric and state that the results of this study favour the view that mental retardation in the child may result from maternal phenylketonuria.

Table X

MATERNAL PHENYLKETONURIA. ANALYSIS OF REPORTED CASES

MOTHER			CHILDREN		AUTHOR
CASE NUMBER	I.Q.	SERUM PHENYLALANINE (mg/100ml)	CASE NUMBER	I.Q.	
MATERNAL PHENYLALANINE 21 mg OR OVER - ALL CHILDREN AFFECTED					
I	96	- 30 20 (cord blood 25)	1 2 3	78 78 76	FORBES
II	96	- 20,30	4 5	MILD BRAIN DAMAGE	COFFELT
III	49	 27	6 7 8 9 10	30 32 30 61 57	MABRY
IV	82	26.5	11	30	
V	27	26.5	12	24	
VI	74	27	13 14 15	48 88 54	ALLAN
MATERNAL PHENYLALANINE 20 mg OR LESS - ALL CHILDREN NORMAL					
VII	102	20.6	16	103	PARTINGTON
VIII	102	8.5	17	95	WOOLF
IX	83	9.9	18	NORMAL	
X	74	3.5	19	NORMAL	ALLAN

Level of phenylalanine

Analysis of the cases where relevant data are available (Table X) reveals an interesting observation.

It appears that brain damage is inevitable if the phenylalanine level is high, but at lower levels there may be no brain damage. The crude indication is that the level of risk is related to the phenylalanine level of 20 mg. Individual brain susceptibility and time of exposure would obviously be other factors concerned. The situation in utero in respect of brain damage in maternal phenylketonuria may be analogous to that of damage arising from conjugated bilirubin in the neo-natal period i.e. at 17·5 mg. bilirubin a small percentage (1/20) of infants are at risk—but one case at least is reported as surviving with no brain damage after a bilirubin level of 52 mg.

The interplay of phenylalanine level, brain susceptibility and time of exposure presumably would explain the difference in I.Q. in the three children under consideration. We observed in our patient that the disappearance from and re-appearance in the urine of the unusual metabolites of phenylketonuria occurred at a blood level of phenylalanine of about 12/15 mg. per cent. Similar levels of overflow are seen in the neo-natal homozygote and in the child not on treatment. Several observers have commented on the tendency for relatively high intelligence to be encountered (in untreated phenylketonuria) in those with relatively low blood levels of phenylalanine. Thus it would appear logical to suggest that in the dietetic control of maternal phenylketonuria, it may not be necessary to maintain a blood level of 2-4 mg. It would probably be safe to keep the level below 10 mg., but levels above 15 mg. are potentially dangerous. One is tempted to consider whether this practice, on the above premise, could, be extended to neonates with phenylketonuria, but clearly until the cause of brain damage at this time is known, such a procedure is not justifiable on this evidence alone.

Mechanism of brain damage

The mechanism of brain damage in phenylketonuria is unknown. The metabolic consequences of the defect in the hydroxylation of phenylalanine are profound. Many of the current theories e.g. deficiency of serotinin—Gaba—or nor-adrenaline are based on the known effect of enzyme depression in phenylketonuria. Information is scanty in respect of the pathological changes in the phenylketonuric brain, but after reduction in brain weight, the change most commonly described is alteration in myelin. As the diet was started in the 5th month and as enzyme creation and myelin are a late event in brain development there are good hopes of avoiding brain damage. We cannot be certain, however, that this is so.

In the light of the observation of Dr. Jacobson (page 4) on the slowing effect at the metaphase stage of cell division in pteridine deficiency on the one hand, and of the importance of pteridine as a co-factor in the hydroxylation of phenylalanine on the other, it is tempting to speculate whether the hyperphenylalaninaemia of the mother could have affected by transplacental transfer the division of the foetal brain cells.

Outlook for future—'at risk' babies

Another important aspect of this subject is the potential number of 'at risk' babies arising as mentioned from 2 sources:

1. The undiagnosed phenylketonuric mother
2. The 'off diet' childhood treated case

For our assessment of this figure we are indebted to Dr. A. E. H. Emery, who reports as follows:

'If the frequency of the gene for PKU is p, and assuming the incidence

of the disease is 1/10,000 then p = 1/100 and q is approximately equal to 1. Considering only the offspring of homozygous (treated) females who have attained adult life and have children, approximately $p^3q + p^2q^2$ of their offspring are genetically not homozygous and therefore should not be affected: they are heterozygous offspring at risk from transplacental passage of noxious substances from the mother. $p^3q + p^2q^2$ is algebraically identical with p^2q. If the birth rate in the United Kingdom is approximately 10^6 then the number of offspring who are born to treated mothers and who are at risk is equal to $10^6 \times (1/100)^2$ or approximately 100 children each year'.

Thus it would appear that in this country, within a decade or so, we can anticipate an annual number of 'at risk' babies in the region of 105.

Other features

1. *Abnormality of lens.* The first child had bilateral cataracts which had been operated on. The second and third showed an identical non-specific increased optical density of nucleii of the lenses of both eyes. The discs and retinae were in all respects normal in all three. Cataracts and other ophthalmological abnormalities are not an unusual feature of metabolic disorders. They occur for example, in the organic acidurias and in galactosaemia. They may also be associated with acquired disease in the pregnancy of the mother e.g. rubella. And finally congenital cataract i.e. cataract of unknown aetiology, but with a genetic distribution, is by no means rare. None of these explanations accounts for the findings in these children. Lens abnormality is not generally considered or described as part of the neural damage in phenylketonuria. However, one case of phenylketonuria with typical bilateral lamelar congenital cataracts has been reported (Parks & Schwilk, 1963)' These authors reviewed the literature and could not find a previous case. None the less, in these three children the evidence is such that we must consider intra-uterine damage arising from the abnormal biochemical environment as the most likely explanation.

2. *Malar flush and dry skin.* The two elder siblings had a marked malar flush resembling that seen in homocystinuria. In addition, all three had unusually dry skins and slight scaliness over the elbows. We have no explanation of these observations. It is possible therefore that these observations have no significance.

3. *The microcephaly and the dwarfism.* Mild microcephaly and shortness of stature are encountered in classical phenylketonuria. The head circumference of the mother was 20½ in. and her height 4 ft. 9 in. The maternal father was 5 ft. 4 in. and the maternal mother 5 ft. It would be impossible to say, therefore, that her relatively short stature was not of genetic origin. The relative microcephaly is presumably more likely to be related to her phenylketonuric state. The first and third sibs were below the 3rd percentile for height, but in each case the degree of microcephaly encountered was

proportionately greater than the height deviation. Thus we consider that in each the microcephaly is most likely related to the adverse intra-uterine environment—an opinion supported by the microcephaly in sibling 2 who was on the 50th percentile for height. We cannot be certain about the aetiology of the dwarfism in siblings 1 and 3. This could be due to an adverse intra-uterine environment, but is presumably more readily attributed, as in the mother, to genetic factors.

Adequacy of control

It is worth noting that, as would be anticipated, the colour of the hair of the mother darkened during the period on diet. We did in fact examine the urinary 24 hour output of 5·OH indole acetic acid and catecholamines during this period and these were within normal limits.

Comment on diet

During the various studies of this patient Winitz *et al.* (1965) published their paper on the use of chemical diets in man. They point out 'that it has been established that diets, purely synthetic in constitution i.e. chemical diets, in studies through several generations in animals (the rat) in respect of long term nutritional investigations, including those bearing on longevity, reproduction and lactation will support normal growth, life span and reproduction.' Furthermore, these investigators found that complete chemical diets in hospitalised patients maintained body weight and health with no untoward psychological or physiological responses and freedom from toxicity and complications.

The diet we used was not entirely a chemical diet, but in view of the fact that Albumaid supplied 60 of the 70 grams of protein, virtually all the carbohydrate, and was supplemented in minerals and vitamins and essential fats, it was virtually fully synthetic. As this woman was maintained in normal health, physically and mentally, before pregnancy, and as all parameters in the metabolic and biochemical field examined remained within normal range, we consider that our experience in respect of chemical diets and adequate nutrition confirms that of Winitz.

As she was also maintained in normal health on such a diet through five months of pregnancy with no deviation from normality in any metabolic or biochemical parameter, we suggest that such diets may be used successfully in the pregnant as well as the non-pregnant human.

Lactation also was established normally. Thus, in this case, a largely chemical diet has sustained nutrition through five months of pregnancy resulted in a normally nourished healthy baby and normal lactation. Winitz *et al.* point out that diets of this type are unique in that the essential and non-essential nitrogen is provided in the form of highly pure l-amino acids. This property, it should be noted, is shared by Albumaid. Diets of this constitution should have an important role to play in the future. It is a

remarkable fact that knowledge of animal nutrition seems to be far in advance of that in human nutrition. For example, more is known of the amino acid and mineral requirements of the battery hen and the buxted chicken than is known of the human female and the human infant. Disorders arising from trace element deficiencies are well documented in the animal and are important, but this field is virtually unexplored in man. Selected chemical diets are being used in the various metabolic disorders e.g. phenylketonuria and maple syrup disease, but the field of potential use is much wider. Should we not have a 'biological' milk for artificial feeding of babies, instead of the somewhat crude commercial preparations widely available and widely used today? Other fields of approach on such lines are many—an adequate dietetic approach to the study and management of uraemia and cholaemia—the application of chemical diets to the study of intestinal absorption and their effect on the bacterial flora of the intestine—the study of carbohydrate and fat metabolism in relationship to degenerative diseases especially coronary thrombosis, as well as base-line studies of nutrition in health and disease.

Concluding Statement

In this paper we have reviewed the subject of maternal phenylketonuria and foetal brain damage, reporting on our observations and making certain suggestions. Perhaps the most important concept arising from our experience is to consider the necessity to screen the mother, not only for phenylketonuria, but for other possible metabolic disorders in the case of any brain damaged infant where explanation is not forthcoming as to the cause. It would appear, in fact, that routine examination of maternal urine during pregnancy should be implemented to this end, initially by the ferric chloride or phenistix test, at least. This procedure has been in operation in our hospitals for the past year, but so far we have not encountered any abnormality. Finally, it seems clear, in the light of present knowledge of maternal phenylketonuria and foetal brain damage and in the light of our experience of dietetic control, that appropriate dietary measures should be implemented at the inception of pregnancy, when the mother is phenylketonuric.

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DISCUSSION OF PRESENTATION BY DR. ALLAN

Woolf (Oxford). Concerning the phenylketonuric mothers we studied some years ago, I think I have been misquoted. I didn't say that I thought maternal phenylketonuria was harmless, but that it was harmless in these cases; the six children were not only normal mentally, but some of them were actually of superior intelligence. They were normal in behaviour and their E.E.G.'s., and they showed no neurological or mental abnormality at all. The eldest of them passed the 11 plus examination most brilliantly and was just about to go to a grammar school. The point I want to emphasise is that these two mothers had a low plasma phenylalanine (under 10 mg./100 ml.). This is really a reinforcement of what Dr. Allan said.

At the present day the latest figures for the prevalence of phenylketonuria is of the order of 1 in 8,000 or 1 in 10,000, but the accepted figure up to 2 or 3 years ago was 1 in 20,000 and the gap between these represents, presumably, people who are walking about normally and leading normal lives, who have the biochemical and genetic error, but do not show much clinical disturbance. Our experience in the past suggests that their blood levels are quite often low and they have, in fact, what we call atypical phenylketonuria and what the Americans are now calling hyperphenylalaninemia. I think that in these cases the children may be quite normal.

As to the question of dietary treatment. First of all the chief thing, as Dr. Allan said, is that we needn't keep the phenylalanine level down to 2-3 mg.% stringently. We can probably get away with it if it is below 10 mg.%. Anyway, I am looking forward to learning about the development of this infant.

Bickel (Marburg). I was struck by two children Dr. Jervis showed recently of a phenylketonuric mother who had had levels of 30-50 mg.% phenylalanine during the pregnancy of her second child. Both children are completely normal, and are now 5 and 7 years of age. Thus, it is not quite so

easy to say that if the level is kept below 20 mg.% there is a good chance of the child being normal. Perhaps there are other quite important differences between these children who stay normal and those who show mental defect. I was also struck by the observation that some of my best patients have remained completely normal, although they are only 6 years old yet, and started treatment with a delay of 6-12 weeks. Is it possible that a child can endure the biochemical lesion for several weeks after birth without any brain damage, when before birth it seems to be already so sensitive to the metabolic error? I always thought it was a question of myelinisation; if the damage can start during pregnancy then we shall have to think again about this question.

Curtin (Limerick). I was interested in Dr. Allan's slide showing a photograph of the infant. Admittedly photographs can be deceptive, but it struck me that this neonate was rather anaemic and the parchment-like skin of the left hand and forearm suggested some degree of placental deficiency, or could it be that this was due to hyperphenylalaninemia?

Allan. On the question of the pallor I actually do not know his haemoglobin level, but this may be due to an iatrogenic cause, the taking of a rather overlarge quantity of blood from him! The mother was most co-operative, except biologically! She had three false labours and this was why at the end she had a rather high phenylaline level of 8 mg.%. She was 7 days overdue, and this may account for the appearance of the baby.

Keidan (Liverpool). At what stage in embryonic or foetal life does phenylalanine develop? Does it cross the placenta either way? In the case of a heterozygote mother bearing a homozygous foetus, would the level of enzyme in her blood control the phenylalanine metabolism of the foetus so that only after birth it got out of control? Is there any evidence that in the homozygote mother, bearing a heterozygous child, that anything happens to her level if she doesn't have the special diet?

Woolf (Oxford). I don't know that anyone knows the answer, I can quote Dr. Kaufman at the Washington conference—he said that his first suggestion that the enzyme was not present until after birth was incorrect, the wrong conclusion was drawn from the results. In fact the apoenzyme is present before birth.

The hydroxlase is only present in liver cells, so it cannot cross the placenta or get into the blood. The phenylalanine is easily transported from blood to cell to blood across the placenta and in this way the liver of the non-phenylketonuric mother metabolises all the phenylalanine for the foetus.

Poley (Zurich). It would be interesting to follow the three or four children reported by Dr. Allan with serial EEG's to see if and what type of abnormalities might develop. There seems to be some evidence, however tenuous, that phenylketonurics who are less severely retarded show less severe EEG-abnormalities.

As direct measurements of phenylalanine-hydroxylase activity in the liver will become available, it is likely to be shown that the activity of this enzyme may vary from patient to patient, and this may be an important factor with regard to development of cerebral damage.

I would like to ask Dr. Bickel about the treated phenylketonurics with normal I.Q's. I wonder if serial E.E.G's have been taken on his patients, whether they have been normal, or whether any abnormalities became evident at, say, the end of the second year of life. A normal IQ does not exclude EEG-abnormalities and the latter may be regarded as evidence of cerebral damage either incurred during foetal life or in spite of treatment.

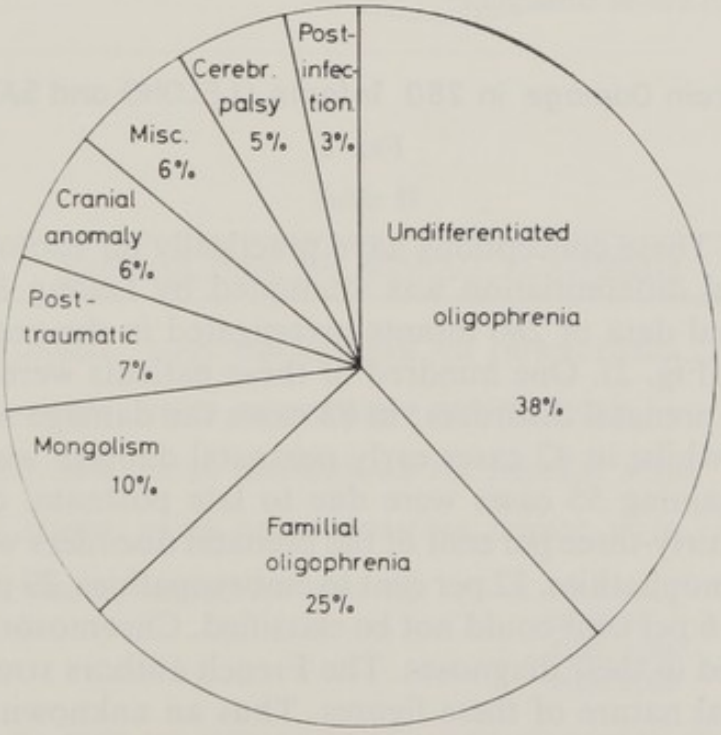
Bickel (Marburg). We follow up all our patients at 4 monthly intervals. These children were started on treatment in the first few weeks of life, all had normal E.E.G's. at the beginning. A few of them afterwards developed perfectly normally, although, as I say, my oldest is only 6 years old. Some few have had occasionally abnormal E.E.G's. and others always had normal ones, and in a few the E.E.G. became normal later. So I don't think there is a very strict parallelism between the development of mentality and the E.E.G. changes.

INBORN ERRORS OF METABOLISM ASSOCIATED WITH BRAIN DAMAGE.—RECENT ADVANCES IN EARLY DETECTION AND PREVENTION OF THEIR MANIFESTATIONS

HORST BICKEL

Frequency and causes of brain damage

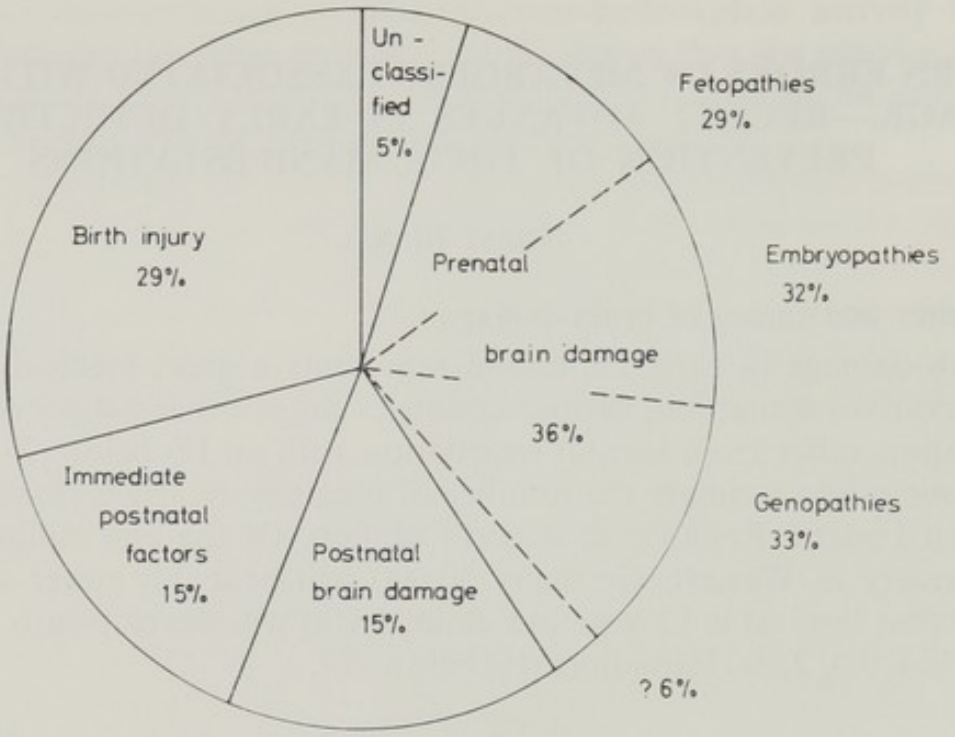
Brain damage in early childhood represents a great medical problem. Conservative estimates in various countries suggest that 3-4 per cent of the population suffer from mental retardation with an IQ below 70. Accordingly one might estimate the number of mentally retarded persons in the German Federal Republic at over $1\frac{1}{2}$ million. Of the one million infants born yearly in Western Germany 30,000 will probably never attain the intellectual level of a 12 year old child, 5,000 will develop into imbeciles (IQ below 50), 2,500 into idiots (IQ below 20).



Oligophrenia-Classification of 12,000 Institutionalized Children (ALLEN and BAROFF 1947-55)

FIG. 1

Research into the causes of brain damage is of decisive importance for diagnosis and prevention. Figure 1 shows how much knowledge is still lacking for an aetiological approach. Allen & Baroff (1955) classified the oligophrenia of 12,000 patients admitted to New York State Schools during the years 1947-55 as 'undifferentiated' in 38 per cent and 'familial'



Causes of Brain Damage in 280 Infants (LELONG and SATGÉ 1950-59)

FIG. 2

in 25 per cent. These conceptions have practically no etiological meaning. A more careful differentiation was attempted by Lelong & Satgé (1961) from the clinical data of 280 infants investigated in the years 1950-59 for brain damage (Fig. 2). One hundred of these patients were thought to be suffering from prenatal disorders; in 83 cases the damage was assigned to birth injuries, whilst in 42 cases early postnatal damage was held responsible. The remaining 55 cases were due to late postnatal causes or were unclassified. Thirty-three per cent of the prenatal disorders were believed to be due to enzymophathies, 32 per cent to embryopathies, 29 per cent to fetopathies, whilst 6 per cent could not be classified. Chromosomopathies were not yet included in their diagnoses. The French authors stressed the tentative, provisional nature of these figures. Thus an unknown proportion of the infants grouped under 'birth injuries' may actually have suffered from prenatal lesions which could not be recognised as such on clinical grounds.

Present-day information suggests that an important, although still uncertain percentage of brain damage in early childhood is due to enzymopathies. Hereditary enzyme defects, usually inherited as an autosomal recessive trait, lead to metabolic errors disturbing cerebral function and development. The error may involve very different pathways of the amino acid, carbohydrate, fat, electrolyte, water, plasma, protein, hormone and bilirubin metabolism. More than 38 examples of such disorders are already known (Table I). Early detection and biochemical characterisation in 17

Table I. *Various forms of inborn metabolic oligophrenia.*
(* Treatment available.)

*Phenylketonuria
*Maple syrup urine disease
Hartnup disease
Argininosuccinicaciduria
Citrullinuria
*Hyperammonaemia

Hyperlysinaemia
*Glycine disease
Cystathioninuria
Homocystinuria
*Methionine malabsorption
Hyperprolinaemia, Hyperhydroxyprolinaemia
Histidinaemia
Indolylacroylglycinuria
Disturbed tryptophan breakdown with autism
*Galactosaemia
*Oculo-cerebro-renal syndrome Lowe
*Valinaemia
*Idiopathic infantile hypoglycaemia
*Wilson's disease
*Renal diabetes insipidus
Pseudohypoparathyroidism
*Cong. disturbances of thyroid hormone synthesis.
Crigler-Najjar's dis.
*Pyridoxine dependency
Amaurotic idiocy (gangliosidosis)
Morbus Niemann-Pick
Morbus Gaucher
Sulfatide lipidosis
Mucopolysaccharidosis
A-beta-lipoproteinaemia
Lahey-Bray syndrome
Sidbury-Harlan-Wittels syndrome
T-substance anomaly
Hydroxykynureninuria
*Idiopathic severe hypercalcaemia

Table II

SCREENING TESTS FOR METABOLIC OLIGOPHRENIA
II-DIMENS. PAPER CHROMATOGRAM FOR AMINOACIDURIA
I -DIMENS. PAPER CHROMATOGRAM FOR MELLITURIA
II-DIMENS. PAPER CHROMATOGRAM FOR PHENOLIC ACIDS
II-DIMENS. PAPER CHROMATOGRAM FOR INDOLIC ACIDS
MICROBIOL. INHIBITION TEST FOR : PHENYLAL. LEUCINE HISTIDINE GALACTOSE
$FeCl_3$ TEST FOR PHENYLPYRUVIC + IMIDAZOLPYRUVIC ACID
CYANIDE-NITROPRUSSIDE TEST FOR HOMOCYSTINURIA
OXYDASE SCREENING TEST FOR WILSON'S DISEASE (AISEN)
IN SELECTED CASES
IN BLOOD : SUGAR - Ca - P - Na - BILIRUBIN - NH_3 - ELECTROPHORESIS -
IN URINE : MUCOPOLYSACCHARIDES - SULPHATIDES
IN INFANTILE CONVULSIONS : PYRIDOXINE

types have enabled the clinician to develop a more or less effective treatment and, better still, prevention of their manifestations (for a detailed account see Bickel & Cleve, 1967). The rapidly growing brain of the young child seems especially liable to be affected by such metabolic deviations, so that early diagnosis is of decisive importance in preventing brain damage before it becomes irreversible.

A screening programme for metabolic oligophrenia

A number of screening tests are now available to detect metabolic oligophrenia. We have used most of the tests listed in Table II to screen a population of 1,400 institutionalised patients and 1,000 patients of various German hospitals including our own. Table III gives the results of this programme, Table IV shows a further differentiation of the metabolic defects detected in the hospitals. The Guthrie test results for phenylalanine, leucine, and galactose are mentioned later in connection with the respective diseases.

The case material of Table III and IV is selected, so the figures give little

Table III. *Results of a screening programme in a population of 1400 institutionalised patients and 1000 patients of various hospitals* (*see text*).
(Results of the Guthrie test for phenylalanine see Table 5.)

	Mental institutions	*Hospitals*
Number of tested cases	1400	1000
Phenylketonuria	19 (1·4%)	97 (9·7%)
Detected in first three months of life	—	13
Other inborn errors of metabolism		46 (4·6%)
Unspecific generalized aminoaciduria	20 (1·4%)	13 (1·3%)
Unspecific generalized mellituria	37 (2·6%)	3 (0·3%)
Isolated glucosuria	25 (1·8%)	4 (0·4%)

Table IV. *Inborn metabolic oligophrenia detected in the 1000 clinical cases* (*see text*).

Number of tested cases	1000
Phenylketonuria	97
Renal diabetes insipidus	9
Galactosaemia	8
Lowe syndrome	8
Maple syrup urine disease	5
Hurler's disease	5
Wilson's disease	4
Severe idiopathic hypercalcaemia	3
Pseudohypoparathyroidism	2
Prolinuria	1
Amaurotic idiocy	1

information about the true frequency of these metabolic oligophrenias. In the institutions only cases with mongolism or definitive postnatal brain damage were excluded, but for external reasons the screening programme had to be confined to paper chromatographic analysis of the amino acids, sugars and phenolic acids excreted in the urine. This fact, as well as the early mortality of numerous metabolic oligophrenias, is probably the reason why in the institutions we failed to find inborn errors other than phenylketonuria, quite in contrast to the clinical screening results. Generalised aminoaciduria and melituria were relatively frequently observed in the institutions and were repeatedly traced back to nutritional deficiencies, to liver and kidney diseases or to other diseases without causal relationship to the brain damage. It is, of course, quite possible that in some of these cases aminoaciduria and glucosuria were secondary manifestations of a primary metabolic error which escaped our detection.

Clinical examples

Of the many oligophrenias listed in Table II there are only a few for which really satisfactory means of early detection and prevention are available. The discussion of three of these, phenylketonuria, maple syrup disease and galactosaemia, might serve as an example for the approach towards the management of the whole group of diseases.

1. *Phenylketonuria.* This enzymopathy shows an autosomal recessive mode of inheritance. The basic lesion is a defect of phenylalanine hydroxylase, resulting in an accumulation of phenylalanine, phenylpyruvic acid and other phenolic and indolic acids in blood, urine and further biological fluids (Fig. 3). The exact cause of the brain damage is still unknown. The damage usually starts after the first three months of life, proceeding rapidly at first, then more slowly until, in most cases, severe oligophrenia results. Infantile spasms and other types of convulsions, a pale complexion

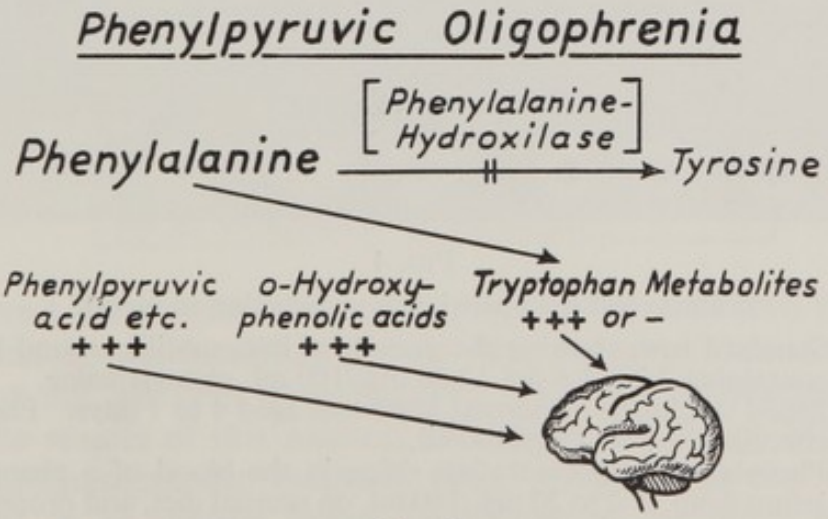


FIG. 3
Metabolic block in PKU.

with fair hair and blue eyes, dermatitis and a phenylacetic acid odour, resembling the smell of horse stables, are other, less obligatory, symptoms.

The simplest diagnostic test in infants is the demonstration of the phenylpyruvic acid excess in the urine by the $FeCl_3$ reaction, either by placing a drop of 10 per cent $FeCl_3$ solution on a wet napkin, or by posting a piece of filter paper soaked in the baby's urine to a central laboratory where the $FeCl_3$ test is performed on this paper. If a green colour develops, which often rapidly fades, the test is positive. Unfortunately this very convenient method has serious drawbacks. The test sometimes becomes positive only some four to six weeks after birth, or it may even later remain negative if the phenylalanine blood level does not exceed 12-15 mg. per 100 ml. Renal clearance factors or a transaminase dysfunction between phenylalanine and phenylpyruvic acid may play a role in producing these 'false negative' results which have led to the serious consequence of young patients being overlooked despite timely testing, while their brain damage could still have been prevented (Boyd, 1961; Farquhar *et al.*, 1962; Carson, 1965). Testing the napkin with 'phenistix' entails a still greater

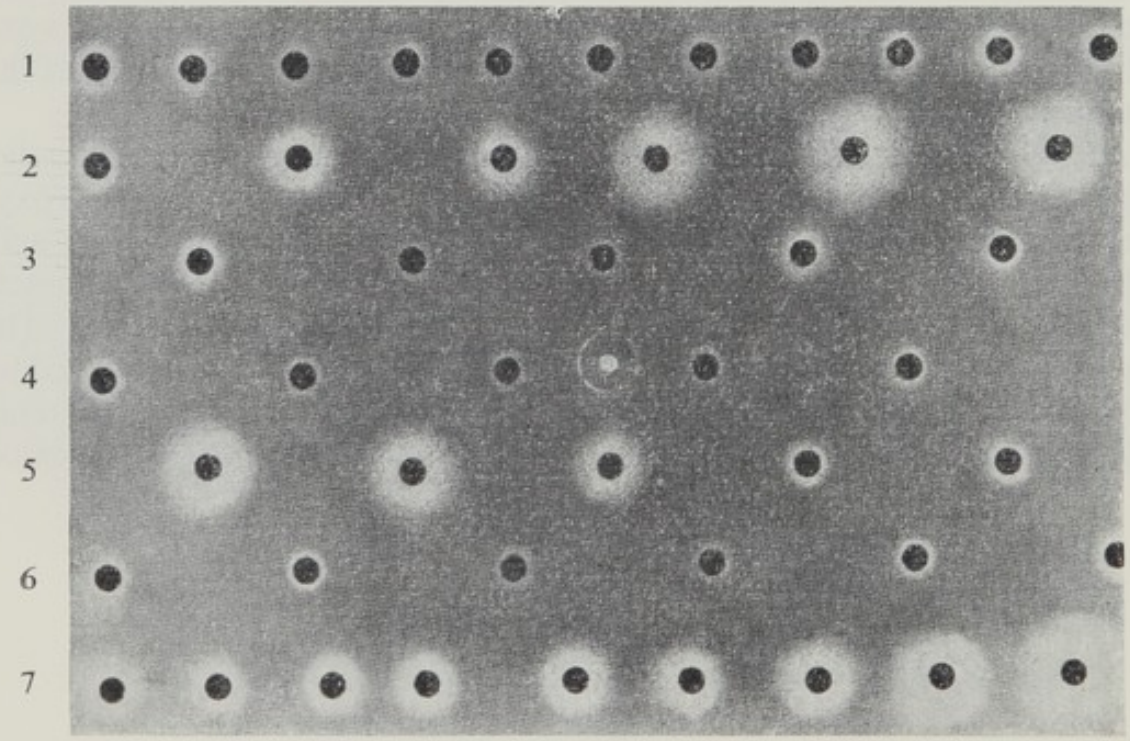


FIG. 4

Guthrie test for phenylalanine detection in blood.

Row 7 = Standard row, showing the growth of bac. subtilis around blood discs containing 2-2-4-4-6-6-8-12-20 mg./100 ml. phenylalanine.

Row 1 = Blood specimens of normal newborns aged 4 to 7 days. Phenylalanine concentration < 2 mg./100 ml.

Row 2-4 = Phenylalanine concentration rising in the blood of a phenylketonuric infant from < 2 to 20 mg./100 ml. on normal diet, and dropping to < 2 mg./100 ml. on a phenylalanine-restricted diet.

Row 5-6 = Another patient with phenylketonuria returning from increased to normal phenylalanine blood levels on a phenylalanine-restricted diet.

risk of false negative results if not enough urine is transferred to the test strip. Less serious are 'false positive' test results due to the $FeCl_3$ reaction with other metabolites or drugs (Gibbs & Woolf, 1959). A more reliable, but also more time-consuming way of diagnosing phenylketonuria in the urine is the chromatographic demonstration of the increased o-HO-phenylacetic acid excretion.

The best screening test available at present is the microbiological inhibition assay by Guthrie & Susi (1963) which measures the increased phenylalanine concentration in a blood drop taken not before the fourth day of life from a heel puncture. The drop is transferred to a filter paper card of standard thickness and sent to a central laboratory where a disc of the blood drop is punched out and placed on a special agar medium which has been inoculated with bac. subtilis. The growth inhibition of the medium, induced by the phenylalanine antimetabolite beta-2-thienylalanine, is overcome by the phenylalanine content of the blood disc, the bacterial growth zone around the disc being the wider, the higher is the phenylalanine concentration. After incubating the medium, the diameter of the growth zones around the blood discs can be compared with standard blood discs of known phenylalanine concentration (Fig. 4). Two hundred and more blood specimens can be tested by one technical assistant per day.

In our experience over the last four years the Guthrie test is highly specific and sufficiently sensitive and reproducible for the purpose. Control tests with various amino acids gave no positive results (Table V), nor did we

Table V

SPECIFICITY OF PHENYLALANINE INHIBITION TEST

THE INHIBITION OF BETA 2-THIENYLALANINE WAS NOT CHECKED BY 20 mg/100ml SOLUTIONS OF :		
ALANINE	GLUTAMIC ACID	ORNITHINE
ARGININE	GLYCINE	PROLINE
ASPARAGINE	HISTIDINE	SERINE
ASPARTIC ACID	HYDROXYPROLINE	TAURINE
CITRULLINE	ISOLEUCINE	THREONINE
CYSTINE	LEUCINE	TRYPTOPHAN
CYSTEINE	LYSINE	TYROSINE
GLUTAMINE	METHIONINE	VALINE
NOR BY EPINEPHRINE OR THYROXINE		

ever observe a 'false positive' result among 94,000 blood tests performed so far. The test is semiquantitative; the best range of measurement being between 1-8 mg. per cent phenylalanine. There is a satisfactory agreement between the results of this method and of the La Du or Moore & Stein column chromatographic methods (Fig. 5), although the Guthrie test values tend to be slightly lower than those of the other two methods (for an explanation and further methodical details see v. Eicken 1966).

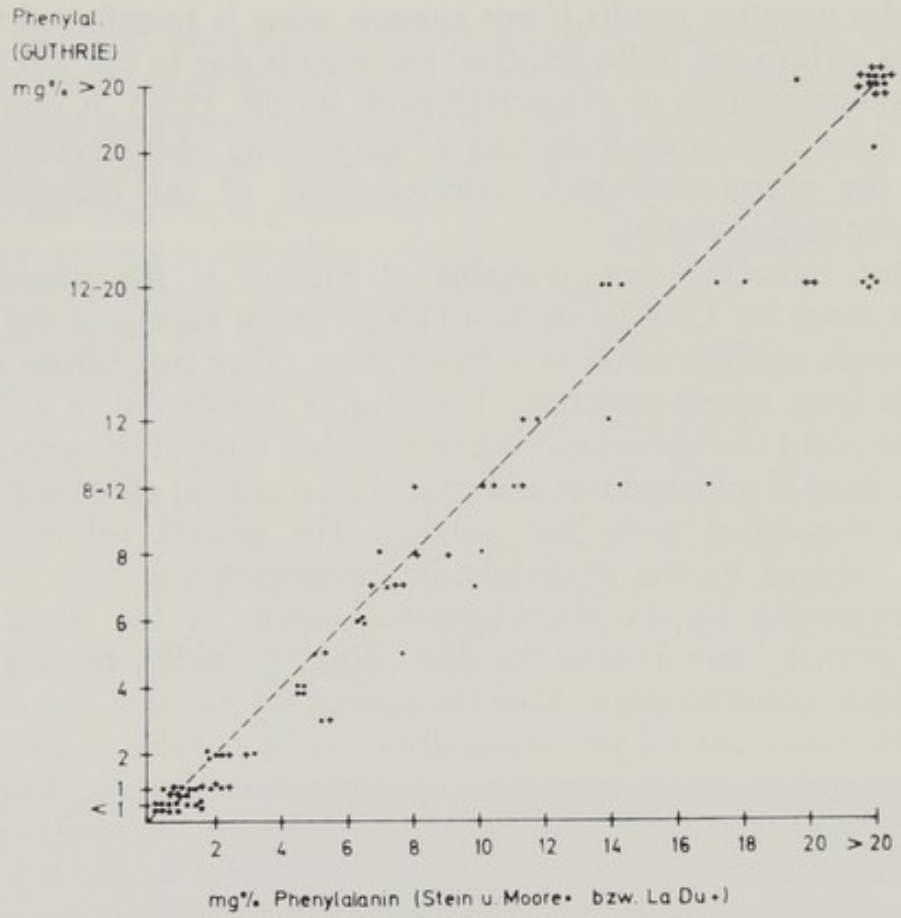


FIG. 5

Comparison of phenylalanine estimation in the blood by the semiquantitative Guthrie test and by the quantitative methods of Stein and Moore or of La Du respectively (see text).

The Guthrie test is well suited, not only for the detection of phenylketonuria, but also for the biochemical control of patients under a phenylalanine-restricted diet, the blood drops being taken by the family doctor, a nurse or even the mother at monthly intervals and sent to the hospital by ordinary letter post.

In screening newborn infants the blood specimens should not be taken before the fourth day of life, preferably a few days later, as the intact enzyme system of the mother keeps the child's phenylalanine blood level normal until birth, and some time will elapse until a pathological rise takes place. Since January 1963 we have tested 94,000 newborn infants in Hessen, Hannover and Bremen and have detected 12 cases of phenylketonuria in families in which this disease was not previously encountered (Table VI). This frequency of 1:7850 is somewhat higher than that found in the much bigger screening programme in the U.S.A., where the corresponding figure is 1:10,862. Assuming a frequency of 1:10,000 for this disease, 100 phenylketonuric children should be born every year in Western Germany alone.

In screening institutionalised, mentally defective patients for phenylketonuria, we found a frequency of 47:15,069 (0·31 per cent) with the

Table VI

RESULTS OF PKU-SCREENING PROGRAMME IN HESSEN, HANNOVER AND BREMEN BY THE GUTHRIE TEST

	NEWBORNS WITHOUT PKU IN FAMILY	PATIENTS IN MENTAL INSTITUTIONS *
NUMBER OF TESTED CASES	94,254	15,069
PHENYLKETONURICS FOUND	12	47
	(0.013%)	(0.310%)

* THE FIGURES INCLUDE PATIENTS WITH VARIOUS PSYCHOSES

Guthrie test and 19:1400 (1·4 per cent) with the $FeCl_3$ test (Bickel 1967). The higher detection rate with the $FeCl_3$ test is due to the fact that in that series the testing was limited to severely defective patients, while the Guthrie test series included psychotic and mildly retarded patients, after a case of phenylketonuria was nearly missed in a patient with so-called schizophrenia. The importance of detecting older cases of phenylketonuria lies in the possibility of advising their families and having every further newborn tested with special care. In view of the recessive inheritance of this disease there is a risk that 25 per cent of the children of the heterozygous parents will be affected.

In the course of our screening programme we have come across six infants whose phenylalanine blood levels were definitely and persistently raised above the upper normal range, but not as high as those of the usual phenylketonuric patients, being somewhere between 4 and 8 mg. per cent, with a negative $FeCl_3$ test in the urine. During loading tests with L-phenylalanine the phenylalanine blood levels rose far above 20 mg. per cent and the $FeCl_3$ test became positive, in marked contrast to healthy children of the same age. These observations raise difficult diagnostic and therapeutic problems. We have so far decided not to treat patients whose phenylalanine blood level does not exceed 8 mg. per cent, but to check their levels at monthly intervals and their mental development at three-monthly intervals. The oldest of these children at the age of 11 months showed a spontaneous decline of his phenylalanine blood concentration to normal levels and may retrospectively be regarded as having suffered from a delayed maturation of the phenylalanine hydroxylase. The other patients continue to have moderately raised phenylalanine blood levels, so far without any retardation in their development. They may be 'formes frustes' of phenylketonuria (Mabry *et al.*, 1962; Mozziconacci *et al.*, 1964; Lund & Ovnbøl, 1966), but it is too early to decide this question. The blood concentration of the other amino acids, especially of tyrosine, is normal. Further biochemical studies, such as the measurement of the o-HO-phenyl-acetic acid excretion and heterozygote tests of the parents, are not yet completed. It should be stressed here that phenylalaninaemia, though

usually a leading symptom of phenylketonuria, may also be observed in other conditions such as tyrosinosis (Gentz *et al.*, 1965) and the disorder described by Anderson *et al.* (1966), so each case deserves a careful clinical and biochemical study before the final diagnosis is established.

The brain damage of the phenylketonuric patients can be prevented by placing them on a phenylalanine-restricted diet within the first three months of life. Detailed descriptions of such diets have been published by various authors (Woolf *et al.*, 1958; Centerwall *et al.*, 1961; Moncrieff & Wilkinson, 1961; Woolf, 1962; Bickel & Gruter, 1963; Lyman, 1963; Blainey *et al.* 1963; Umbarger *et al.*, 1965; Wilson & Clayton, 1965; Bickel & Bremer, 1967; Bickel, 1967). Of the phenylalanine-low protein hydrolysates commercially available we have principally used 'Cymogran', but we have also had excellent results with 'Lofenalac','Albumaid' and with two Japanese preparations, 'Lophemilk' and 'Phenytol'. In addition to the hydrolysates it is important to provide a mixed diet with a balanced vitamin intake (Wilson & Clayton, 1965) and additional phenylalanine, mainly in the form of proteins of high biological value such as milk, to cover the reduced, but still essential phenylalanine requirement of these patients. Two specimen diets are shown in Table VII and the phenylalanine requirement of different age

Table VIIa and b. *Specimen diets for a 10 week and a 13 month old patient with phenylketonuria.*

a

Phenylketonuria	*10 weeks, 5.100 g. BW*	
60 g. Cymogran 100 g. milk 35 g. sugar 15 g. cornflour 150 g. carrots 10 g. margarine 30 g. carrot juice	*44 mg. phenylalanine* 4·3 g. protein 3·6 g. fat 17 g. carbohydrate 126 Cal	per kg. BW

b

Phenylketonuria	*13 months, 10·7 kg. BW*	
100 g. Cymogran 100 g. milk 30 g. sugar 5 g. cornflour 150 g. carrots 50 g. potatoes 25 g. margarine 150 g. apples 50 g. cornflour bread 20 g. marmalade 100 g. apple juice 30 g. orange juice	*29 mg. phenylalanine* 3·2 g. protein 3·2 fat 15·1 g. carbohydrate 108 Cal	per kg BW

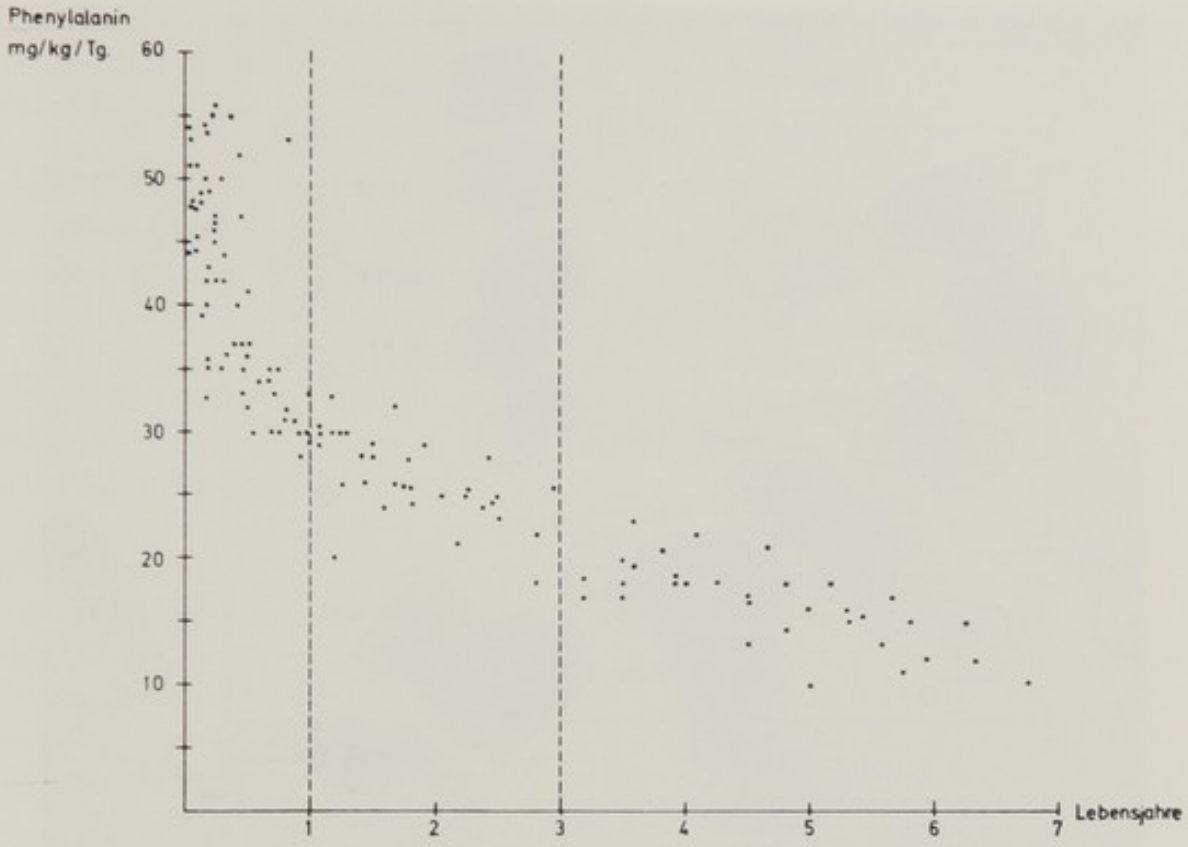


FIG. 6

Phenylalanine requirement of phenylketonuric patients of different age groups, as measured by the phenylalanine intake in mg./Kg./24 hrs. The data were taken only from patients on a well controlled phenylalanine-restricted diet, with phenylalanine blood levels between 1-4 mg per cent and with a satisfactory gain in weight. In the first year of life the requirement shows a broad scatter between 30-55 mg./Kg./24 hrs., whilst in the second and third year the requirement ranges between 20-30, thereafter between 10-20 mg./Kg./24 hrs.

groups in Figure 6. We try to keep our patients' phenylalanine blood concentrations as closely as possible to between 1-2 mg./100 ml., and tolerate only temporarily an upper limit of 4 mg./100 ml. No definite proof has yet been submitted that a higher phenylalanine blood level may be maintained over a longer period of time without harm to the patient.

Symptoms of protein or phenylalanine deficiency may occur if the patient's phenylalanine intake is restricted too rigidly or if he refuses to accept the unpleasant flavour of the hydrolysates. In our experience such symptoms and signs have been relatively mild, consisting of generalised aminoaciduria (Fig. 7), reversible osteodystrophy and, in some cases, a failure to grow and gain weight normally (Dittrich, 1965). Since we increased the daily phenylalanine intake to the figures shown in Figure 6 these symptoms have become much rarer.

Within the last 14 years we have treated some 60 patients over periods of ~~from~~ one to six years. In 20 of these patients treatment was started within the first three months of life. Figure 8 shows an attempt to present our results in diagramatic form. The development quotient (DQ) remains

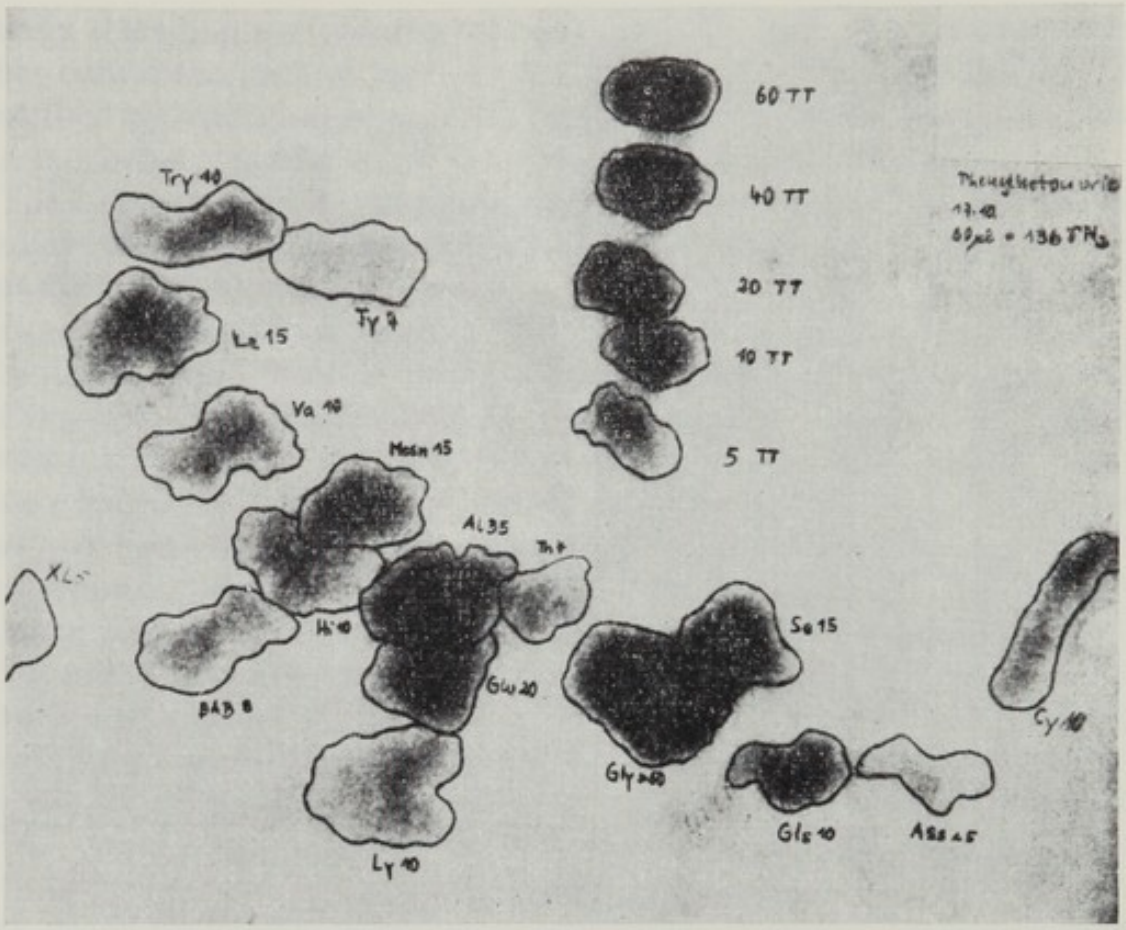


FIG. 7

Two-dimensional paper chromatogram showing generalised aminoaciduria in 'overtreated' PKU.
Cy=cystine as cysteic acid, Ass=aspartic acid, Gls=glutamic acid, Gly=glycine, Se=serine, Th=threonine, Al=alanine, Glu=glutamine, Ly=lysine, Mesn and Try=methionine (as sulphone) and tryptophan, from additions to the caseine hydrolysate, Hi=histidine, βAB=beta-amino-isobutyric acid, Va=valine, Le=the leucines, Ty=tyrosine. 5-60 TT=test spots with 5-60 μg. taurine. X=unknown ninhydrin reacting substance.

normal if the diet is started in the first three months of life whilst delayed treatment encounters an increasing degree of irreversible brain damage. Little or no improvement is observed in patients whose treatment is started after the fifth year of life (Bickel & Gruter, 1963). A boy whose treatment was begun at the age of 2 months and who has now at the age of $5\frac{1}{2}$ years a DQ of 104 is shown in Figure 9, in contrast to an idiotic untreated patient aged 12 in Figure 10.

There are empirical and theoretical reasons suggesting that strict dietary treatment can be terminated at the age of 7 to 8 yrs. and be substituted by a normal diet of relatively low protein content (Bickel & Gruter, 1963). An important decision will, however, soon have to be faced when young phenylketonuric women, kept normal by the diet, reach child-bearing age. Will the phenylalanine-restricted diet have to be reinstituted during pregnancy? Present-day evidence supports this policy. Through various authors'

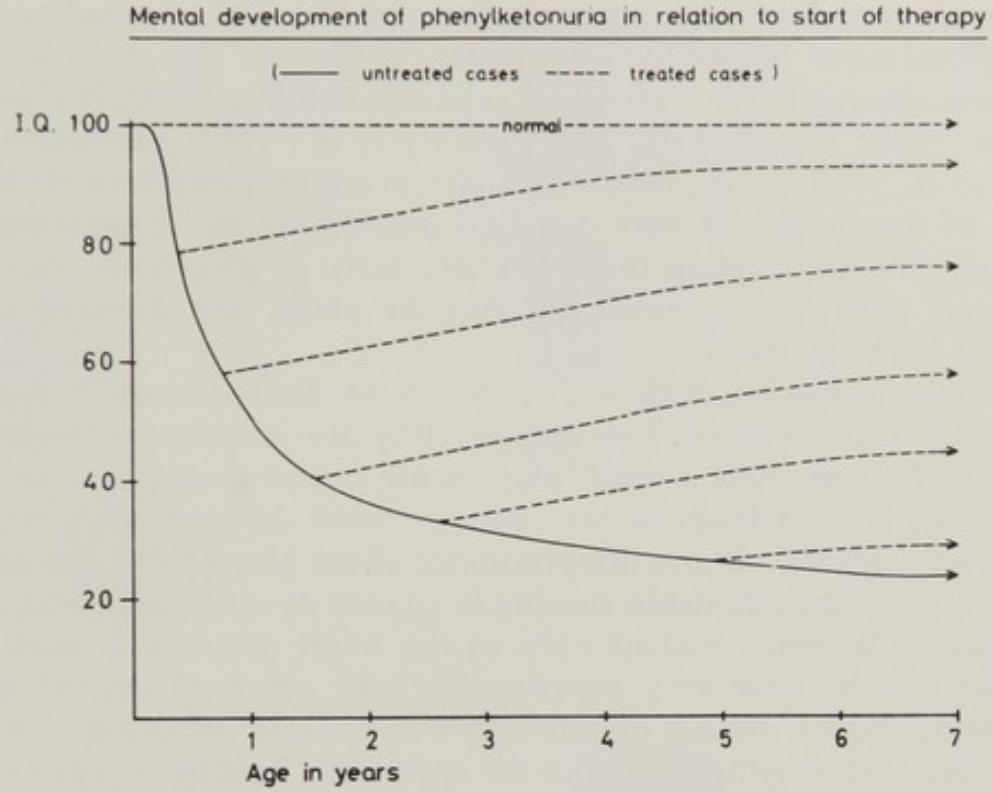
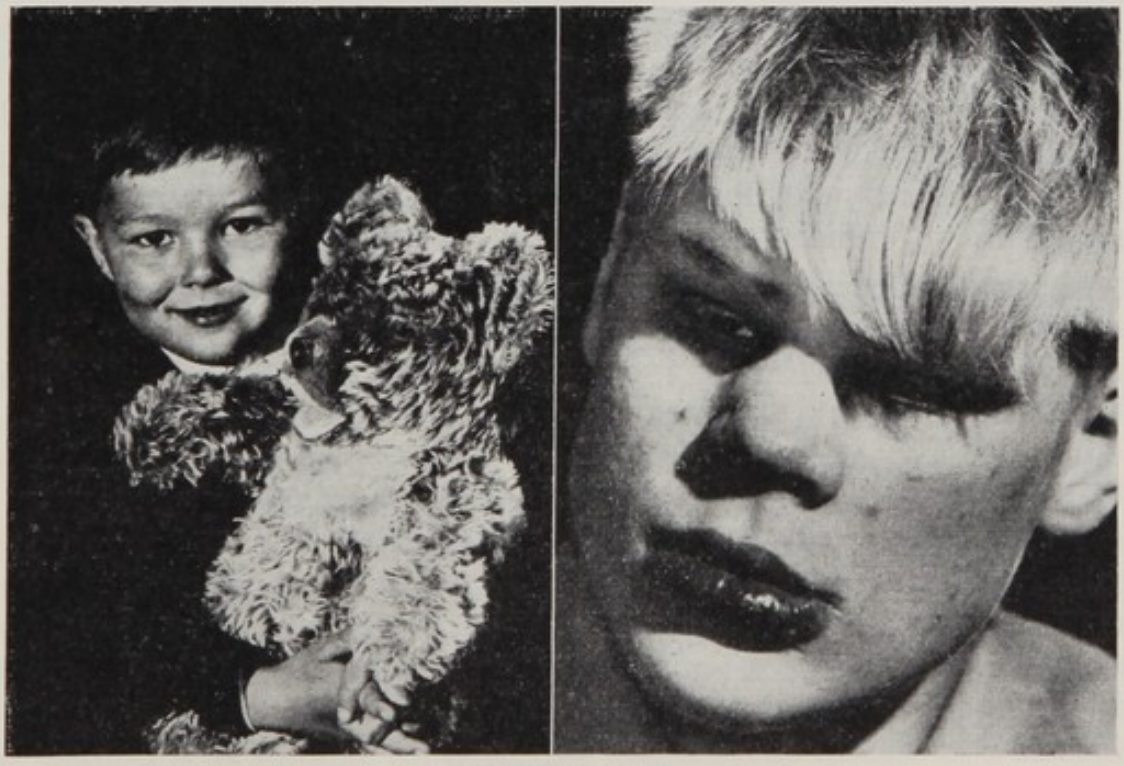


FIG. 8

Mental development of PKU in relation to start of therapy. (——— untreated cases, — — — — — — treated cases, for further description of the diagram see text.)



FIGS. 9 and 10

LEFT: 5½ year old patient whose treatment was started soon after birth and who has remained normal. RIGHT: 12 year old idiotic patient with PKU who remained untreated.

publications and private communications we know of a limited number of phenylketonuric mothers who have produced non-phenylketonuric children, about a third of whom suffered substantial prenatal brain-damage (Jervis, 1954; Woolf *et al.*, 1961; Mabry *et al.*, 1963; Denniston, 1963; Fisch *et al.*, 1966; Jervis, 1966). Though it is not yet possible to explain why some of these children were damaged and others not, experience so far strongly favours treating these mothers early in pregnancy with a well balanced phenylalanine-restricted diet to avoid any damage to their infants' brain in utero.

(Ed.: See also Allan & Brown's paper in this Symposium).

2. *Maple syrup disease.* This enzymopathy, also inherited in an autosomal recessive fashion, runs a much more acute course than phenylketonuria. Generally, it soon leads to very serious brain damage, with attacks of dyspnoea, convulsions and disappearance of the Moro reflex some days or weeks after birth, with death ensuing in infancy or early childhood. Mental retardation becomes evident only in the more protracted cases, whilst 'formes frustes' have very occasionally been observed beyond infancy, presenting sudden attacks of coma, torsion spasms, deviation of the walk and other central symptoms in so far apparently healthy children (Morris *et al.*, 1961; Lonsdale *et al.*, 1963; Kil & Rokkones, 1964; Morris *et al.*, 1966; Müller & Bickel, 1966). The disease was first described by Menkes *et al.* in 1954 in North America and derives its name from the odour of the patients' urine, resembling that of the syrup of the maple sugar tree.

The disease is due to a metabolic block in the oxidative decarboxylation of the alpha-keto acids of leucine, isoleucine and valine (Fig. 11). The keto acids and amino acids accumulate in the blood and urine a few days after birth and can be demonstrated in the urine by paper chromatography (Fig. 12). An early diagnosis is of special importance, as the brain damage progresses so rapidly, and protective treatment is now available. In

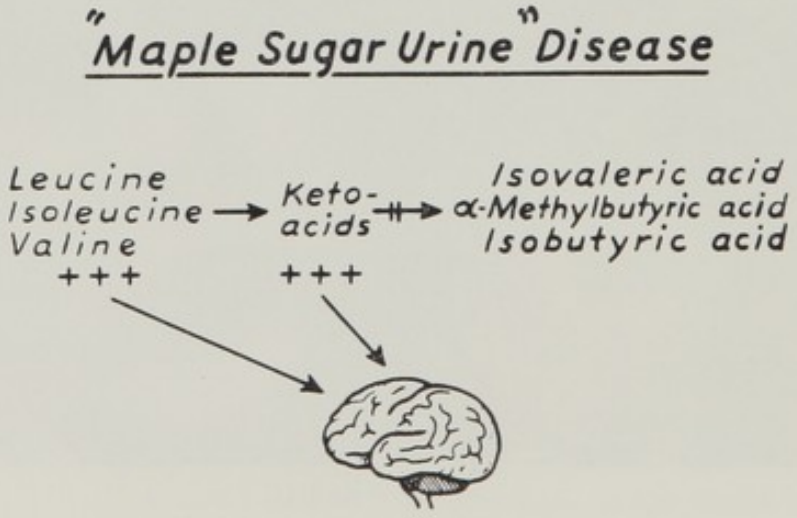


FIG. 11
The metabolic block in maple sugar urine disease.

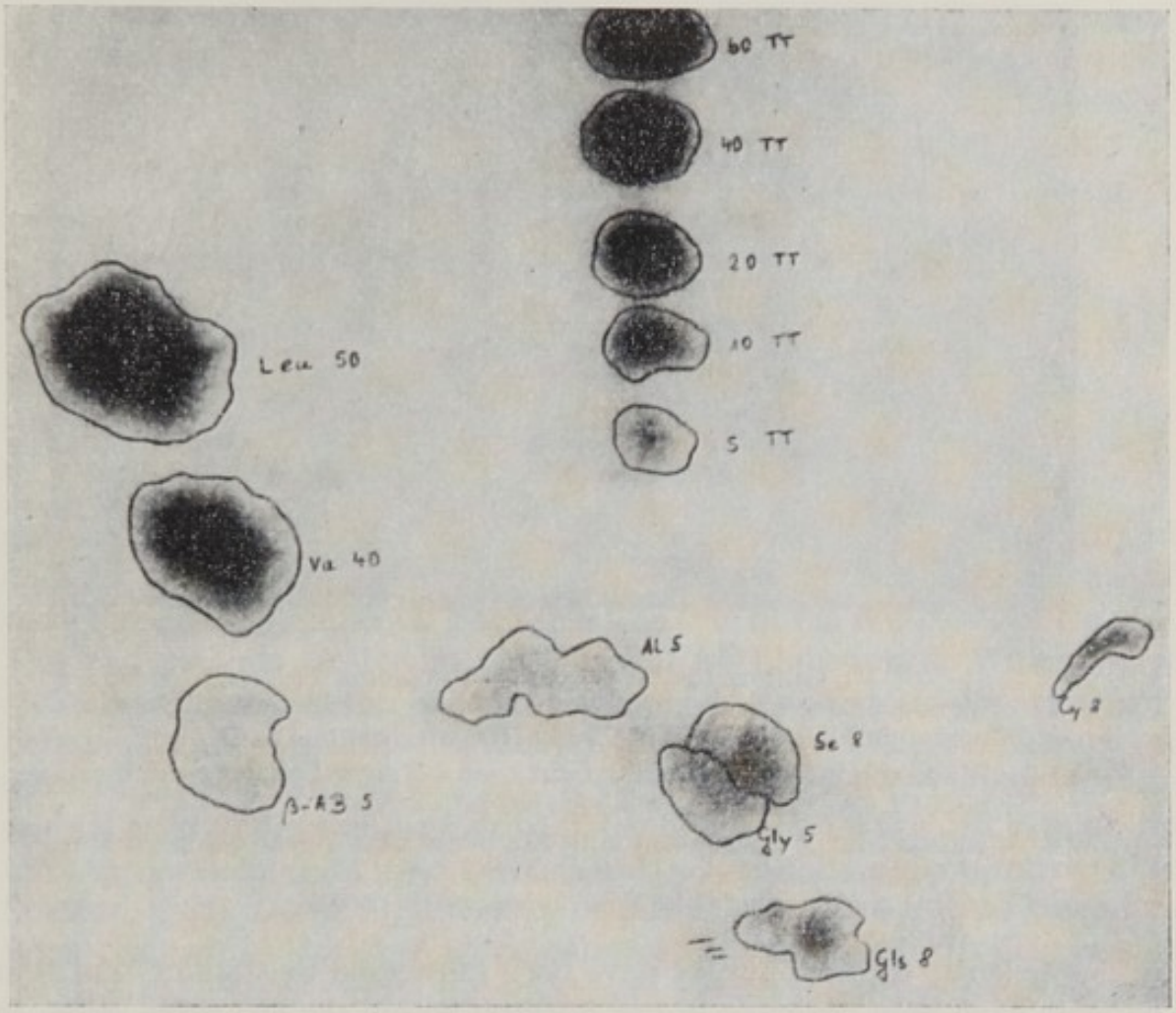


FIG. 12

Two-dimensional paper chromatogram showing the strong increase of valine and the leucines (leucine and isoleucine) in the urine of maple sugar urine disease. (For abbreviations see Fig. 7).

analogy to the inhibition test for phenylalanine, Guthrie (1964) described a similar inhibition test for leucine, using as the only modification another antimetabolite, 2-methyl-leucine, for the leucine inhibition. The blood specimen for this test can be collected on the same filter paper card as for the phenylalanine test.

The leucine inhibition test was introduced into our laboratory in 1964 and has proved to be as specific and sensitive as the phenylalanine test. So far 3,016 newborn infants, a small number of mentally retarded patients and a 2 year old boy with the 'forme fruste' of maple syrup disease have been tested. An increased blood concentration for leucine before and after a loading test was observed only in this patient (Fig. 13), suggesting that the method is as suitable for detecting maple syrup disease in a screening programme as is the phenylalanine test for phenylketonuria. Every newborn infant in the population should have a routine test for leucinaemia just as for phenylalaninaemia. No data are yet available as to the frequency

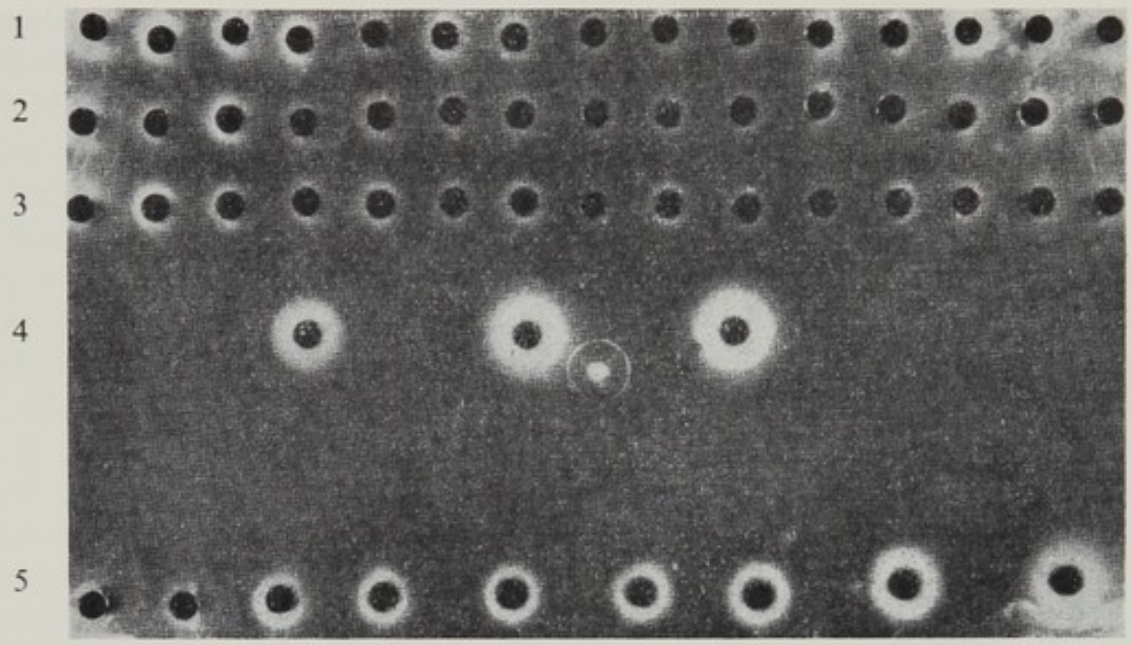


FIG. 13

Guthrie test for leucine in the blood.

Row 5 = Standard row, showing the growth of bac. subtilis around blood discs containing 2-2-4-4-6-6-8-12-20 mg./100 ml. leucine.

Row 1-3 = Blood specimens of normal infants aged 4-7 days. Leucine concentration about 2 mg./100 ml., max. 4 mg./100 ml.

Row 4 = Raised leucine concentration in the blood of a 2 year old patient with mild maple sugar urine disease, fasting (left), 2 hrs. (center) and 3 hrs. (right) after loading with 1·5 g. L-leucine by month.

of maple syrup disease, but we have been impressed by the fact that four patients have been observed in our hospital within the last three years. In the United States routine screening has revealed maple syrup disease in a newborn infant (personal communication, Ashley, 1966).

In the acute form of the disease the metabolic deviation must be corrected within the first seven to ten days of life if brain damage is to be fully or at least partially prevented (Westall 1963; Snyderman *et al.*, 1964; Linneweh & Willenbockel, 1965; Ireland, 1965). Treatment is still more complicated than in phenylketonuria, as the intake of three essential amino acids, leucine, isoleucine and valine, has to be restricted to the small amounts tolerated by the individual patient. This can be achieved only with a synthetic diet, providing protein mainly in the form of pure amino acids (Table VIII). It is thus possible, although sometimes difficult, to reduce the blood level of the branched-chain amino acids to normal. Good biochemical control is essential, and is achieved by measuring the plasma concentrations of the amino acids at regular intervals by the quantitative column chromatographic method.

The success of the therapy is still difficult to assess, as the number of cases treated over a longer period of time is still small. It will depend largely on the age at which treatment is started and on the severity of the enzyme defect. Whilst Westall succeeded in keeping a now 5 year old girl mentally

Table VIII

SPECIMEN DIET FOR A 7 MONTH OLD PATIENT WITH MAPLE SYRUP DISEASE

MAPLE SYRUP DISEASE	7 MONTHS, 7.0kg BW
200g WHEY	
20g L-AMINOACIDS	
30g CORNFLOUR	
40g SUGAR	LEUCINE 30 mg/kg BW
60g BUTTER	ISOLEUCINE 22 mg/kg BW
150g CARROTS	VALINE 22 mg/kg BW
5g YEAST	
600g TEA	
VITAMINS & MINERALS	

normal by beginning the diet on the sixth day of life, Snyderman and co-workers were unable completely to prevent brain damage in two of their three treated patients. The same was true of the two cases of Ireland. The two patients treated in our hospital by Linneweh & Willenbockel showed an unusually low leucine tolerance and died from a severe erythrodermia at 4 and 9 months respectively, despite the early start of therapy on the seventh and tenth day of life. A remarkable autopsy finding was the normal myelogenesis in the treated patient, whilst an untreated case of the same age exhibited a very marked lack of myelinisation (Linneweh & Solcher, 1965). Despite the severity of this disease there is good reason to hope that with increasing therapeutic experience and early diagnosis it will become possible in future, to prevent the brain damage more successfully than has been possible so far.

3. *Galactosaemia*. The enzymatic defect of this recessively inherited error of carbohydrate metabolism is located in the transformation of galactose-l-phosphate to glucose-l-phosphate (Fig. 14). Galactose and galactose-l-

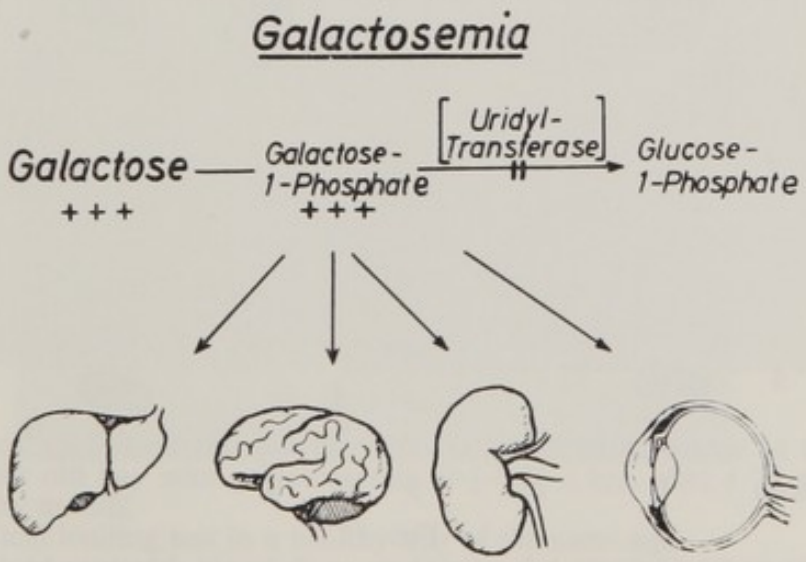


FIG. 14
The metabolic block in galactosaemia.

phosphate accumulate before the block and can be traced in blood and urine in very high concentrations, leading soon after birth to damage of the liver, brain, of the eyes, lenses and kidneys. In the British Isles the frequency of the disease was estimated to be 1:70,000, with a gene frequency of 1:268 (Schwarz *et al.*, 1961).

In view of the excellent work of Holzel and his co-workers (for reviews see Holzel, 1957; Holzel & Schwarz, 1957) the clinical features of glactosaemia are so well known that I need comment only on the relative ease with which serious brain and liver damage, cataracts and early death from malnutrition, infections or electrolyte and water imbalance can be prevented by early diagnosis and the introduction of a galactose-free diet. For this disease also, routine screening of every newborn baby is of the utmost importance.

Galactosuria should be suspected if the urine shows a positive reduction test together with a negative glucose oxidase reaction. More specific and

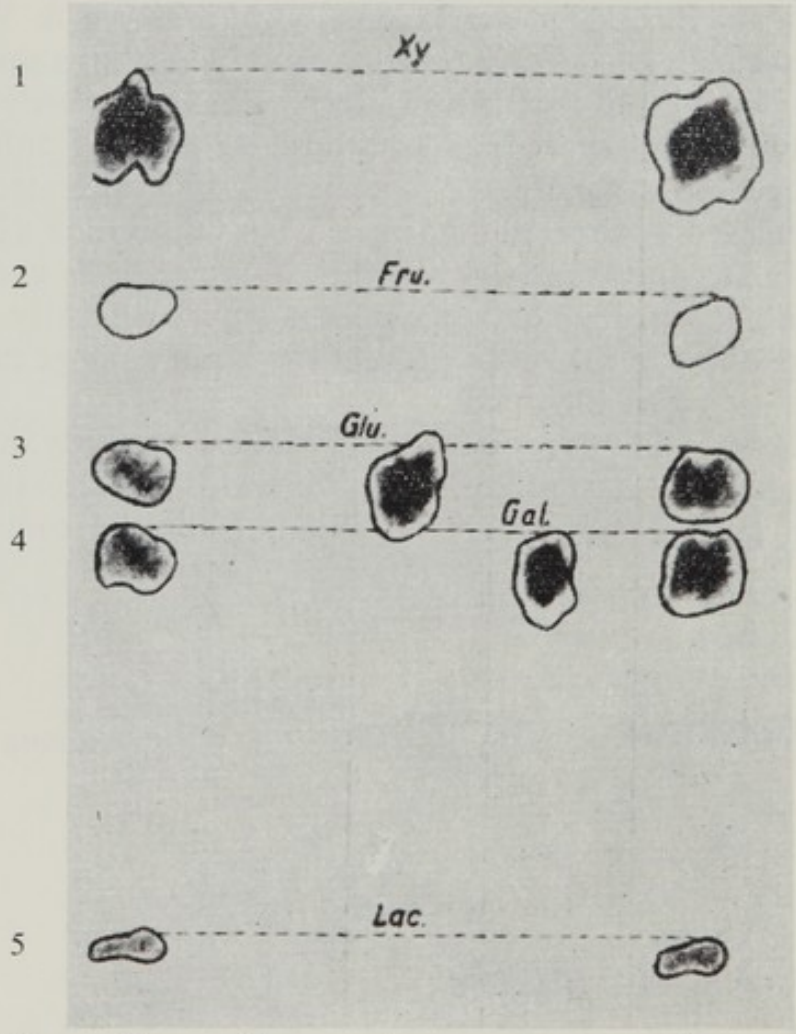


FIG. 15

One-dimensional paper chromatogram with normal urine (column 2), with urine of a glucosuria (column 3) and urine of a galactosaemia (column 4). Columns 1 and 5 show test runs with pure lactose, galactose, glucose, fructose and xylose.

sensitive methods of detection are paper or thin-layer chromatography demonstrating galactose in quantities of several gamma within a few hours of birth (Fig. 15). Special techniques to measure the reduced activity of galactose-l-phosphate-uridyl-transferase in the patients' erythrocytes have been established and some of these have been adapted for screening purposes (Schwarz, 1960; Beutler *et al.*, 1964; London *et al.*, 1964).

A microbiological inhibition test has been described by Guthrie (1964) which is well suited for large scale screening. In contrast to the phenylalanine and leucine test, this method does not use an antimetabolite, but a mutant coli strain W-3101 which lacks uridyltransferase activity, just like the patient. One or two drops of blood are taken from a heel prick, deposited on the same filter paper card as for the phenylalanine and leucine tests and sent to a central laboratory. The punched-out blood discs are then placed on a special agar medium which has been inoculated with the coli mutant. After incubation the growth of the bacteria will be inhibited around those blood discs which contain galactose, the inhibition zone correlating roughly with the galactose concentration of the specimen (Fig. 16).

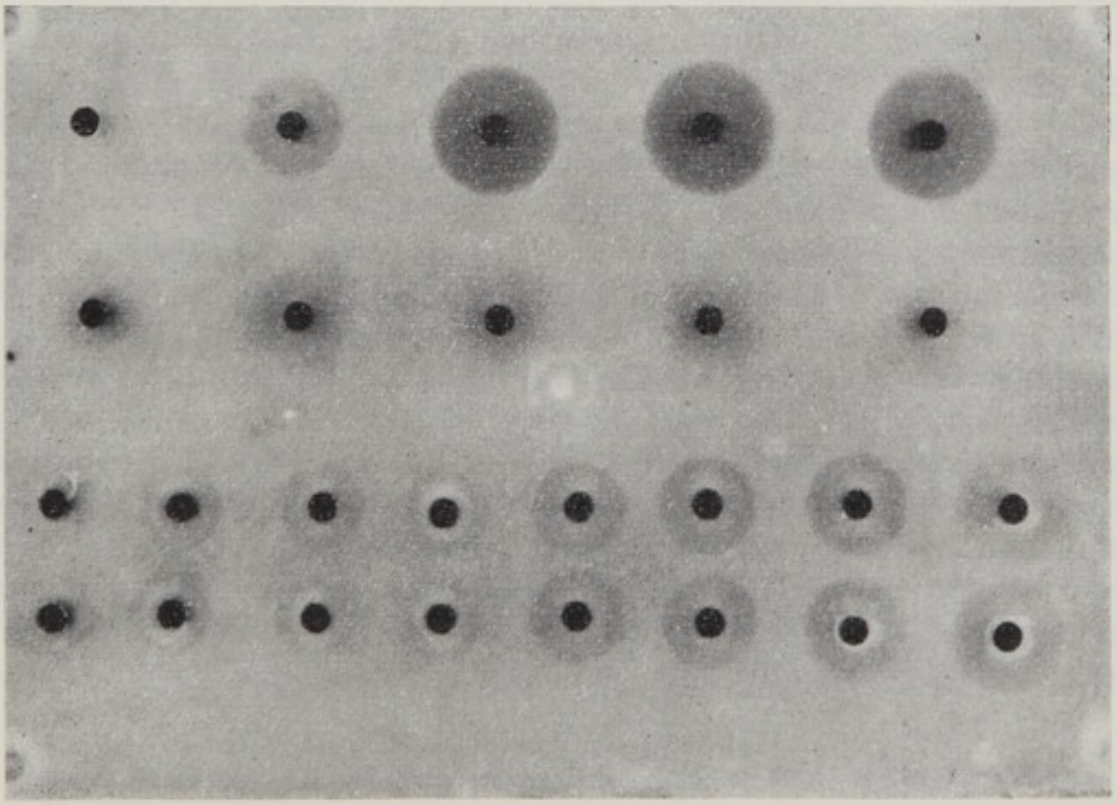


FIG. 16

Microbiological inhibition test for the demonstration of galactose in the blood.
Row 1: 1½ year old boy with galactosaemia, 0-½-1-1½-2 hrs. after 1·75 g. D-galactose by mouth.
Row 2: Identical loading test in a healthy boy of the same age.
Row 3: Standard row with blood discs containing 6-6-10-10-30-30-50-100 mg./100 ml. galactose.
Row 4: Standard row identical to row 3.

As galactose and galactose-l-phosphate may be expected to increase in the blood of a patient soon after birth, this test can be performed at the same age as the test for phenylalanine and leucine, namely on the fourth or fifth day of life. It will easily disclose any pathological galactosaemia above 10 mg./100 ml. Specificity tests carried out in our laboratory by Gross (1967) revealed that valine and glycine also gave growth inhibition in this system, the inhibition of valine in concentrations of 8 mg./100 ml. corresponding to the inhibition of galactose in concentrations of 10 mg./100 ml., whilst more than 16 mg./100 ml. glycine had to be present to give a very small inhibition corresponding to less than 6 mg./100 ml. galactose. A positive 'galactose' test should, therefore, always be differentiated from valinaemia and glycinaemia by other suitable methods (for details of this test and of Guthrie's leucine test see Gross, 1967). So far we have tested 5,097 newborn infants for galactosaemia with negative results. Galactosaemic newborn babies have already been discovered in screening programmes with this test in the United States, Denmark and Switzerland (personal communications Ashley, 1966; and Poley, 1966).

Conclusions

The examples of phenylketonuria, maple syrup disease and galactosaemia give an idea of how brain damage caused by inborn errors of metabolism can be prevented in future. Other possibilities, such as routine heterozygote testing of prospective parents and eugenic counselling if they carry the same mutant gene, have not been discussed here because of the complicated problems inherent in such procedures (see also Evans, 1965). Even in the case of known heterozygosity of the parents, medical advice is difficult to give and should be carefully individualised. Heterozygous parents have a right to know the facts about the risks involved in having further children, but they should be absolutely free to make their own decision. It is the doctor's task to have the means of early detection and therapy available if further affected children are born. In these high-risk individuals, as in the general population, the expenditure of time and money for a preventive programme bears no relation to the great financial burden and amount of care required by the late or undiagnosed patients, nor to the emotional stress which they inflict upon their families.

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EEG—FINDINGS IN PATIENTS WITH PHENYLKETONURIA BEFORE AND DURING TREATMENT WITH A LOW-PHENYLALANINE DIET AND IN PATIENTS WITH SOME OTHER INBORN ERRORS OF AMINO ACID METABOLISM

J. R. Poley and G. Dumermuth

Introduction

Inborn metabolic errors have become a field of paramount interest for two main reasons: (1) most of the known entities are caused by a genetically determined enzyme defect and are frequently associated with mental retardation; and (2) early diagnosis followed by correct treatment will prevent the development of mental retardation. This is known for phenylketonuria (Horner, 1961; Horner, *et al.*, 1962; Carson & Neill, 1962; Murphy, 1963; Sutherland, *et al.*, 1966), maple syrup urine disease (Guthrie, 1965; Ireland, 1965) and may also hold true for galactosemia.

The sequelae of the biochemical lesion in inborn metabolic errors are detrimental to the normal development of the brain especially in the newborn period and in infancy and, as in the case of maternal phenylketonuria brain damage does occur in utero (Allan, 1966; Fisch, *et al.* 1966; Mabry, *et al.* 1966).

Cerebral lesions induced by metabolic disorders will give rise to qualitative and quantitative changes in the EEG. Lesions of different aetiologies, however, will produce EEG abnormalities which are not typical for the underlying disease, but depend largely upon the state of cerebral maturation (Gibbs & Gibbs, 1964). In spite of difficulties which may arise in the interpretation of certain pathological changes, the EEG examination has become an indispensable means for the assessment of cerebral function. Repeated EEG examinations are of great importance for the follow-up of patients with those inborn metabolic errors amenable to treatment.

Since there is a need to correlate long-range therapeutic results with electroencephalographic findings, we have put major emphasis on the modification of EEG abnormalities in patients with phenylketonuria undergoing treatment with a low-phenylalanine diet. Investigations on this subject were carried out by Woolf, Griffiths & Moncrieff 1951, Armstrong & Tyler 1955, Horner & Streamer 1956, Low, Bosma & Armstrong 1957, Geisler & Stroeder 1958, Hsia, Knox, Quinn & Paine 1958, Pampiglione 1961, Stadler 1961 and Stemmermann 1965, but the study periods were, in general, too short for definite conclusions. The EEG findings in untreated phenylketonurics are extensively discussed in the papers of Low, Bosma & Armstrong (1957), Paine (1957), Hsia, Knox, Quinn & Paine (1958),

Pampiglione (1961), Stadler (1961), and Fisch, Sines, Torres & Anderson (1965), to which the reader is referred for special details.

Among the group of inborn errors of amino acid metabolism treated in this paper, we also have included homocystinuria, hyperglycinemia and the cerebro-oculo-renal syndrome (Lowe). Since there is little detailed information in the literature concerning EEG findings in these disorders, we take the opportunity to report our observations, although their interpretation remains still rather uncertain.

Phenylketonuria

Methods and Patients

EEG technique. EEGs have been recorded either with a Grass 8-channel electroencephalograph or with an Offner 16-channel apparatus. Each examination included bipolar as well as unipolar montages. In infants and children above one year of age, electrodes were set according to the 10-20 international system (with the exception of electrodes F7 and F8 positioned more laterally, Dumermuth, 1965), whereas in newborns and young infants a reduced montage of 12 electrodes was used. Recording was performed with a paper speed of 30 mm./sec. and usually with a setting of 7 mm pen deflection for 50 microvolts amplitude.

Determinations of serum amino acids. Phenylalanine and other amino acids in the serum were determined by column chromatography according to the method of Spackman, Stein & Moore (1958). To follow the effect of the low phenylalanine diet phenylalanine in the serum was also determined by the bacterial inhibition assay of Guthrie & Susi (1963).

Assessment of intelligence levels. Intelligence and developmental quotients were determined by the tests of Brunet-Lézine, Kramer, Stanford-Binet, the Hamburg-Weschsler intelligence scale for children and the Schweizer Test (Biäsch).

Patients. Between 1950 and 1966, 34 patients (18 males, 16 females), aged 2 weeks to 19 years were observed at the Kinderspital Zürich. Of the 31 patients who had either one or more EEGs, 18 did not receive any treatment, either because a low-phenylalanine diet was not available or because the patients were already at an age where a therapeutic success could no longer be expected. The mean age at the time of diagnosis in this group was 3 1/12 years (range 1 3/12 to 5 11/12). With the exception of five patients, the first EEG was done at the time of diagnosis. The remaining 13 patients received a low-phenylalanine diet supplemented with a low-phenylalanine protein hydrolysate (Lofenalac, or Albumaid XP, or Cymogram) according to Table I. The mean age at the time of diagnosis in the treated group was 1 3/12 years (range 6 days to 3 1/12 years). The beginning of the treatment ranged from 2 weeks to 3 3/12 years and the duration of treatment from 2 months to 9 6/12 years. All 13 patients are presently still on the dietary regimen.

Table I

PHENYLKETONURIA TREATMENT : DAILY PHENYLALANINE (Phe) and PROTEIN REQUIREMENTS

AGE	PHE, mg/kg/24h	PROTEIN, g/kg/24h
2 WEEKS TO 3 MONTHS	45 - 60	3.0
4 TO 5 MONTHS	30 - 45	3.0
6 TO 17 MONTHS	25 - 30	2.8 - 3.0
18 MONTHS TO 3 YEARS	18 - 25	2.6 - 2.8
3 TO 5 YEARS	15 - 20	2.2 - 2.4
5 TO 10 YEARS	14 - 17	2.0

Table II

EEG ABNORMALITIES, I.Q. AND BLOOD-PHENYLALANINE LEVELS IN UNTREATED PATIENTS WITH PHENYLKETONURIA

NO. & SEX	INITIAL INVESTIGATION				FOLLOW-UP INVESTIGATION			
	AGE YR MO	EEG	I.Q.	PHE mg/100ml	AGE YR MO	EEG	I.Q.	PHE mg/100ml
1 M	1 3	HYPS [1]	ID-IM	-	-	-	-	-
2 F	1 4	HYPS [1]	ID	-	1 8	HYPS/SSW	ID	-
3 F	1 7	HYPS [1]	ID	-	2 [8] 3	SSW	ID	-
4 F	1 7	HYPS [1]	ID	-	14 4	DS* β	ID	30
5 F	1 11	HYPS [1]	ID	-	3 [10] 7	HYPS/SSW	ID	-
6 M	2 11	HYPS/SSW, DS* [2]	ID	-		-	-	-
7 F	5 4	SpWP, MEF [3]	ID	-	-	-	-	-
8 F	5 11	SpWP, RHY	59	-	13 1	FLSpWP, DS*	60	12-15 [11]
9 F	10 11•	SpWP	ID-IM	-	-	-	-	-
10 M	2 6	DS*	ID?	-	-	-	-	-
11 M	4 12	DS* β [4]	ID	-	19 1	FLSpWP, EF [5]	ID	20
12 M	5 6	DS*	ID	-	-	-	-	-
13 M	5 7	β [6]	ID	-	-	-	-	-
14 M	18 8•	β	ID	20	-	-	-	-
15 F	1 9	N	ID-IM	-	16 6	FLSpWP	ID	20
16 F	17 5•	N	42	30	-	-	-	-
17 M [7]	13 11•	DS* β	IM	16-17 [11]	-	-	-	-
18 M [7]	13 11•	DS*, β	IM	16-17 [11]	-	-	-	-

• DIAGNOSED EARLIER.

Key to Symbols: Key to EEG findings see Table III.

1. Infantile spasms.
2. Normalization after a 10-day treatment with ACTH.
3. Only discrete bioccipital spike/wave-paroxysms and multifocal sharp waves.
4. Seizures, having occurred during the third and fourth year of life, are reported without details.
5. Epileptogenic focus in the posterior temporal region.
6. Infantile spasms during the first three years of life.
7. Identical homocygotic twins.
8. Death at 4½ years after a severe gastroenteritis.
9. Died of a cerebral hemorrhage secondary to a status epilepticus.
10. Self-strangulation at 4 years.
11. Low-protein diet (no strict low-phenylalanine diet plus hydrolysate).

ABBREVIATIONS
ID—Idiot. IM—Imbecile. N—Normal.

Table III

KEY OF ABBREVIATIONS OF EEG FINDINGS

EEG ABNORMALITIES	SYMBOLS
1. NON-SPECIFIC ABNORMALITIES :	
11. DIFFUSE SLOWING OF BACKGROUND ACTIVITY	DS
DEGREE : (DS) BORDERLINE	
DS * SLIGHT	
DS ** MODERATE	
DS *** SEVERE	
12. FOCAL SLOW WAVES (DELTA-FOCUS)	FS
13. INCREASED FAST ACTIVITY (RELAT. INCREASE OF β-ACTIVITY WITHOUT EFFECT OF MEDICATION)	β
14. INTERMITTENT DELTA-RHYTHMS	RHY
15. PAROXYSMAL SLOW WAVES	PSW
2. SPECIFIC HYPERSYNCHRONOUS ABNORMALITIES :	
21. HYPSARHYTHMIA	HYPS
22. SHARP AND SLOW WAVE-PATTERN (PETIT MAL VARIANT)	SSW
23. SPIKE-WAVE PAROXYSMS (GENERALIZED PAROXYSMS OF IRREGULAR SPIKE AND WAVE COMPLEXES)	SpWP
SPIKE-WAVE PAROXYSMS FOLLOWING PHOTIC STIMULATION ONLY	FLSpWP
24. SPIKE AND WAVE RHYTHM (RHYTHMIC 3/SEC-SPIKES AND WAVES EQUALS CLASSICAL PETIT MAL)	SpWRHY
25. EPILEPTOGENIC FOCUS (FOCAL SHARP WAVES)	EF
26. MULTIPLE EPILEPTOGENIC FOCI (MULTIFOCAL SHARP WAVES	MEF
(SYMBOLS IN PARENTHESIS: QUESTIONABLE OR ONLY DISCRETELY PRESENT)	

* Indicates severity of diffuse slowing in E.E.G.

Prior to 1960, the diagnosis was established by a positive $FeCl_3$-test in the urine, after 1960 in association with the determination of the serum-phenylalanine*. One patient was discovered in early 1966 during the routine newborn screening program with the bacterial inhibition assay (Guthrie & Susi, 1963).

* We are indebted to Dr. I. Antener, AFICO Laboratoires de Recherches, La Tour-de-Peilz, for the determinations of the serum-phenylalanine (Udenfriend, S. & Cooper J. R., 1953) from 1960 to 1962.

Results

Electroencephalographic findings in untreated patients. The EEG findings together with data of the serum-phenylalanine concentrations and the IQ (whenever done) are summarized in Table II.

Electroencephalographic findings in patients treated with a low-phenylalanine diet. The initial EEG findings as well as the follow-up examinations are presented together with other clinical data in Figures 1, 2 and 3. Before therapy, three patients presented with hypsarhythmia, one with multiple epileptogenic foci, four showed increased, diffuse beta-activity, whereas the remaining five had a normal EEG. In an attempt to follow the changes of the EEG findings, the patients will be discussed according to the pre-treatment pattern of the EEG.

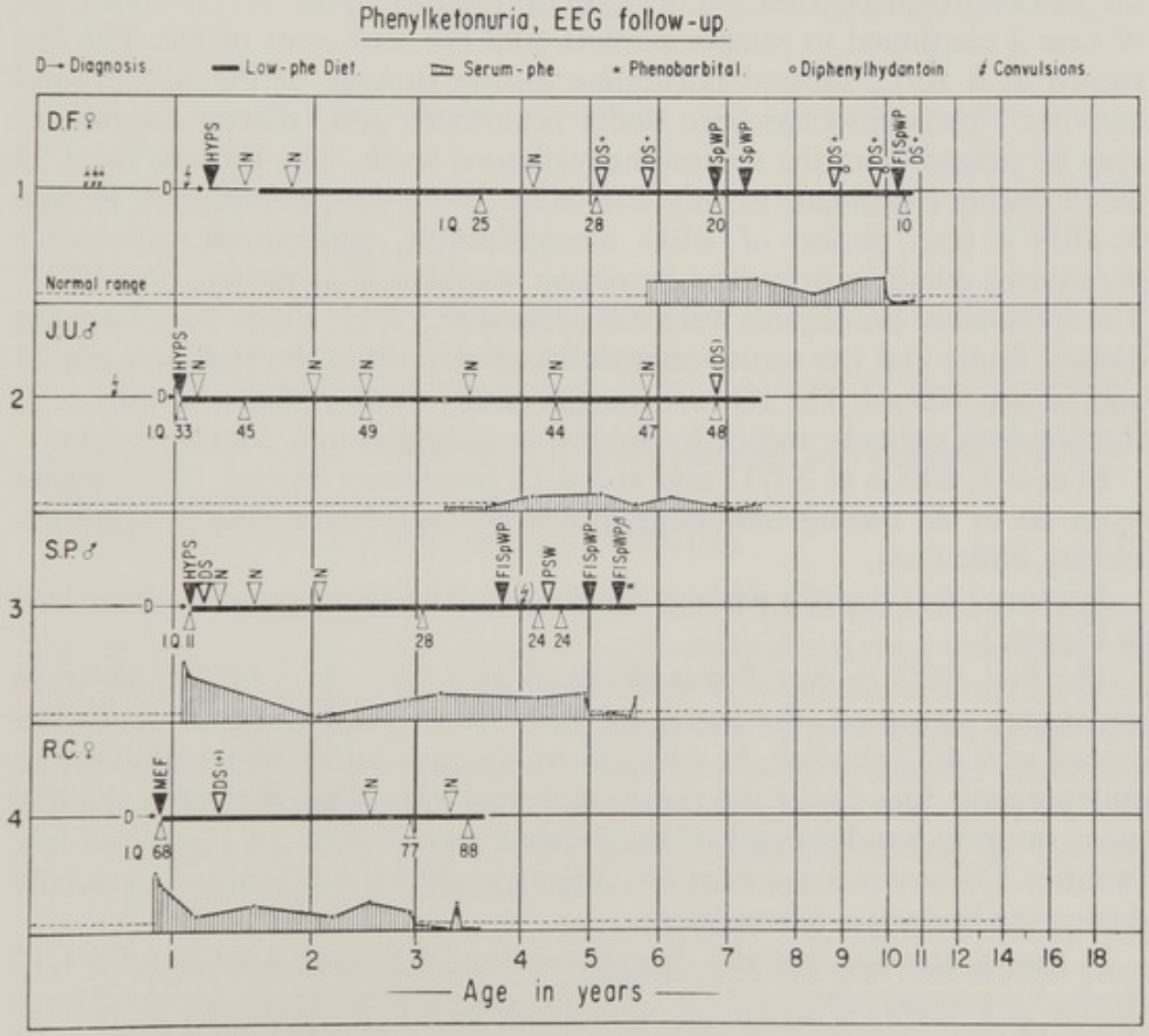

FIG. 1

EEG follow-up in phenylketonurics undergoing treatment with a low-phenylalanine diet.

Patients with specific, hypersynchronous abnormalities prior to treatment.

(Key for abbreviations of EEG findings see Table III).

Hypsarhythmia and multiple epileptogenic foci. The findings are summarized in Figure 1. Interestingly enough, in case 1 the EEG had become normal spontaneously when it was repeated at the age of 1 6/12. The child was then started on a low-phenylalanine diet and the EEG remained normal until her fourth year of life. During the sixth year of life slight diffuse slowing of the background activity was noted, followed by spontaneous spike-wave paroxysms at 6 10/12. The last recording, at 10 4/12 (without anticonvulsive medication) showed slight diffuse slowing of the background activity as well as spike-wave paroxysms following photic stimulation. Dietary management was difficult and consequently, the serum-phenylalanine was elevated between 10 and 12 mg./100 ml. over long periods of time. Her IQ was never higher than 28.

Cases 2 and 3 were diagnosed at approximately the same age. In both, the EEG normalized after a short term of treatment with ACTH. The EEG of case 2 continued to remain normal until the sixth year of life. The last record at 6 10/12 showed borderline diffuse slowing of the background activity. This patient has been under reasonably good dietary control, as may be judged from the serum-phenylalanine levels. The IQ rose from 33 to 48 during treatment, mainly due to better verbal performance. In case 3, after a brief period of EEG normalization, generalized spike-wave paroxysms could be provoked by photic stimulation at the age of 3 10/12. The spike-wave paroxysms were still present at 5 5/12. There were frequent dietary faults and the serum-phenylalanine was often elevated between 10 and 12 mg./100 ml. The IQ rose insignificantly during treatment but, as in the previous patients, motor hyperactivity subsided to a notable extent.

In case 4, EEGs at 2 7/12 and at 3 4/12 years were normal. The IQ rose from 60 to 88 during treatment, however, there were also intermittent dietary difficulties.

Increased fast activity without medication. The findings are summarized in Figure 2.

The first EEG in case 5 was obtained at 3 3/12. At 3 6/12 spike-wave paroxysms appeared, the increased beta-activity still being present. The record at 9 4/12 showed, in addition to the preexisting abnormalities, an epileptogenic focus over the right precentral area. This patient is the one most severely handicapped of the treated group, with an IQ in the low twenties. Dietary management was often fraught with difficulties, especially during the first years of treatment.

In case 6, the beta-activity disappeared and EEGs at 4 1/12 and 5 1/12 showed a normal pattern. At 6 1/12, an epileptogenic focus appeared over the right temporal area, changing its localization in subsequent EEGs and disappearing again at 8. The serum-phenylalanine, which initially was well over 20 mg./100 ml., remained at rather high (10-20 mg./100 ml.) levels for longer periods of time due to feeding difficulties. The IQ remained unchanged around 50. In case 7, bilateral posterior delta-rhythms were recorded,

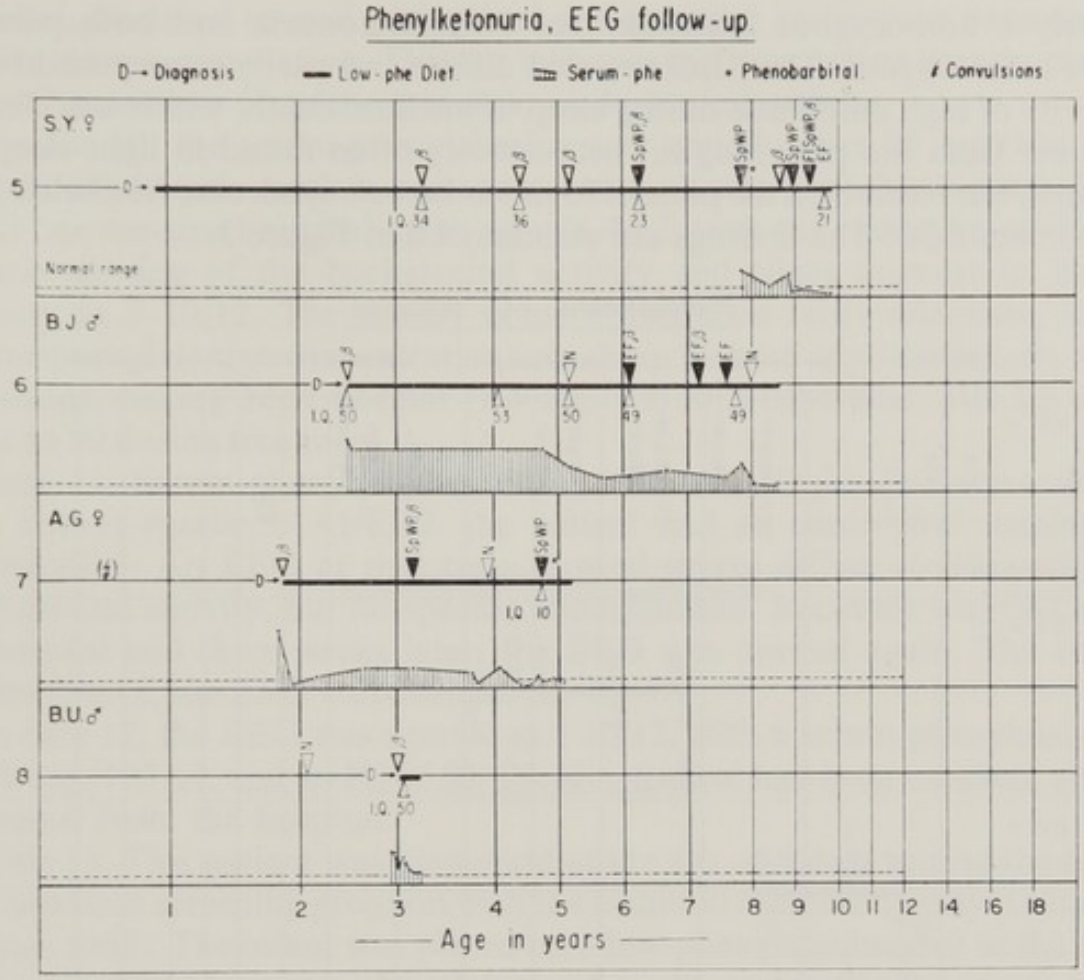

FIG. 2

EEG follow-up in phenylketonurics undergoing treatment with a low-phenylalanine diet.
Patients with non-specific EEG abnormalities prior to treatment.
(Key for abbreviations of EEG findings see Table III.)

in addition to the increased beta-activity. At 3 4/12, in spite of a low-phenylalanine diet for 20 months, irregular spike-wave paroxysms were observed over the posterior regions during light sleep. With phenobarbital, a normal EEG was obtained at 3 11/12, whereas at 4 9/12 generalized spike-wave paroxysms were again present. The initial serum-phenylalanine concentration was over 20 mg./100 ml. and remained moderately elevated (4-9 mg./100 ml.) with occasional normal levels, especially during the last two years. The IQ is at the idiot level.

Case 8. Even though mentally retarded (IQ of 50), repeated $FeCl_3$-tests in the urine failed to show positive results during the first and second years of life. It happened to be positive when tested at 2 11/12. The serum-phenylalanine, checked repeatedly over a period of four weeks while on a regular diet, ranged between 6 and 20 mg./100 ml. with an average of 12 to 15 mg./100 ml. To determine his zygosity, a phenylalanine loading test (Anderson *et al.*, 1962) was carried out in the patient, in his parents and in normal controls. According to the serum-tyrosine response the patient was

clearly a homozygous individual for phenylketonuria and both parents were heterozygous. The EEG revealed diffuse and partly paroxysmal beta-activity of high amplitude during sleep of medium depth, which was clearly distinct from the physiological beta-activity often found in light sleep of infants, but resembled the pattern found in Lowe's syndrome (see below).

Normal EEG. The findings are summarized in Figure 3.

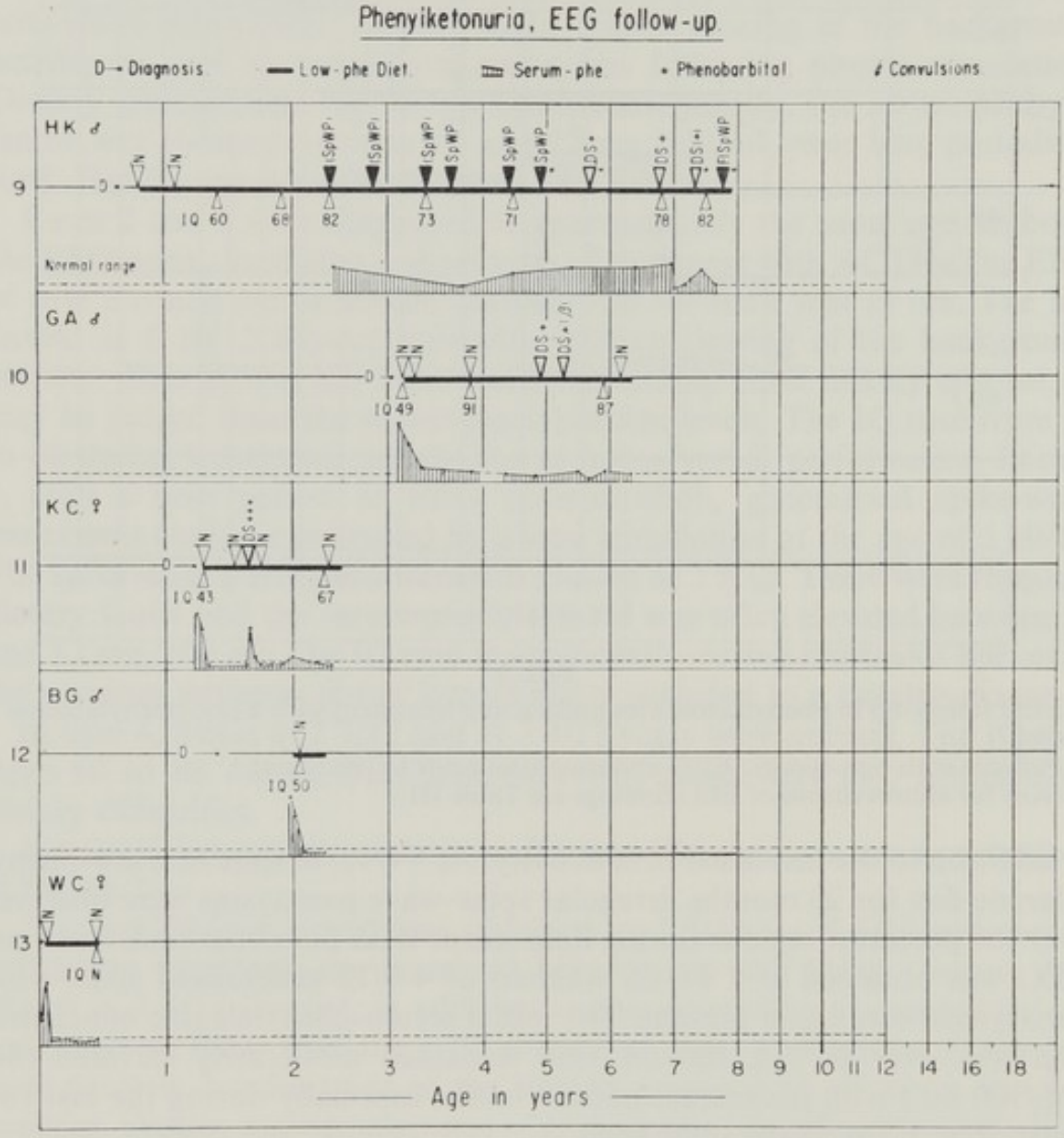

FIG. 3

EEG follow-up in phenylketonurics undergoing treatment with a low-phenylalanine diet.
Patients with a normal EEG prior to treatment.
(Key for abbreviations of EEG findings see Table III).

Case 9 showed questionable spike-wave paroxysms during the third year of life, which became distinct at 3 9/12, although the background activity showed a more mature pattern. The last EEG, at 7 9/12, in spite of pheno-

barbital, disclosed several generalized and irregular spike-wave paroxysms during photic stimulation. Dietary management was intermittently poor. The IQ rose from 60 to the low eighties during treatment.

In case 10, the serum-phenylalanine was initially over 20 mg./100 ml. and the IQ 49 (this result is open to question, it was probably higher). The EEG has remained normal up to now (6 4/12) with the exception of slight diffuse slowing of the background activity and slight increase in beta-activity at 5 10/12. The dietary control had been fairly adequate, with serum-phenylalanine concentrations reaching 5 and 6 mg./100 ml. on two occasions, having been normal (1-4 mg./100 ml.) otherwise. His IQ had risen to 90 during treatment.

Case 11. Serum-phenylalanine 39 mg./100 ml., IQ 43. Dietary control was always excellent. At 8/12, the patient had an acute viral meningo-encephalitis. An EEG at that time showed severe diffuse slowing of the background activity, but no specific abnormalities. Recovery was fast and uneventful and three weeks later, the EEG was normal again. The child is now 2 6/12, the EEG still normal, the IQ 67.

In case 12, the EEG was normal at 1 10/12, with a serum-phenylalanine of 30 mg./100 ml. and an IQ of 50. Dietary control had been excellent since dismissal from the hospital.

Case 13. This patient was diagnosed at the age of 6 days during the routine newborn screening program with the bacterial inhibition assay (Guthrie & Susi, 1963). The infant was placed on a low-phenylalanine diet at the age of 2 weeks and the serum-phenylalanine has always been in the upper normal range since. The psychomotor development at the age of 6 months was normal at all levels. A repeat EEG was also normal.

Comment

The EEG findings in our patients with phenylketonuria without, or prior to, treatment with a low-phenylalanine diet correlate well with those published in the literature (Fig. 4). A high percentage of patients show hypersynchronous abnormalities: Fois, Rosenberg & Gibbs (1955) 95 per cent, Low, Bosma & Armstrong (1957) 91 per cent, Stadler (1961) 85 per cent, Hsia, Knox, Quinn & Paine (1958) 63 per cent, Loesch (1965) 58 per cent, Paine (1957) 58 per cent, Fisch, Sines, Torres & Anderson (1965) 57 per cent and 48 per cent of our patients. Figure 4 also shows that the more severe abnormalities like hypsarhythmia and generalized sharp and slow waves occur in infants and young children, whereas generalized spike-wave paroxysms are predominantly present in older children. The incidence of normal EEGs after two years is considerably lower in the untreated than in the treated patients (Fig. 4). In all of our patients, the pattern of hypsarhythmia as well as multiple epileptogenic foci disappeared following the initiation of the low-phenylalanine diet (Fig. 5). Spike-wave

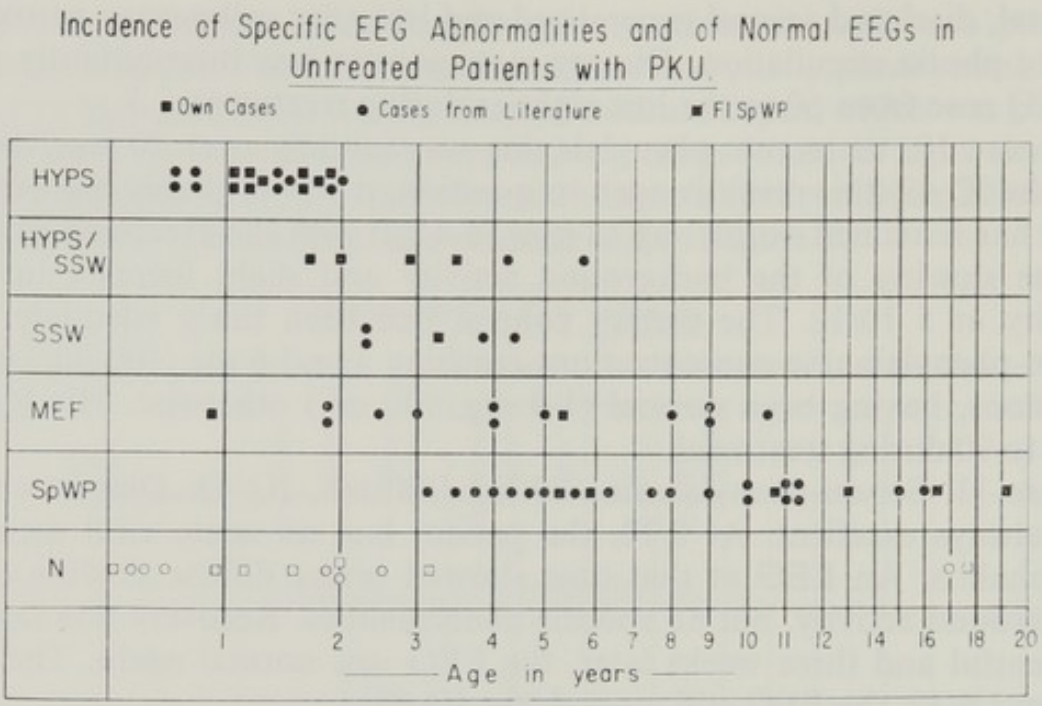

FIG. 4
(See text and Table III)

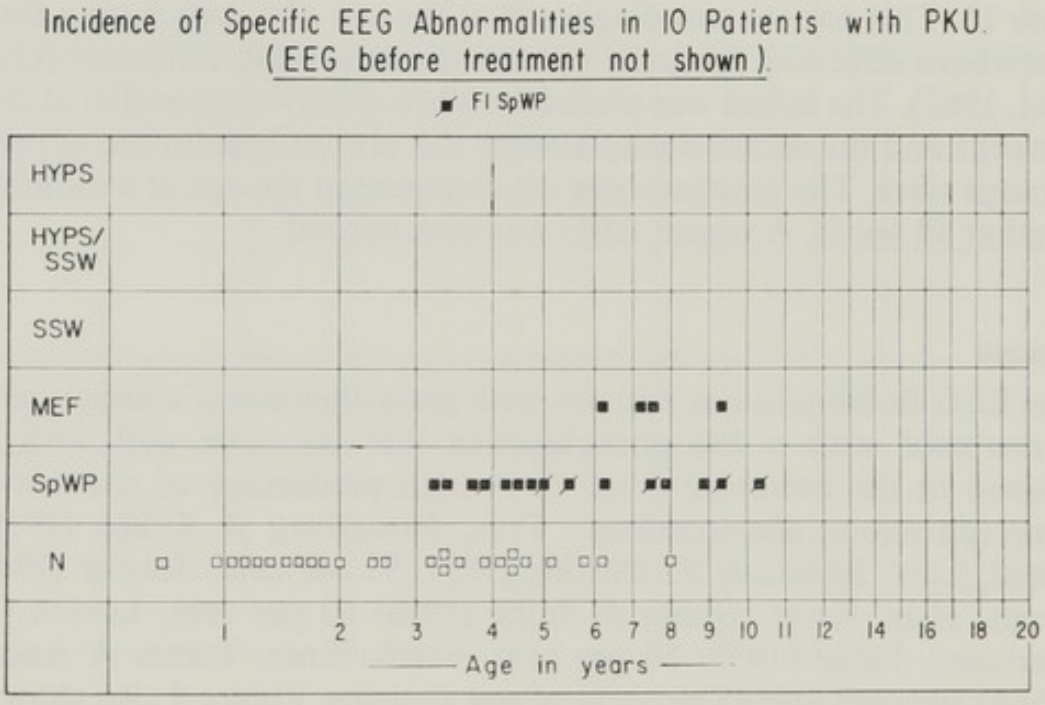

FIG. 5
(See text and Table III)

paroxysms were the predominant findings in children after three years (also in the treated patients).

The expression of the sequelae of the biochemical lesion seem to vary widely with regard to the degree of EEG abnormalities, the degree of mental retardation, the serum-phenylalanine concentration and the excre-

tion of phenylpyruvic acid and other metabolites in the urine (Armstrong & Low, 1957; Stadler, 1961). For a better understanding of the variety of clinical and EEG findings, the biochemical lesion (as far as is known) will be discussed briefly.

Primarily, phenylketonurics lack a functional hydroxylating system, which includes the phenylalanine-4-hydroxylase and the enzyme that hydroxylates tryptophan to 5-hydroxytryptophan (Bessman, 1964). The phenylalanine-hydroxylase consists of two fractions, of which fraction I is the important principle and only present in the liver (Mitoma *et al.*, 1957; Wallace *et al.*, 1957). In addition, an unconjugated pteridine, dihydrobiopterin is required as co-factor to mediate the hydroxylation of phenylalanine (Kaufman, 1963) together with dihydrofolic reductase and NADPH.

In homozygous individuals, the phenylalanine-hydroxylase block is about 95 per cent effective (Bessman, 1964) and phenylalanine will be metabolized to various hydroxylated and non-hydroxylated compounds which, together with the elevated phenylalanine are implicated in a series of disturbances which lead to cerebral damage: Excess phenylalanine prevents the transfer of essential amino acids into the brain cells, thus causing a deficiency situation which affects the brain in an important phase of its functional differentiation (Linneweh, *et al.* 1963). Excess phenylalanine also interferes with myelin formation (Chase & Obrien, 1966).

Phenylalanine and/or some of its metabolites inhibit various decarboxylases, causing a deficiency of the primary amines dopamine, serotonin and gamma-aminobutyric acid (Allan & Moss, 1964). Serotonin deficiency in early infancy seems a very important etiologic factor for the causation of the mental defect (Pare *et al.*, 1957; Pare *et al.* 1958; Perry *et al.*, 1964; Woolley & Van der Hoeven, 1964). The primary amines mentioned are known to act as synaptic transmitter substances (Allan & Moss, 1964) and have inhibitory properties. Relatively large amounts of gamma-aminobutyric acid are present in the normal brain (Tsukada *et al.*, 1963; Elliot, 1965) and especially in increasing amounts during development (Baxter & Roberts), 1961). In phenylketonuric animals, the gamma-aminobutyric acid content in the brain is markedly reduced (Tsukada, 1966). Depletion of gamma-aminobutyric acid of the brain was shown to be associated with convulsions (Elliott, 1965; Scriver *et al.* 1966).

By the action of L-aromatic amino acid decarboxylase, the synthesis of toxic amines from phenylalanine is encouraged: o-tyramine and phenylethylamine (Bessman, 1964). Phenylethylamine is synthesized in increased amounts in phenylketonurics and has vasoactive and convulsive properties (Oates *et al.* 1963).

Hyperphenylalaninemia results in a defective intestinal transport of tryptophan (Yarbro & Anderson, 1966). The tryptophan metabolic abnormalities seem to be, in part, secondary to a transport defect of this amino acid.

There is an enhanced formation of phenylacetaldehyde from phenylalanine or from phenylpyruvic acid. Phenylacetaldehyde may exert toxic effects by its reaction with SH-groups. The latter are also present in co-enzyme A, which is involved in synaptic transmission of nerve impulses (Seidenberg *et al.* 1962).

Phenylalanine and/or some of its metabolites inhibit the enzyme tryptophan pyrrolase leading to a deficient synthesis of nicotinic acid, which may be implicated in symptoms referable to the central nervous system (Bessman, 1964).

The pathological changes in the brain of phenylketonurics are of two types: status spongiosus and demyelinization (Malamud, 1966). Some of the older patients show changes indistinguishable from non-inflammatory sudanophilic leucodystrophy (Crome, 1964). Furthermore, marked changes in the Betz cells and certain other types of nerve cells in the brain stem and in the spinal cord have been found (Corsellis, 1953).

If the complexity of the biochemical lesion and its secondary effects are taken into consideration and the possibility entertained that the metabolic block of hydroxylation may only be partial (Hudson *et al.*, 1963), and/or the secondary effects of the phenylalanine metabolites on various other enzyme systems only active to a certain degree, it may become more understandable, why, for instance, a phenylketonuric child of 3 years (without treatment) is only moderately or slightly retarded or may even have an IQ of 120 (Sutherland, Berry & Shirkey, 1960), or why some children with phenylketonuria at 1 3/12 have an abnormal EEG, whereas others at the same age have hypsarhythmia and are severely mentally retarded. Very important are the observations that siblings and parents of phenylketonurics show a higher incidence of abnormal EEGs than the general population (Fisch *et al.*, 1965) and that different EEG abnormalities may be encountered in identical phenylketonuric twins (Herrlin, 1962).

One could expect that a considerable increase in EEG abnormalities occurs in phenylketonuric children during a phenylalanine loading test and it is interesting that the children who only showed mild or negligible changes in the EEG during a phenylalanine load were those, whose IQ at the time of the test were around normal (Clayton *et al.*, 1966).

The evolution of EEG abnormalities in patients during treatment with a low-phenylalanine diet is not clearly understood, but is most likely enhanced by factors that compromise the delicate balance of the phenylalanine-hydroxylating activity. In our experience the EEG abnormalities found in phenylketonuric children seem to follow a similar evolutive pattern as in those children with diffuse, respectively generalized epilepsy without associated metabolic errors.

The most challenging and interesting patients will be those in whom the diagnosis can be made in the newborn period. Patients, in whom excellent dietary control with normal serum-phenylalanine concentrations

throughout the first four to six years of life can be maintained, should theoretically always show normal EEG activity in correlation with a normal psychomotor development. If this postulation proves to be incorrect in spite of every effort towards an optimal control, two possibilities have to be entertained: the time between diagnosis and beginning of treatment (in newborns and young infants) was sufficient to induce a cerebral lesion, or the brain was already damaged in utero. The latter has to be expected when the mother is a phenylketonuric herself, albeit of normal or near-normal intelligence (Coffelt, 1964; Allan, 1966; Perry & Tischler, 1966).

Homocystinuria

Homocystinuria is a well-defined inborn error of sulfur-containing amino acids associated with mental retardation (for a recent review see: Werder *et al.*, 1966). However, in distinction to phenyl-ketonuria, the evolution of cerebral damage may be less progressive (Carson & Neill, 1962). The aetiology is a deficiency of the enzyme cystathionine synthetase, which forms cystathionine from homocysteine and serine. The relationship between the enzyme defect and the cerebral damage is not known. In the literature, the EEG findings are described as being normal (Carson & Neill, 1962; Komrower & Wilson, 1963) or abnormal (Carson *et al.*, 1965). No observations in infants or young children are available as yet.

Patients. Two female patients with the classical features of this metabolic syndrome were first seen at the age of 5 8/12 and 6 3/12 years. In both children, the methionine and the homocysteine concentration in the serum as well as the methionine concentration in the cerebrospinal fluid were markedly elevated. Both excreted also very large amounts of homocystine in the urine. Both had an IQ in the imbecile range.

EEG findings. The EEG of the younger patient showed a slight diffuse slowing of the background activity with bilateral intermittent delta-rhythms in the parieto-temporal regions. At 6 11/12, there were also discrete spike-wave paroxysms during drowsiness. The older patient showed a similar pattern: slight diffuse slowing of the background activity with bilateral intermittent delta-rhythms over the temporo-occipital regions as well as sporadic generalized, irregular spike-wave paroxysms.

The EEG findings in these patients indicate that structures of the brain stem were predominantly affected. Interestingly enough, the most conspicuous lesions in the case reported by Carson and her co-workers (1965) were focal necrosis and gliosis, mainly in the midbrain. A low-protein (low-methionine) diet enriched with cystine (Brenton *et al.* 1965) given to one of our patients did not correct the biochemical or alter the EEG abnormalities to a significant extent.

Hyperglycinemia

The enzyme defects of the acidotic and the non-acidotic types of this

disease are not yet known (Rampini *et al.*, 1967). It is probable that different enzyme blocks can lead to similar hyperglycinemic syndromes. The EEG reports in patients with hyperglycinemia contain little detailed information. EEGs in the newborn period may either be markedly abnormal (Childs *et al.*, 1961; Balfe *et al.*, 1965), or normal (Schreier & Müller, 1964; Corbeel, 1966).

Patients. Two patients with hyperglycinemia could be studied. The first (female) with the acidotic type of the disease was admitted at the age of 7 weeks with severe ketosis, a strongly elevated glycine in the serum and with marked hyperglycinuria. There were no convulsions, but during acidosis occasional myoclonic jerks could be observed. For several weeks, the patient did fairly well on a diet containing 1 g. of protein/kg. body weight/day. Following an increase of the dietary protein to 3·3 g./kg. body weight/day, there was a severe relapse into marked ketosis associated with stupor, hyperglycinemia and hyperglycinuria. The patient died at the age of $4\frac{1}{2}$ months due to a generalized septicemia.

The second patient (male) was hospitalized at the age of 2 months and was found to have the non-acidotic type of hyperglycinemia and hyperglycinuria associated with frequent and severe convulsions. A reduction of the dietary protein to 1 g./kg. body weight/day did not have any effect upon the clinical course and the low-protein diet was therefore discontinued. The patient is now 1 5/12 years old, shows severe psychomotor retardation, but no convulsions with antiepileptic therapy.

EEG findings. The EEG in the first patient (7 weeks) merely showed moderate diffuse slowing of the background activity. A repeat EEG five days prior to the exitus showed marked diffuse slowing of the background activity with the intermittent flattening of the record known as an ominous sign. The initial EEG in the second patient (non-acidotic type of hyperglycinemia with convulsions) at the age of 2 months disclosed multiple epileptogenic foci, especially over the posterior regions. The reduction of the protein intake (see above) and an antiepileptic therapy lead to transient normalization of the EEG as well as to a reduction of the frequency and severity of convulsions. At 4 months, multiple epileptogenic foci reappeared again over the posterior regions and at 5 months, in spite of the strictly low-protein diet, the record showed hypsarhythmia. In the last EEG at 1 1/12 (antiepileptic therapy) there were discrete spike-wave paroxysms during light sleep.

No explanations can be given concerning the relationships between the acidotic and the non-acidotic types of hyperglycinemia and the degree of cerebral damage. The patient with the acidotic type had a severe clinical course and demonstrated only non-specific EEG abnormalities, whereas marked hypersynchronous abnormalities were found in the patient with the non-acidotic type. The EEG findings in the two patients cannot, in view of limited experience, be called pathognomonic for either type of hypergly-

cinemia. The changing EEG patterns in the second patient (multiple epileptogenic foci-brief normalization after beginning of low-protein diet-hypsarhythmia-spike-wave paroxysms) are also encountered in other types of metabolic errors (phenylketonuria for instance) and have no bearing as to the course, but are the expression of the altered response of the brain according to the increase in age and functional maturation.

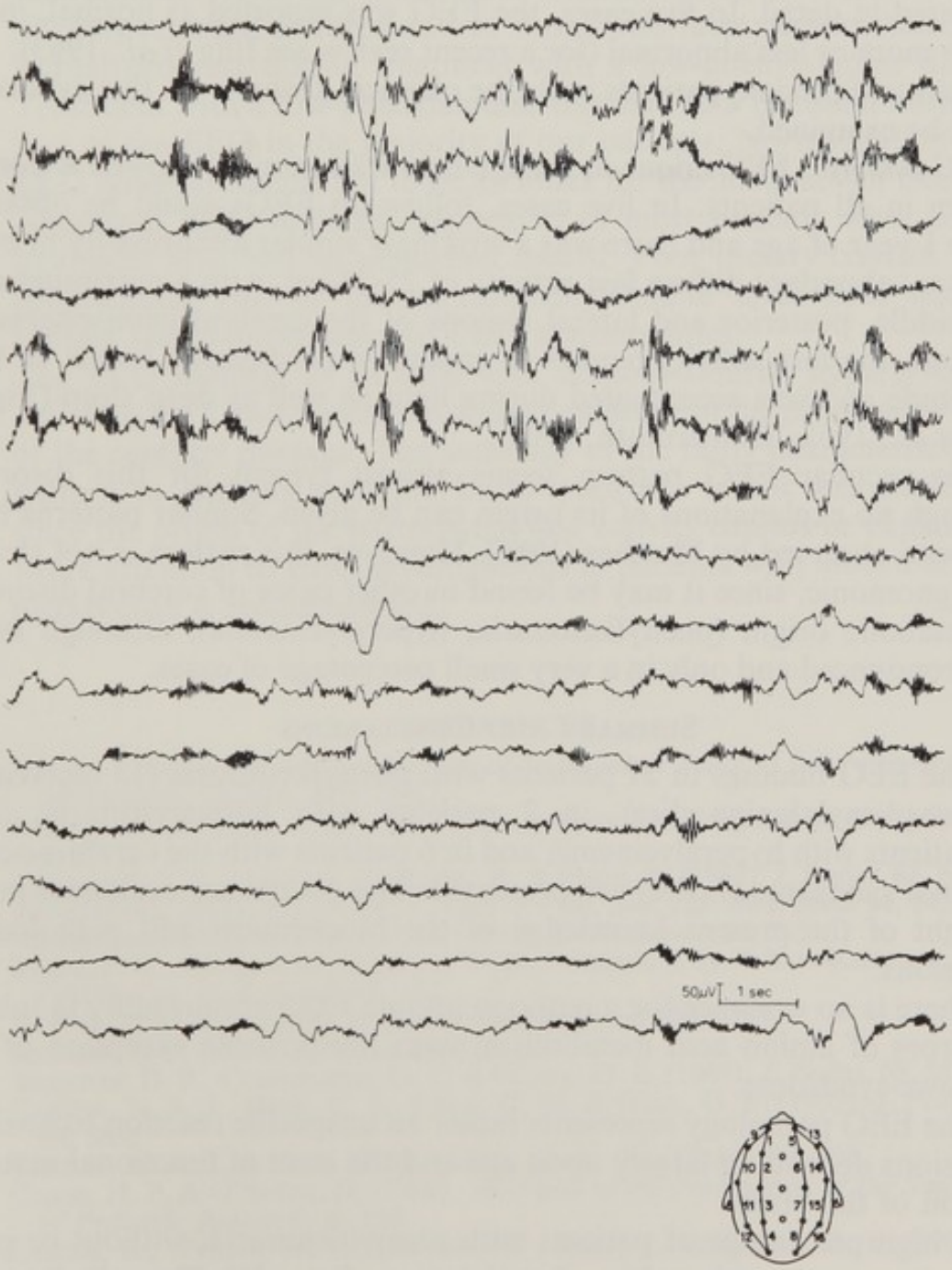

FIG. 6

E.H., 4 years: Cerebro-oculo-renal syndrome (LOWE). EEG: Diffuse beta-activity, appearing in paroxysmal bursts of high amplitude (sleep). EEG Nr. 10515a, 23.4.63, ZK 0·1/F70.

Cerebro-oculo-renal syndrome (Lowe)

The cerebro-oculo-renal syndrome (Lowe) is a well known clinical entity, though nothing is known of its etiology. The disorder has been described only in males and its principal features include: psychomotor retardation, cataracts and/or glaucoma, hyperaminoaciduria and proteinuria, as well as extreme muscular hypotonia, the deep tendon reflexes often being absent. The EEG findings are known from 23 cases, but not always presented in detail. In five cases, the EEG was reported as normal, in the rest as more or less abnormal (for a recent review see Illig *et al.*, 1963).

Patients. Six male patients with the classical features of this syndrome could be examined.

EEG findings. EEGs done at an age below 10 months showed a normal pattern in all patients. In five cases, follow-up EEGs could be obtained above 1 year of age and there was a strikingly similar abnormality in every instance: abundant diffuse fast activity of 25-32/sec. with a maximum over the middle, posterior and lateral regions of the cerebral hemispheres. In addition, the fast activity tended to appear in paroxysmal bursts of high amplitude and was accentuated during light as well as deep sleep (Fig. 6, Illig *et al.*, 1963; Dumermuth, 1965).

This peculiar EEG pattern seems almost typical for this disorder, although no explanations of its origin can be given. Similar patterns have also been observed by Matthes (1966). However, this pattern is not strictly pathognomonic, since it may be found in other cases of cerebral disorders of metabolic origin (phenylketonuria, hyperglycinemia), although much less pronounced and only in a very small percentage of cases.

Summary and Conclusions

1. The EEG findings in 31 patients with phenylketonuria (13 receiving a low-phenylalanine diet), in 2 patients with homocystinuria, in 2 patients with hyperglycinemia and in 6 patients with the cerebro-oculo-renal syndrome (Lowe) are presented. The results are evaluated in the light of the present knowledge of the biochemical and pathological lesions.
2. There is no evidence for a pathognomonic EEG abnormality in inborn errors of amino acid metabolism, with the possible exception of the Lowe-syndrome.
3. The EEG pathology represents rather an unspecific reaction to noxious lesions depending largely upon age and the state of functional maturation of the brain.
4. A high percentage of patients with phenylketonuria, without or prior to treatment with a low-phenylalanine diet, show so-called hypersynchronous abnormalities in the EEG.
5. Whereas hypsarhythmia as well as sharp and slow waves (petit mal variant) occur in infants and young children, generalized spike-wave

paroxysms are the prevalent findings in older children. The typical pattern of rhythmic 3/sec. spikes and waves (classical petit mal) was never observed.

6. The background activity was disturbed in over half of the patients studied.
7. There is no significant correlation between the type of EEG abnormality, the serum-phenylalanine concentration and the degree of mental retardation. However, EEG abnormalities seem less severe in less severely retarded children and in those who later demonstrated intellectual progress during treatment with a low-phenylalanine diet.
8. Treatment with a low-phenylalanine diet lead to a transient normalization of the EEG in the majority of our patients. Normalization of the EEG however, seems not to be an index for the adequacy of treatment. Abnormal EEG findings reappeared in most patients who, prior to treatment also had an abnormal record. Poor dietary control with a concomitant rise in the serum-phenylalanine level does not fully explain the re-appearance of EEG abnormalities.
9. The EEG findings in patients with homocystinuria, hyperglycinemia and with the Lowe-syndrome present evidence of the progressive course of the cerebral damage. The aetiology of the fairly characteristic EEG pattern in patients with the Lowe-syndrome is not well understood since the origin of the paroxysmal fast activity cannot be explained.
10. More attention to EEG investigations in patients with inborn errors of metabolism is recommended.

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SOME INBORN METABOLIC DISORDERS AFFECTING CEREBRAL ELECTROGENESIS

G. PAMPIGLIONE

OUR current concepts of the central nervous system have evolved slowly over the last few centuries. It was postulated in the Middle Ages that different parts of the brain, or its ventricles, had different functions, but only over the last hundred years or so has there been a rapid accumulation of data, not only of the anatomy and histology of the brain, but also of its many functional aspects, including all those biophysical phenomena that may be recorded with appropriate electronic equipment.

The recent anatomical and biochemical advances in the study of the developmental aspects of the central nervous system have stimulated a revision of our views on cerebral function and higher nervous activities.If we consider the brain as a complex organ with a variety of systems, it seems probable that each one of these systems may mature independently of the others at either foetal or post-natal ages. During normal development, both in man and in animals, the evolution of each system probably runs parallel with the normal evolution of particular enzymatic constellations. When we study pathological processes, however, a number of problems arise which do not appear to be dealt with by the ever increasing literature on the subject.

For example, there may be discrepancies in the presentation of inborn errors of metabolism in theory and in practice. Theoretically, a given inborn error of metabolism should give rise to a chain of events underlying the presentation of particular groups of clinical phenomena, and such syndromes should appear at one particular age rather than at another. In practice this is only partly true as there are great individual differences in the presenting symptomatology of patients with apparently identical inborn errors of metabolism.

Often the patient is taken to hospital because of a number of apparently non-specific complaints and a considerable delay might occur before the correct problem is put to the biochemist. In paediatric centres with active biochemical and histological departments the chances of a correct classification of the metabolic disorder are usually much greater than in other centres where the study has to be limited to clinical observation alone. However, our knowledge of the reasons why particular clinical symptoms or physical signs appear in a given patient and not in another is only speculative, even when we have obtained all the chemical and histological evidence. Moreover, both histological and chemical investigations on brain tissue are possible only when the portion of tissue to be studied is separated from its biological site of function. Such studies, therefore, imply an

interruption of the investigations on the intact brain and give values which are related to a frozen moment in a continuously variable metabolic condition.

Since the early days of experimental neurophysiology and clinical electroencephalography it has been recognised that the morphology of the electrical activity of the brain is largely dependent on the metabolic state of the nervous tissue. In contrast with anatomical and direct chemical investigations, the study of the electrical activity of the brain may be considered as a kind of running commentary about the continuously varying metabolic state of neuronal aggregates. With appropriate techniques, various aspects of normal and abnormal cerebral function can be studied throughout the course of a variety of clinical syndromes by recording through the scalp the continuously variable electrical activity from the surface of the brain, without interfering with the brain tissue itself.

We still know very little about the mechanisms that underlie cerebral electrogenesis either in health or in disease. We know even less about the reasons why, during development in man and animals, a particular series of normal changes occurs in the electrical patterns of activity of the maturing brain. When we study the EEG of young children we are faced with the evaluation of the intrinsic, normal, developmental changes in cerebral function and also with the recognition of those deviations from the normal which might be due to intercurrent, or pre-existent, metabolic disorders.

From a careful study of the course of a metabolic disorder affecting the brain we may be able to learn the limits of cerebral tolerance or homeostasis, and which are the important factors in the evolution of cerebral electrogenesis.

In paediatric neurophysiology, and particularly in electroencephalography, there is a great need for standardisation of basic techniques. In addition to those technical aspects dealing with frequency characteristics of the apparatus, amplification factors and type of display, it is essential to standardise the criteria underlying electrode placement and recording montages if we wish to facilitate a comparison of data between observers, as well as for repeated investigations of the same patient over periods of months or years.

Since the opening of the neurophysiological department at Great Ormond Street at the end of 1956, a uniform system of electrode placement has been utilised based on measurements from bony landmarks. This method (Pampiglione, 1956) is now in use in several other laboratories in England and its anatomical validity in various age groups has been verified. Knowledge of the site of placement of electrodes in EEG bears the same importance to the interpretation of the results as the knowledge of the precise site of sampling in histology. This basic topographic precision becomes even more relevant when, during development, changes occur in the distribution of both normal and abnormal electrical phenomena

recordable from various regions of the brain over periods of only a few months.

In our approach to metabolic alterations that may affect the electrical aspects of brain function two points should be kept in mind. Firstly, the help that neurophysiological investigations might give in as yet undiagnosed metabolic disorders, and secondly, the experimental opportunities offered by some neurometabolic disorders to study both normal and abnormal maturation of cerebral function.

Infantile Spasms

At any given age there are electroclinical syndromes which, with individual variability, correlate with particular types of metabolic disorders. For example, babies between 6 and 12 months presenting with a syndrome of mental and motor retardation and short lived massive spasms of the limbs, neck and trunk (West's Syndrome, or infantile spasms), usually show gross EEG abnormalities. The traces from the various regions of the brain are of large amplitude, with nearly continuous irregular slow activity, multi-focal spikes or sharp waves and an obvious poverty of rhythmic activity. The overall amplitude of the traces might be of the order of 500 to 1,000 microvolts. This grossly abnormal EEG pattern was recognised long ago and given the now widely used name of 'hypsarhythmia', by Gibbs & Gibbs (1952). Such features are substantially different from the normal rhythmic activity of healthy children of the same age group, where the amplitude of the traces is of the order of 50 to 200 microvolts, and all activities have a well defined topographic distribution (Gibbs & Gibbs, 1950; Dreyfus-Brisac *et al.*, 1961; Pampiglione, 1965; Dumermuth, 1965).

Over the last nine years some 350 children with these electroclinical features have been seen, and with the help of Drs. Crichton, Gregoriades, Harris, Krauthammer and Papatheophilou some of the suspected aetiological factors have been analysed. In the literature a common grouping of children with infantile spasms is between 'secondary' and 'idiopathic,' forms, based on a history of pre-existing neurological or paranatal illness or otherwise (Jeavons & Bower, 1964; Gastaut *et al.*, 1964). It is difficult, however, to accept these classifications in view of the subsequent evolution of the disorder in many children.

Taking all our 350 children, no reasonable aetiopathogenetic classification could be made in nearly three quarters of them. A small group, of the order of 1 in 20, showed alterations of tryptophane metabolism (Bower *et al.* 1965). Another group of similar size consisted of phenylkentonuric children. The size of these groups is given tentatively as over the last nine years the material kept changing as soon as the phenylketonuric children were on dietary treatment. A third and more substantial group, of the order of nearly 1 in 10, was eventually classified amongst the tuberous sclerosis

complex. A fourth and much smaller group, of the order of 1 in 50, consisted of patients with Down's Syndrome (Gregoriades & Pampiglione, 1966). Amongst all the 350 children there was no proven case of any other type of recognisable metabolic disorder, such as, metachromatic leucodystrophy, Tay-Sachs, glycogen storage disease, Gaucher or Niemann-Pick disease, nor did any have a history of poisoning.

In the four groups of recognisable and apparently dissimilar pathological processes mentioned above, some of the symptomatology, both from the clinical and EEG aspect, was similar during a particular phase of the evolution of the respective disease process in the second semester of extrauterine life. On follow up of these patients, it was found that both the clinical and EEG features evolved differently in each of the four groups. In fact, after five years most of the mongol children with infantile spasms had died, whereas a much smaller proportion of the children with the tuberous sclerosis complex and with an abnormal tryptophan metabolism had died. The mortality amongst the phenylkentonuric children was negligible whether or not adequate treatment had been given. The evolution of the EEG features in the 18 phenylketonuric children with infantile spasms was rather similar to that of the other 52 phenylketonuric children in our series who never developed spasms. The 26 surviving children with infantile spasms in whom the tuberous sclerosis complex had appeared, showed the greatest fluctuations in the EEG abnormalities as they grew older, with a fairly good preservation of the responses to photic stimulation.

Does some common mechanism operate in a similar way upon the central nervous system of patients affected by these four apparently dissimilar conditions? Why should this occur over a particular, limited, period of time? Should we consider instead the possibility that these four conditions, as well as the larger undiagnosed group, have in fact some common metabolic inborn aetiological factor? In our present state of ignorance it would not be particularly informative to divide the infantile spasms syndromes into 'idiopathic' and 'secondary' forms.

Phenylketonuria

The evolution of the EEG features in the 70 phenylketonuric children was complex. For a variety of reasons, it was not possible to carry out systematic EEG studies at six monthly or yearly intervals on each child and, particularly before 1960, several of the referred children were already a few years old. It was possible, however, to collect a total of 214 EEGs from the 70 children at various ages. Some of our findings parallel the published data of Fois, Rosemberg & Gibbs (1955), Low, Bosma & Armstrong (1957), Stadler (1961), Herrlin (1962) and Blehova (1963), but in these papers the emphasis is not on individual follow up studies.

In our material the severity of the clinical symptomatology, the blood level of phenylalanine, and the developmental quotient were not closely

related with the type and severity of the EEG abnormalities. Individual variabilities were great, even in children on diets and with either persistently low or with variable phenylalanine levels in their blood. However, when the 70 children were considered as a group, a general trend emerged in the evolution of the EEG patterns. In the first few weeks of life the phenylketonuric babies showed relatively minor EEG abnormalities. Those who were treated early and maintained on diet showed less severe EEG abnormalities in the following months and years in comparison with the phenlketonuric babies in whom the diagnosis was made late, and had been left untreated for several months. Those children who were first referred between the age of 6 months and a year, usually showed a severe generalised EEG abnormality with multi-focal discharges, large amplitude irregular slow activity, and poverty of the normal rhythmic components (Fig. 1). Between 6 months and a year of age the responses to photic stimulation were only just recognisable and there was no increase in discharges that might be attributed to photic stimulation. A comparison of these EEG features with those of untreated older babies, would suggest that the metabolic alteration which affected cerebral electrogenesis was not a static one.

As the patients grew older, whether untreated or irregularly treated, the abnormal EEG patterns showed some degree of improvement with a diminution in the disorganisation of the traces, particularly after the age of 2 years. Between 2 and 4 years of age, the EEG abnormality was greater during sleep than during the waking state; the responses to photic stimulation were clearly recognisable, and only exceptionally was there an increase in discharges evoked by photic stimulation. However, there was usually an increase in asymmetry between the activities of the two hemispheres in terms of lateralisation of the excess of irregular slow components and of the occasional discharges. Some rhythmic activity at about 6-8 c/s could be recognised over the middle and posterior third of the head, but usually an excess of 4-5 c/s activity was seen over the anterior third of the two hemispheres.

In older children between 4 and 12 years of age, some unstable alpha rhythm was often recognisable, but irregular slow waves and sharp components, or spikes, tended to occur at irregular intervals mostly over the posterior temporal regions, often lateralised. In the anterior frontal regions near the midline, runs of rhythmic 6 c/s waves reaching 100 microvolts were often seen lasting 1-2 seconds, even when the patient's eyes were open. Photic stimulation in this group of children evoked large amplitude irregular sharp waves, spikes and slow components, particularly after eye closure (12-18 flashes per second), often without any recognisable concomitant clinical change. Abnormal responses to photic stimulation were also seen in the cases of similar age on dietary treatment. When, in addition to the conventional type of recording (with a time constant of either 0·3 or 1 second), steady potential changes were also studied, small but definite DC

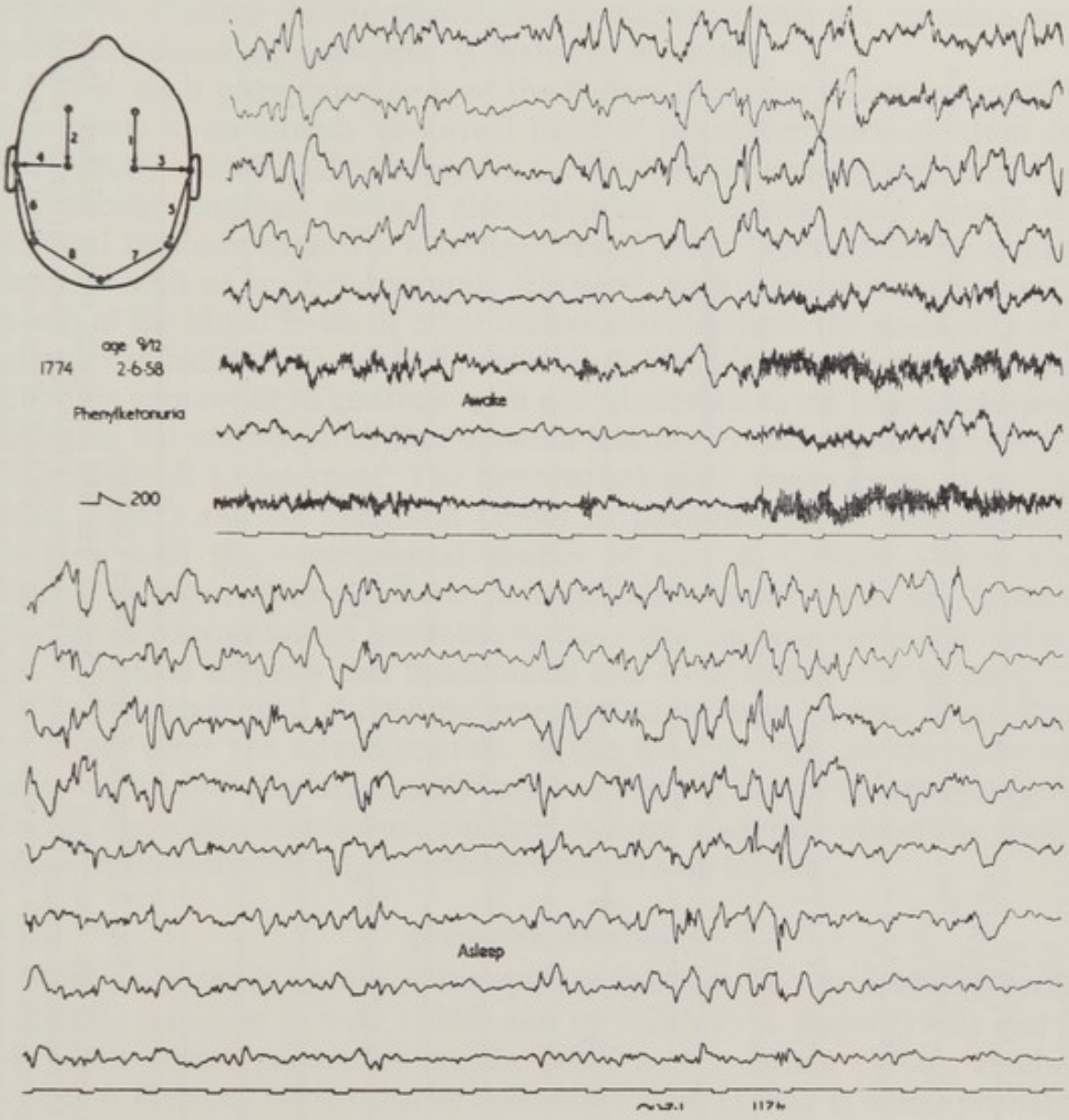

FIG. 1

Phenylketonuria (Baby)

This baby had been suffering from the syndrome of infantile spasms, mental retardation and some regression of motor skills since the age of 6 months. The diagnosis of Phenylketonuria was made at the age of 9 months. He made some slight progress on diet, but the developmental quotient remained in the 50's. These two portions of the EEG were taken when the child was first admitted at the age of 9 months. The large amplitude, irregular slow activity, and multi focal discharges persisted during the waking state and during sleep. Some of the discharges reached an amplitude of nearly 1 millivolt.

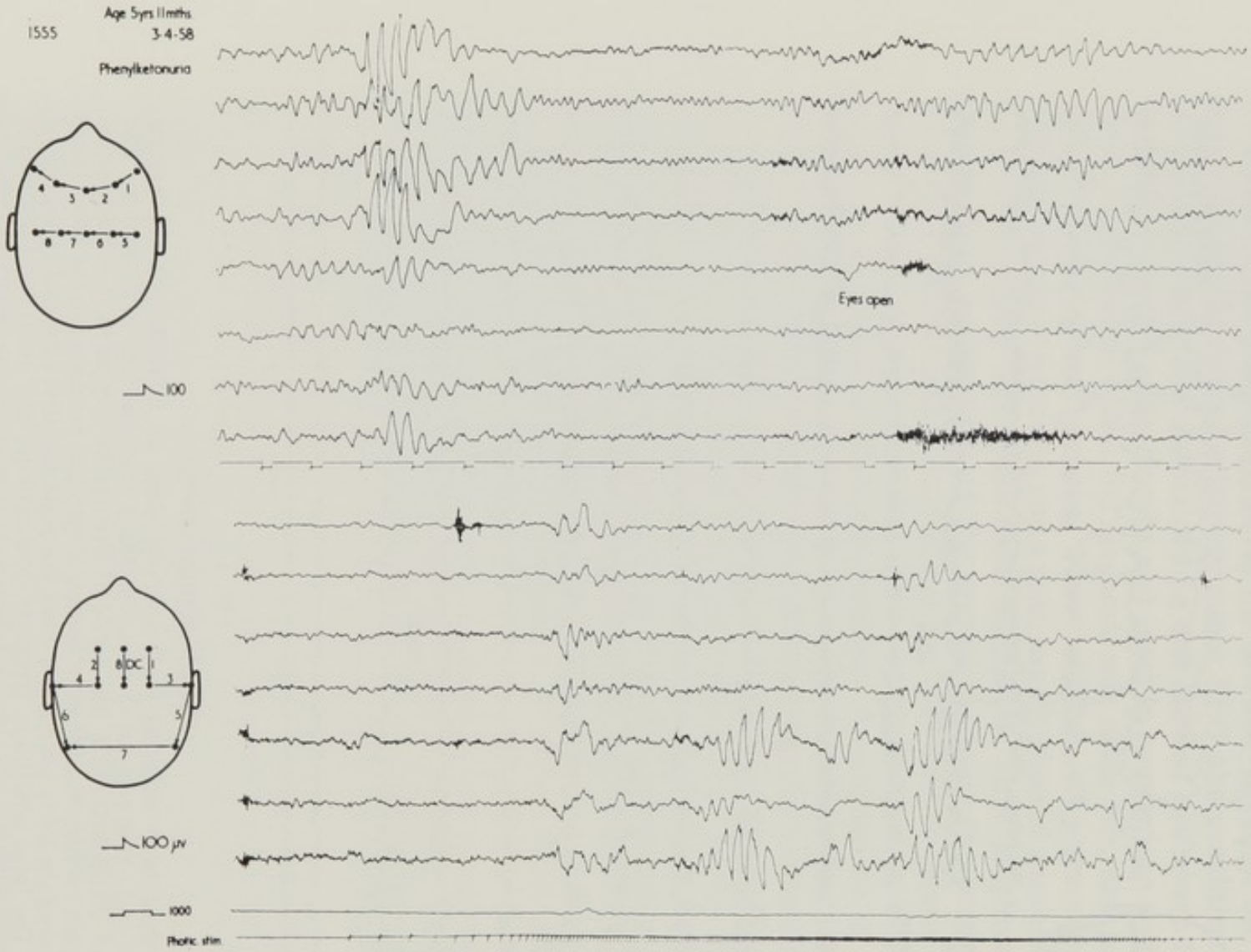

FIG. 2—Phenylketonuria (Older Child)

This was an untreated boy nearly 6 years of age discovered in E.S.N. School. He had infrequent minor seizures. There was only a slight improvement on diet and the I.Q. remained around 40-50. These two portions of EEG were taken during the waking state. In the first portion (above) some runs of large amplitude slow waves were seen over the anterior half of the head mixed with few sharp elements. In addition, some runs of 6 per second waves were seen about the mid frontal electrode in between the bursts of slower waves (whether or not the boy's eyes were closed). In the second portion (below) irregular slow components and small sharp elements were elicited over the posterior half of the head by photic stimulation.

shifts could be recognised at the time of the appearance of the large discharges elicited by photic stimulation (Fig. 2).

The relationships between the biochemical and EEG findings in phenylketonuric children during phenylalanine tolerance tests are puzzling, and have been described elsewhere. (Clayton *et al.*, 1966).

Following a phenylalanine load the factors that may determine marked alterations in an already abnormal EEG of phenylketonuric children are not directly related to the changes in the levels of serum phenylalanine. Our unexpected findings during phenylalanine tolerance tests should be checked by more extensive investigations in other laboratories on treated cases as well as on heterozygous 'carriers', with simultaneous measurements of the blood levels of other amino acids to check the possibility of a complex imbalance. The experimental work of Cadell, Harlow & Waisman (1962) is somewhat in contrast with our observations on phenylketonuric children, but comparative data between laboratory and clinical studies are often difficult to interpret. The histopathological observations of Crome *et al.* (1962) and of Malamud (1966) in phenylketonuric patients is in keeping with the experimental studies of Liss & Grumer (1966) suggesting that permanent cellular damage might occur when the concentration of phenylalanine available to the tissue exceeds particular values. Dr. Jacobson suggests that biochemical and EEG studies during phenylalanine tolerance tests should be extended to at least 12, and if possible, 24 hours after the administration of the load (p. 10). At the moment, although we can offer apparently logical criteria in favour of continuing the diet in some phenylketonuric children, we are uncertain about the choice of measurements that might indicate when to stop the diet.

Sphingolipidoses

Reviews of the literature of the sphingolipidoses may be found in books edited by Aronson & Volk (1962) and by Folch-Pi & Bauer (1963) and in the more recent papers by Austin (1965 a, b,), Brady (1966), Bischel, Austin & Kemeny (1966) and by Sourander *et al.* (1966). It seems probable that within each group of the sphingolipidoses, several sub-groups will eventually be recognised. The variety of age groups involved, of progression of the clinical symptomatology, and of individual life expectancy within each recognised group should encourage a revision of our ideas about the metachromatic leucodystrophies, Krabbe's and Tay-Sachs' diseases, the forms of amaurotic family idiocy under various eponyms, as well as the various forms of Gaucher's and Niemann-Pick's diseases with CNS involvement. Some experimental studies are now beginning to be reported on various pathogenetic mechanisms (Sourander *et al.*, 1966; Brady 1966; Bischel *et al.*, 1966), although the EEG studies on children with various forms of spingolipidoses are limited to small groups (Cobb *et al.*, 1952; Radermecker, 1952; Rosembaum & Stein, 1953; Rohmer *et al.*, 1954;

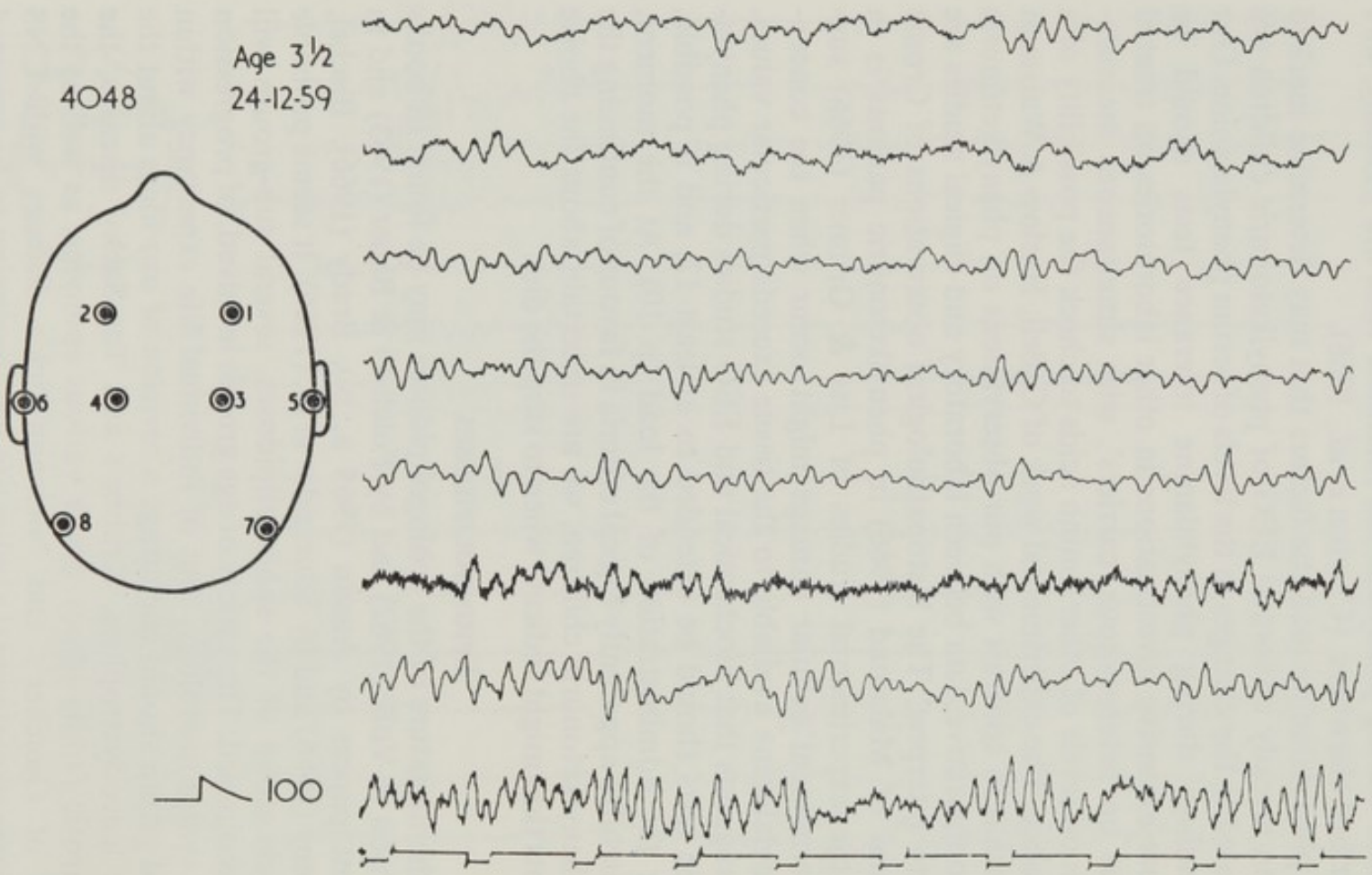

FIG. 3

This boy, age 3½ years, was considered to be retarded from the age of 10 months. He acquired speech, but he lost it at the age of about 2½ years. He became clumsy and his ability to walk diminished from the age of 2 years. No seizures had occurred. This EEG was taken with a common average reference. The rhythmic activity is mostly at 5-7 c/s and is of higher amplitude over the posterior than over the anterior portion of the two hemispheres. Some slower components are also present, but no spikes or complex wave forms appeared at any time.

Crawley, 1957; Morell & Torres, 1960; Carels, 1960; Herrlin & Hillborg, 1962; Hagberg *et al.*, 1962, 1963; Isler *et al.*, 1963). The EEG studies, however, are of importance in the differential diagnosis of progressive neurological disorders in infancy and childhood.

Metachromatic leucodystrophy

A diagnosis of metachromatic leucodystrophy (MLD) was proven in 20 children. Unexpectedly the EEG features of these children showed a considerable degree of similarity, particularly in the early phases of the disease. Regardless of the age at which the condition began the EEG showed rhythmic activity with topographic distribution similar to that of normal children which was fairly well preserved for several months, in contrast with the obvious simultaneous clinical deterioration. Usually, however, after a few months the frequency of the rhythmic components became slower than that seen in a normal child of similar age. For example, with our electrode placement the alpha rhythm is normally well established by the age of $2\frac{1}{2}$ years, when its frequency over the posterior third of the head is of the order of 8-9 c/s. In children with MLD, already suffering from gross motor disorder at that age the activity over the posterior half of both hemispheres, although rhythmic, was at only 5-7 c/s (Fig. 3). This activity disappeared on eye opening. As the MLD children grew older, in addition to an increase in the rhythmic, rather slow activity, there was also an increase in irregular slower components over both hemispheres, and the normal differences between the anterior and the posterior portions of the two hemispheres disappeared. An excess of rhythmic faster activities at 12-20 c/s usually became prominent over both the anterior and posterior portions of the hemispheres, superimposed upon the slower components. In general, spikes or complex wave forms were only exceptionally seen in the early and middle phases of MLD. It is difficult to assess whether the occasional late appearance of paroxysmal features might have been related to a number of complicating factors such as the child's deteriorating respiratory and nutritional conditions. However, occasional spikes and sharp waves were present in one child in whom the histological and chemical studies were said to show co-existent MLD and gangliosidosis (Lake 1966 personal communication).

In addition to EEG studies, polygraphic records were made, whenever possible, of such phenomena as the peculiar tremors of the initial phases, the pendular movements of the eyeballs (Fig. 4), and at later stages, the complex alterations of muscle activity in agonist-antagonist groups during voluntary action and in response to a variety of stimuli.

Krabbe's disease

There is currently a renewed interest in Krabbe's disease as a separate entity from MLD, and it is probable that EEG investigations would be of

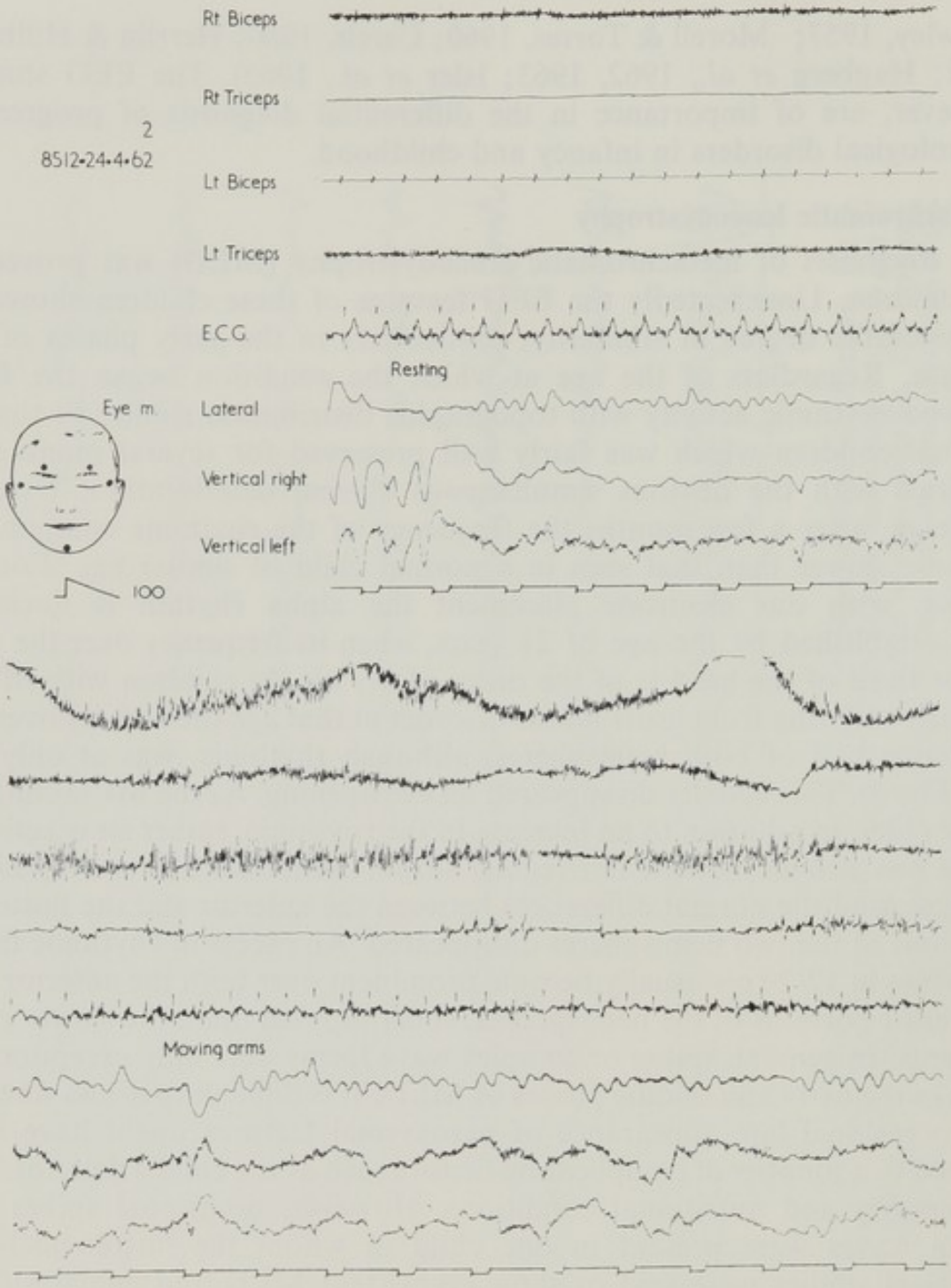

FIG. 4

M.L.D.: This child's symptomatology started at the age of 1½ years when she appeared to be tremulous in her movements and became increasingly clumsy. This polygraphic record was taken at the age of 2 years. In the first section (above) the patient was at rest and able to relax fully both the right triceps and the left biceps although their antagonists could not be fully relaxed. The record of the eye movements shows a somewhat irregular pendular nystagmus at about 4-5 per second, mostly horizontal. In the second section (below) during voluntary movements the discontinuous activity of agonist and antagonist groups of muscles is recognizable at the rate of about 4 per second, particularly in the left biceps and left triceps. There is also a fairly marked horizontal rhythmic oscillation of the eyeballs at about 4-5 per second, persisting throughout the period of voluntary activity of the arms. There is a number of slower more irregular vertical movements of both eyes together.

value in the differential diagnosis even at an early phase. However, my material on verified Krabbe's disease with EEG studies amounts to only three cases. Irregular slow components, sharp waves and occasional spikes were seen in these three young patients, in contrast with the absence of discharge in the children of similar age groups with MLD.

Is this because Krabbe's disease begins much earlier? Or is it because the processes affect different neuronal mechanisms? These limited EEG observations could lend support to the experimental work of Sourander *et al.* (1966), who added cerebrosides and sulfatides to cell cultures from the rat retina and cerebellum: the mesenchymal cells were transformed into cells with morphological and histochemical properties similar to those of the globoid cells of Krabbe's disease and of the metachromatic granular cells of older cases of metachromatic leucodystrophy respectively.

Tay-Sachs' disease

In Tay-Sachs' disease and allied forms of neuronal lipidosis a peculiar ganglioside accumulates in many of the neurones. The EEG features in these patients are substantially different from those seen in MLD at a moderately advanced stage of either condition. However, following earlier papers (Cobb *et al.*, 1952; Pampiglione, 1961), I have examined, two apparently clinically normal babies who later developed proven Tay-Sachs' disease. The gross EEG abnormality which is usually seen in these patients during the second year of life was not present in the first few months of extra-uterine life. In a brief communication, Rohmer and his colleagues (1954) had mentioned an apparently normal EEG in a baby of 5 months with probable Tay-Sachs' disease.

These findings might be parallel to the observations that some phenyl ketonuric babies in the first few weeks of extra-uterine life do not show the gross EEG abnormality that appears later if they are not treated (Pampiglione & Papatheophilou, 1961). It is tempting to speculate that in children with the infantile forms of neuronal lipidosis there might be a gradual build-up of abnormal metabolites which eventually alters the electrogenetic mechanisms in a manner comparable to the early evolution of untreated phenylketonuric children. Is the occurrence of abnormal clinical and EEG features in patients with Tay-Sachs' disease directly related to an accumulation of unwanted metabolites? Or is it due to a gradual diminution of other utilisable substances to the neuronal aggregates? Some interesting speculations as to the possible treatment of patients with sphingolipidoses have been put forward recently by Brady (1966), but at present we have not even begun to try out a dietary treatment at a very early age on siblings of patients with known Tay-Sachs' disease before the appearance of either clinical or EEG abnormalities.

Other neuronal lipidoses

The EEG features in children who eventually developed a verified neuronal lipidosis, showed a peculiar evolution. Those children who could be examined in the first few months of life before the development of any clinical symptoms or signs showed very little, if any, abnormality in their resting record, whether during the waking state or asleep. In particular, their responses to photic stimulation were normal. However, a few weeks or months after the appearance of motor, or sensory symptoms, or seizures, the EEG became rapidly abnormal, usually more so than would have been expected from the slow clinical deterioration. The excess of slow activity and the occasional mono or polyphasic spikes were usually more prominent over the posterior than over the anterior half of the head, particularly in the forms other than Tay-Sachs' (amaurotic family idiocies of the Batten, or Spielmeyer-Vogt, or Bielschowsky groups).

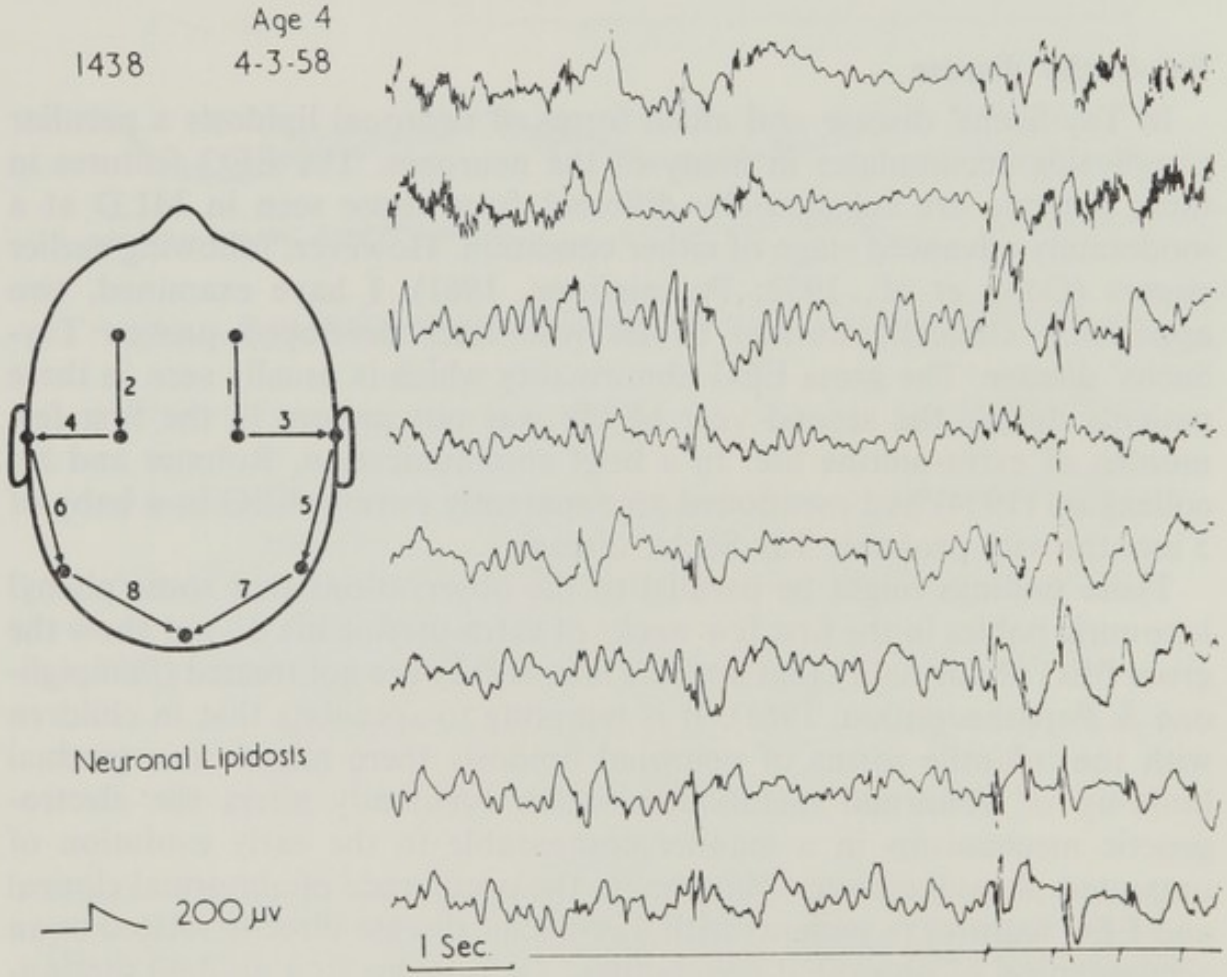

FIG. 5
Neuronal Lipidosis

This girl, aged 4, developed normally until the age of 3½ years when she became clumsy in her movements, occasionally falling about, and began to have infrequent seizures. This EEG, taken only a few months after the beginning of the clinical symptomatology, shows an excess of irregular slow components, no alpha rhythm and large amplitude, irregular spikes, occasionally polyphasic. Photic stimulation at low rates of flickering evoked large discharges even after the first flash.

Although there were considerable individual variations, the rhythmic activity appropriate to the child's age was disrupted by irregular slower components and occasionally by runs of faster elements. Over a period of a few months the overall amplitude of the traces increased to two to three times the normal, and photic stimulation, particularly at low rates of flickering, evoked large single or polyphasic spikes (Fig. 5) as described by Carels (1960). Often at this stage the clinical diagnosis of neuronal lipidosis was not yet made, and the peculiar EEG abnormality was of help in the differential diagnostic problem, being the first pointer to a severe disturbance of cerebral electrogenesis.

In those patients who survived a few years the amplitude of the discharges diminished gradually (Fig. 6).

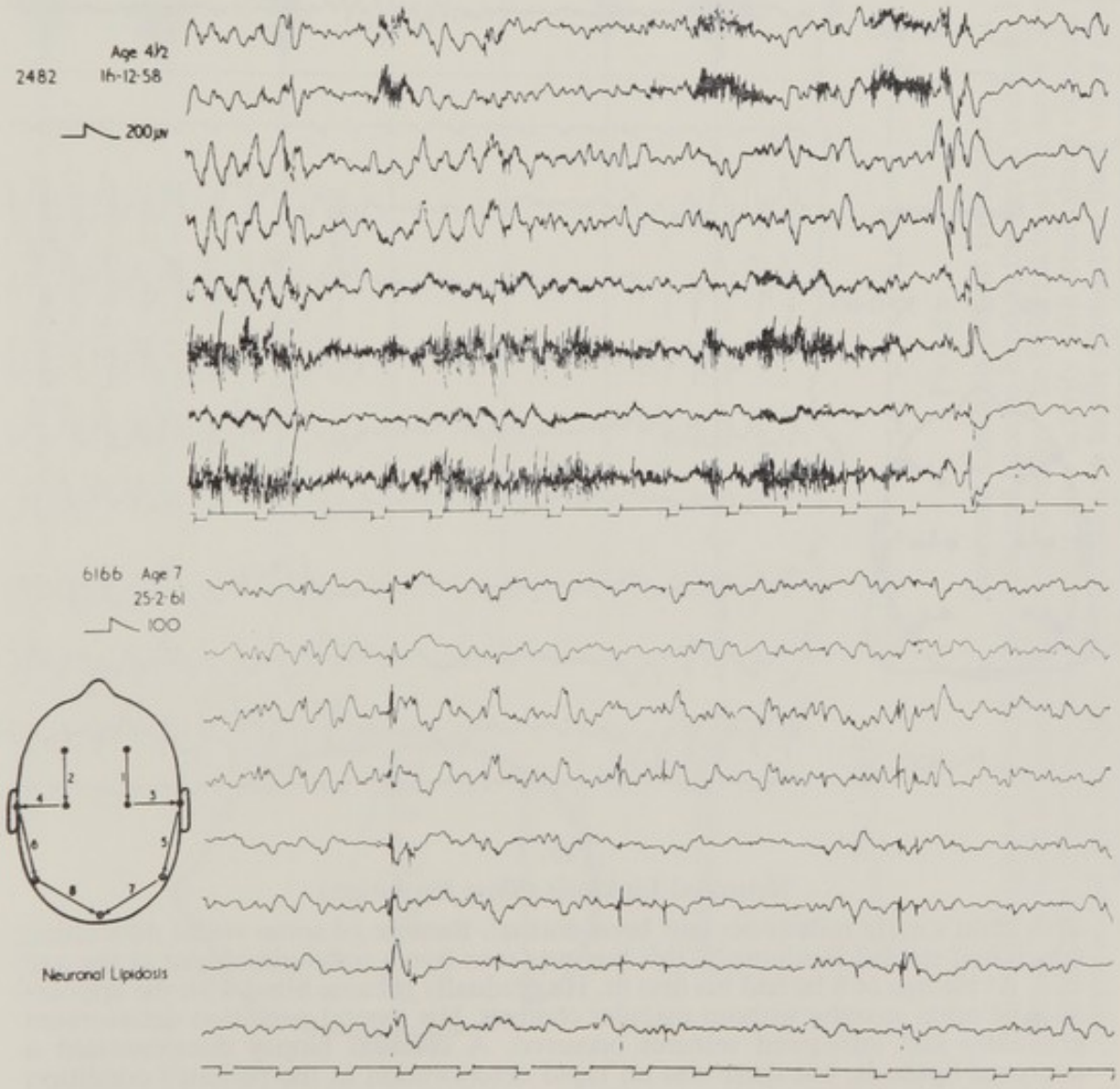

FIG. 6

The same child in Figure 5 seen at the age of 4½ years and 7 years. The diminution in the amplitude of the slow components and of the discharges should be compared in relation to the size of the calibration signal on the left.

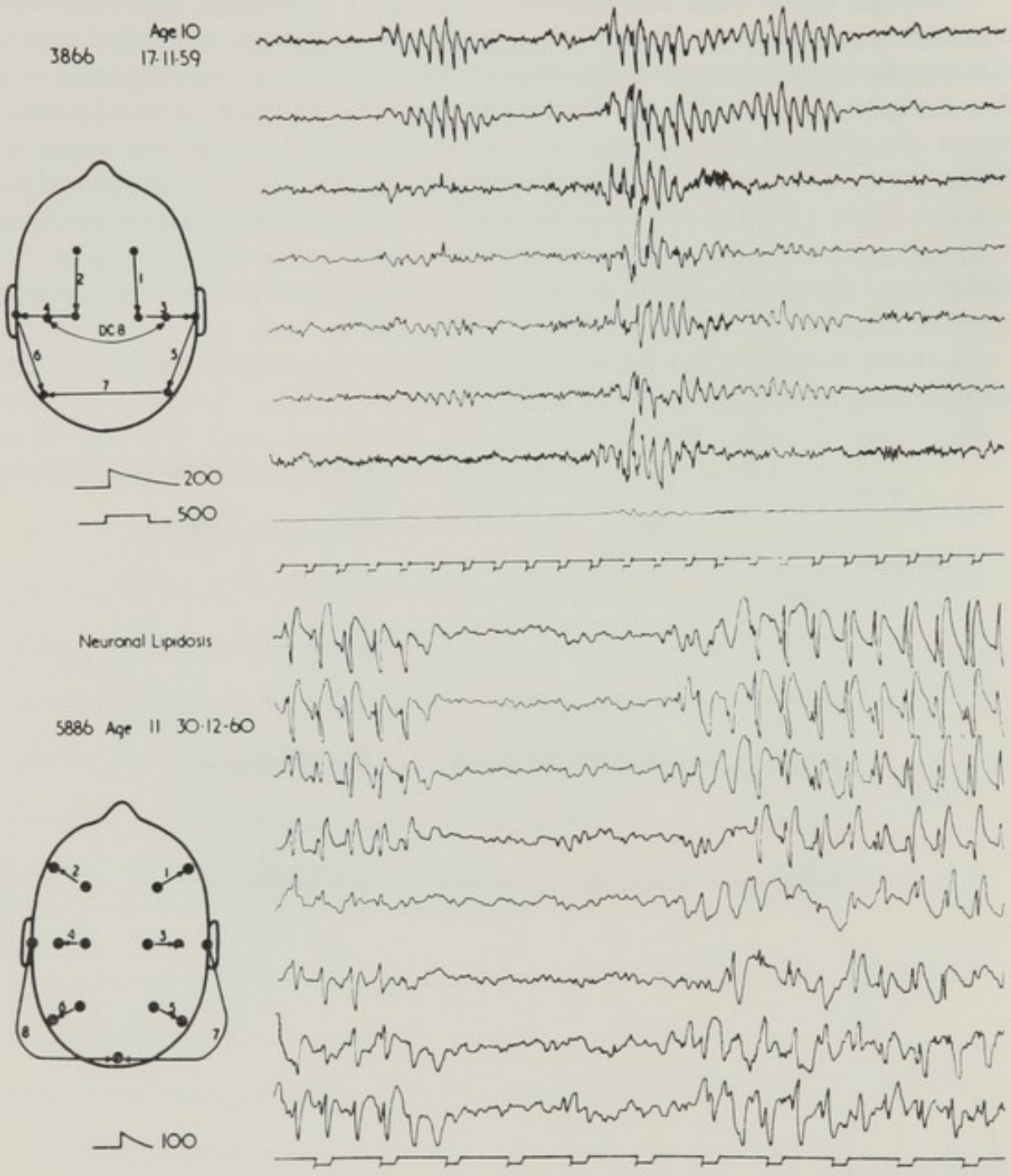

FIG. 7

Neuronal Lipidosis (Slow Evolution)

This child's early milestones had been normal. Because of some visual difficulties, which had started insiduously, this boy was seen by an ophthalmologist at the age of 7. At the age of 8 he had his first fit. He gradually became blind with the appearance of optic atrophy without macular changes. His mental condition deteriorated gradually and infrequent seizures occurred. A cerebral biopsy demonstrated a neuronal lipidosis, but there was no rapid deterioration in the patient's condition in the following 4 years. These two EEGs were taken at the ages of 10 and 11 years. The bursts of fairly rhythmic complex discharges are recognizable in the two EEGs taken at different paper speeds. There was no marked D.C. shift (see portion above) at the time of these bursts. The child was still alive at the age of 14 when he was transferred to an adult hospital.

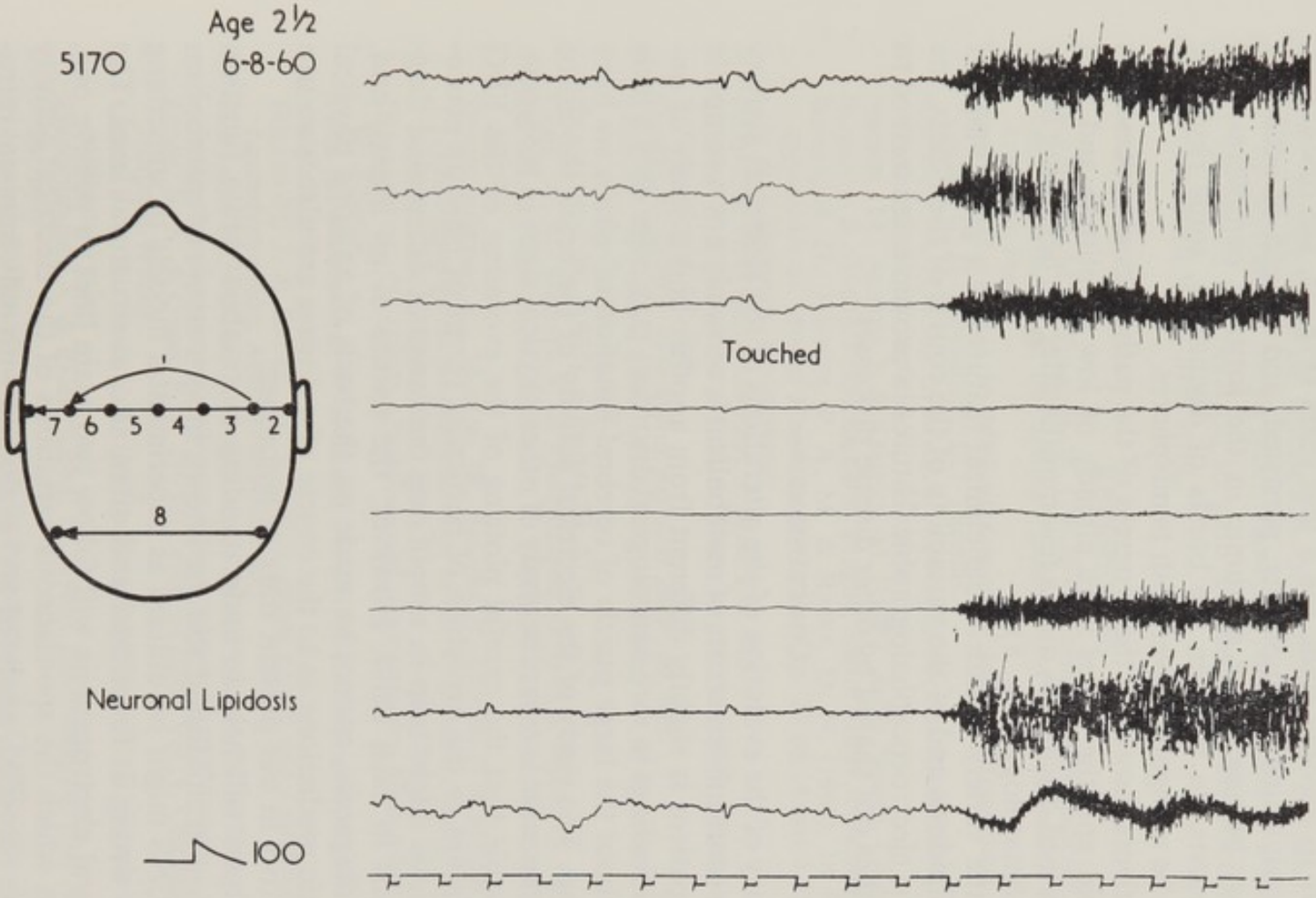

FIG. 8

Neuronal Lipidosis (Rapid Evolution)

This child regressed rapidly in his skills and became blind at the age of one year. He had only an occasional seizure. He had a tetraparesis with optic atrophy. The EEG taken at the age of 2½ years shows a total absence of rhythmic activity and some irregular slow activity with small sharp elements persisting in the posterior temporal and sylvian region. In response to a tactile stimulation there was a prolonged generalised spasm. (See appearance of muscle action potentials superimposed upon the EEG traces.)

In the terminal stages of the fatal cases the discharges decreased in spite of the increase in myoclonic jerks. The irregular low amplitude slow activity was not affected by a variety of sensory stimuli which, however, still elicited myoclonic phenomena. In one patient in whom apparently the disease seemed to become static for a few years (Fig. 7), the discharges, rather than diminishing in amplitude, persisted, and instead of occurring irregularly they became more rhythmic in the form of complexes, and occurred in bursts. In between the bursts of discharges, some rhythmic activity could be recognised over both hemispheres.

In other patients, however, the progress of the pathological process was extremely rapid and the traces were already of low amplitude and with very poorly formed discharges only a few months after the beginning of the clinical symptomatology (Fig. 8).

In spite of the variety of individual clinical features at a given stage of the neuronal lipidosis and of the variability of the course of the illness, the evolution of the electroencephalographic features in proven cases seemed to show peculiarities not shared by other disease processes.

Conclusions

This summary of the evolution of the alterations of the electrical activity of the brain in some inborn errors of metabolism is certainly not a complete review. Each patient is slightly different from another and a study of uncommon EEG findings in rare neurological disorders may offer only a little information about the large puzzle of cerebral function of which we have no definite map. The study of the electrical activity of the brain should be considered at the same level as the study of other physical signs in medicine. It seems probable that the careful plotting of the evolution of the EEG features in relation to the evolution of particular forms of obscure neurological conditions might help in simplifying the range of differential diagnostic problems in each child. However, the nature of an illness, and, therefore, its diagnosis, cannot be made on the basis of any one physical sign. Prognostic predictions as to the course of a disease are also a source of frequent errors in our present state of ignorance.

New techniques will help in understanding more about brain function and its evolution in childhood and the known inborn errors of metabolism affecting the CNS might be utilised as experimental models of something that has gone wrong at functional and often also at structural level. The study of cerebral electrogenesis will follow two main lines: a passive one, so to speak, in which the spontaneous variations of the electrical activity of the brain are recorded, analysed and evaluated with only minimal intrusion into the patient's spontaneous state. Another approach is that of inducing by either sensory or biochemical means (including dietetic and pharmacological ones) particular changes in the evoked activity at cerebral level. Once the range of normal variations and their predictability in each

age group is known, we shall be able to recognise alterations in latency, quantity, and quality of the induced changes that might be determined by the course of neuro-metabolic disorders. Through specific neurophysiological measurements in well documented cases we might be able to provide an early recognition of alteration of function at cellular, fibre, white matter, or peripheral nerve level. However, we still have a long way to go before we are in the position to gain a little more knowledge about what makes the brain work and why.

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DISCUSSION OF PRESENTATIONS BY PROFESSOR BICKEL, DR. POLEY AND DR. PAMPIGLIONE

Hudson (Liverpool). I am sure it is right to go on taking recordings of these patients and it is important that we do it regularly.

I wish to describe my observations on four of my patients with phenylketonuria who have been followed after returning to normal diets. Out of 12 infants who started treatment before 5 weeks of age, three have returned to normal diets because it was impossible to continue the dietary treatment.

Two of these children started treatment at the ages of 10 days and 24 days, both of them had satisfactory biochemical control for at least $4\frac{1}{2}$ years, but then they broke the diet so consistently that it became quite impossible to keep them on the treatment, and reluctantly, I agreed to stop it. Both of them had I.Q's. measured over the years which fluctuated between 75 and 85, and these levels corresponded with their cultural backgrounds. Their E.E.G. readings were recorded annually and all were normal or occasionally border-line normal, and none showed any significant abnormality (Dr. John Roberts). One of the children has now been on a normal diet for 2 years, and the other one for $4\frac{1}{2}$ years. Both have maintained their I.Q. levels, both are learning something in school, and neither has shown a deterioration in their E.E.G.

The third child, who is a brother of one of these first two children, started treatment at the age of 10 days. His treatment was very satisfactory for the first 14 months. Unfortunately, we did not get an E.E.G. until he was 14 months old. At that time he appeared to me to be developing absolutely normally, but the E.E.G. report was grossly abnormal and hypsarrhythmic. The child continued on his low phenylaline diet and

3 months later the E.E.G. was reported to have improved. When he was three years and four months old his mother said it was no good treating him any more as he was doing perfectly well and even better than his healthier brother so she stopped his diet. After he had been on a normal diet for 12 months he looked to be a normal child, his I.Q. was unchanged and his E.E.G. had become completely normal. I find it difficult to explain these facts.

The fourth child started treatment at the age of 14 months, she was moderately retarded with a measured I.Q. of approximately 50. She had satisfactory blood levels for about 4 years, normal E.E.G's. and her I.Q. rose to 80. She returned to a normal diet and shortly afterwards an irritable spike focus appeared in the left occipital area and this is still seen in some of the E.E.G. recordings, but whether this was the result of returning to a high phenylalanine intake or not is uncertain.

There is possibly a difference between the fourth case and the other three. There may be a difference between the phenylketonuria child who returns to normal diet after starting treatment early, and the child who started treatment later when brain damage had already occurred.

Jacobson (Cambridge). I should like to ask the previous speakers if there is any information available whether characteristic changes could be observed in the E.E.G., if e.g. noradrenaline function is interfered with by giving methyldopa, or if a drug is given which leads to the discharge of serotonin, like lysergic acid. I think we forget that the primary defect involved in the central nervous system may not be caused by phenylpyruvate, lactate or acetate or their derivatives, but may be due to a true deficiency of the nerve cells at their synapses; e.g. a deficiency of noradrenaline at the adrenergic synapses, or serontonin at others. Careful clinical observations on these patients may allow interpreting the significance of changes observed in the E.E.G.

Poley. I am afraid I don't have anything to add to what has been said by Dr. Hudson. We have not yet enough experience with E.E.G. abnormalities in phenylketonurics on a low-phenylalanine diet—especially in those children who were treated since the early newborn period.

We have not done any studies which involved serotonin congeners or decarboxylase inhibitors or competitors and I fear that any metabolic reactions or changes secondary to the administration of these drugs Dr. Jacobson has referred to would be difficult to interpret electroencephalographically.

Pampiglione. In reply to Dr. Jacobson, I do not recollect any experiments in which these particular substances have been administered to young animals at a comparable level of cerebral maturity.

The literature on the administration of lysergic acid to mature animals and adult human volunteers, agrees on the occurence of minor E.E.G. changes which are compatible, as far as we can tell, with the slightly

altered state of awareness or consciousness and with the presence of abnormal impressions or sensations or hallucinations. These features are quite different from the type of E.E.G. seen in young children with phenylketonuria, either treated or untreated, or during a tolerance test. This might be due to the fact that we are dealing with a considerably different age group and a different type of enzymatic constellation in terms of brain maturation.

In reply to Dr. Hudson, the E.E.G. has to be considered as a physical sign, not as a diagnostic device. It is only a physical sign, so we don't want to utilise one particular sign as a measure of all the other phenomena that might occur in a patient.

If the problem is to study our patient's cerebral function in terms of electrical activity of the brain, the E.E.G. will give us data to be utilized in a clinical context. I am not prepared to say that if the E.E.G. is not showing any gross abnormality then the patient is well.

Bickel. I would like to raise two practical questions. Firstly, there are phenylketonuric patients who have infantile spasms and who do not respond promptly to dietetic treatment so that the spasms continue. We have tried A.C.T.H. in these cases because in the literature there are reports saying it is valuable in phenylketonurics. Unfortunately, in our experience this drug did not seem to have any beneficial effects in addition to the diet.

The second point concerns the question of when to stop the diet without risk to the patient. May it not be that the E.E.G. is of some help in this difficult decision? I have seen one patient who before stopping the diet had a normal E.E.G. for years, but a few weeks after stopping the diet had a convulsion at the age of 7 years with corresponding changes in the E.E.G.

Pampiglione. The problem that you pose is to show whether stopping the diet is going to alter the cerebral electrical activity in a given person. This can be easily demonstrated in E.E.G. terms, but the interpretation of the E.E.G. findings depends clearly on other factors. You may decide that if convulsions occur the patient should be put back on the diet, but would you put him back on the diet because of the convulsions or because of the E.E.G. deterioration?

We know that the E.E.G. has changed and that some metabolic changes must have occurred because of the interruption in the diet but should we wait until some clinical changes have occurred or are we sufficiently knowledgeable to utilize only the E.E.G. data?

DISACCHARIDE INTOLERANCES

A. Holzel

Some disorders arising from inborn enzyme defects demonstrate most strikingly the interplay between hereditary and environmental factors. In a small number of them the course of the disease and the consequences of the errors of metabolism can be modified by altering the environmental element with the introduction of special diets eliminating the components that cannot be metabolized in a physiological way. This has been carried out more or less successfully in galactosaemia, phenylketonuria, and fructosaemia. In many of these conditions damage to the body results from the accummulation of harmful intermediate metabolites, but for the past seven years a great deal of attention has been paid to another aspect of deficient intracellular enzyme activity which leads, not to the amassing of noxious metabolites, but to a failure of the absorption of important nutrients. Deficient disaccharidase activities have been recognised only very recently, but it has nevertheless, lead to the rapid expansion of this field of medical knowledge.

The realization that disaccharidase activities might be inhibited in unrelated disturbances of the gastro-intestinal tract brought about an intensive search for their presence in a large number of alimentary diseases. These efforts led to the extension of our still limited knowledge of disaccharidase deficiencies from the group of apparently inherited conditions to the acquired ones. For example, in the case of lactase deficiency, an isolated deficit in the activity of this enzyme has been described in a variety of intestinal disturbances, but has also been encountered as a chance finding in adults. (Cuatrecasas *et al.*, 1965; Littman & Hammond, 1965; Haemmerli *et al.*, 1965). The question is whether the absence of lactase in an otherwise healthy adult can be regarded as a persisting congenital state, or as the permanent sequela of a healed injury to the intestinal mucosa. The lack of a relevant history in early infancy is against, but a high racial incidence is in favour of a congenital defect.

The hereditary disaccharidase deficiency syndromes have a great deal in common since the most important manifestation, namely diarrhoea, occurs in all of them.

Congenital lactose malabsorption

Congenital lactose malabsorption was first recognised in 1959 by Holzel *et al.* in two siblings; since then 13 cases have been described in the literature and we have had the opportunity of investigating an additional 5 cases. The history is fairly characteristic. There is diarrhoea from a very early

date in life, generally as soon as milk feeding has become well established. It is likely to be more marked in the breastfed baby, and in the artificially fed baby whose cow's milk formula is augmented with lactose. The lack of lactase activity leads to the retention of the sugar in the gut. Due to the change in intraluminal osmotic pressure there may be considerable influx of water into the intestine and, as a result of fermentation short chain fatty acids accumulate which have an irritating effect on the intestinal mucosa and also induce the evacuation of frothy sour smelling thin stools. The high content of organic acids in the faeces frequently provokes extensive excoriation of the buttocks which is resistant to treatment until the metabolic upset is remedied.

Babies suffering from lactose malabsorption are restless, irritable infants who cry a great deal, partly because of hunger and partly because of recurrent intestinal colic. The commonly used formulae do not satisfy them since they lose approximately 40 per cent of the calories offered to them. They are often considerably underweight and fail to thrive.

Among the 18 cases so far described there were 3 pairs of siblings, but consanguinity in the parents has not been noticed. The familial occurrence points to a hereditary factor and one is probably justified in the assumption that lactase deficiency is also a recessively transmitted inborn error of metabolism.

Treatment of the condition consists of replacing the lactose by another carbohydrate and only in the cases in which vomiting is a marked feature is one forced to replace the ordinary formula by a low lactose food. These infants often lose their symptoms and gain weight rapidly when given a soya bean preparation or one of the low lactose proprietary brands.

Improvement also takes place very quickly and can be easily maintained when mixed feeding is introduced early.

Severe infantile lactose intolerance

Another condition which was described by Durand in 1958 as idiopathic lactosuria, and subsequently under a variety of other names, has been attributed to lactase deficiency and lactose malabsorption. There have been well over a dozen similar cases reported and we had the opportunity of studying an additional three in the past two years.

We prefer the designation: 'Severe infantile lactose intolerance' for these cases. The condition becomes manifest somewhat later than lactase deficiency. Vomiting is a more prominent symptom than diarrhoea; dehydration develops very quickly. There are frequently signs of renal involvement such as renal acidosis and aminoaciduria. Lactosuria may be severe and is an almost constant finding, and is occasionally accompanied by sucrosuria and glucosuria. Haemorrhagic manifestations have been observed and also pyloric stenosis in two cases. Mild steatorrhoea has been a feature in two of them, as well as an appreciable reduction in xylose excretion. Removal

of lactose from the diet leads to prompt improvement; however a return to even less than 1 per cent of lactose in the food can precipitate a catastrophe and death. Nearly half the total number of cases recorded in the literature died. In the survivors spontaneous recovery occurred after varying intervals from 7 to 18 months, after which lactose can be tolerated without any discomfort or sign of ill health.

Table I compares the two conditions: both start in the first few weeks of life; severe lactose intolerance on the whole a little later. Vomiting and diarrhoea is common to both. Lactosuria is absent in lactase deficiency except to a slight degree during lactose loading tests, and is almost always present in lactose intolerance, often accompanied by other forms of melituria and aminoaciduria. Signs of general impairment of the absorptive capacity are unknown in lactase deficiency, but are not unusual to some degree in lactose intolerance.

Hereditary factors may play a part in both conditions. Consanguinity has been found among the parents of children with lactose intolerance, and there is a familial incidence in lactase deficiency. In our opinion these two diseases are distinct entities probably with different pathogenesis, pathophysiology, prognosis and to some extent treatment.

Sucrase-Isomaltase defect

The most common of the hereditary disaccharidase deficiencies is the sucrase-isomaltase defect. Prader & Auricchio (1965) referred to 63 cases described in the literature, 34 of whom were males and 29 females. The time at which symptoms appear varies considerably depending on the introduction of sucrose and starch into the infant formula. Here diarrhoea is also the leading manifestation; the stools are frothy, loose and of low pH. Minor degrees of starch intolerance have been an associated manifestation in all the examined cases. Occasionally refusal of sucrose-containing foods, abdominal distension, colicky pain and even malnutrition may occur, but on the whole symptoms are less severe than those encountered in lactose malabsorption. Isomaltose, which consists of two molecules of glucose joined by a 1, 6 α bond, forms the branching links in glycogen and amylopectin and comprises only a very small proportion of the total amount of maltose formed in the digestion of the starch in our daily diet.

The co-existence of the two enzyme deficits in the children studied has aroused a great deal of interest from the genetic point of view. In our present limited knowledge absence of the two enzyme activities seems to contradict the 'one gene one enzyme' theory; hypotheses adduced to explain the state of affairs propose the presence of a common regulator gene which may be defective or the defect of a single structural element with separate active side chains for different substrates. No definite proof has yet been provided for the recessive autosomal nature of the disorder. Anderson

Table I

	CONGENITAL LACTASE DEFICIENCY OR CONGENITAL LACTOSE MALABSORPTION	SEVERE INFANTILE LACTOSE INTOLERENCE
FEATURE		
ONSET	WITH MILK FEEDING DURING FIRST WEEK	IN THE COURSE OF THE FIRST MONTH OF LIFE
HEREDITARY FACTOR	FAMILIAL	CONSANGUINITY OF PARENTS
DIARRHOEA	SEVERE	MILD - MODERATE
VOMITING	MILD - MODERATE	SEVERE
FAILURE TO THRIVE	PRESENT	PRESENT
EXCORIATION OF BUTTOCKS	PRESENT	
OTHER FEATURES		
	-	HAEMORRHAGIC MANIFESTATIONS
	-	MELITURIA: LACTOSURIA
		SUCROSURIA
		GLYCOSURIA
	-	AMINOACIDURIA
	-	RENAL ACIDOSIS
		SLIGHT STEATORRHOEA
LACTASE ACTIVITY	ABSENT (PERMANENTLY)?	DIMINISHED DURING ILLNESS
		NORMAL AFTER RECOVERY

et al. (1963) observed two siblings and investigated the parents without being able to find convincing evidence of their heterozygosity. The children's father had a history of diarrhoeal episodes following the intake of large quantities of sucrose and starch and a flat sucrose absorption curve, but on qualitative assessment of invertase activity in a peroral biopsy specimen there was no definite decrease of the enzyme. The mother's sucrose absorption seemed to be reasonably normal. Sucrose malabsorption tends towards spontaneous improvement although in some patients symptoms may appear throughout life with the ingestion of larger quantities of sugar and starch.

Differential diagnosis

The diagnosis of the hereditary disaccharidase deficiencies should be considered whenever there is a history of diarrhoea with onset in early infancy. Marked malnutrition is a feature of only the more severe forms and is more commonly seen in lactase deficiency. The pH of the stools can be used as a screening test, but is not very reliable since it may also be lowered in infective forms of gastroenteritis. The pH of the normal cow's milk stool is between 6·5 and 7, whilst in the fermentative diarrhoeas it is below 6 and sometimes closer to 5. Since the fall in the pH is the result of the formation of large quantities of low molecular organic acids, the estimation of lactic acid has been recommended as a useful test. It can be determined by enzymatic as well as chemical methods and a simple new technique has recently been developed (Clarke & Podmore, 1966). Increase in lactic acid excretion may occur in other forms of diarrhoea and cannot be regarded as characteristic although it adds to the diagnostic evidence.

Sugar loading tests carried out in the absence of diarrhoea are extremely helpful in the assessment of disaccharidase activity. The dose of sugar to be administered has been more or less arbitrarily recommended as 2 g./kg. bodyweight or 50 g./m^2 surface area. It seems to make little difference which of the two standards one accepts provided they are maintained.

To enhance the value of the lactose tolerance test Fischer & Zapf (1965) recommended the administration of 0·5 g. of aethanol per Kg. bodyweight 10 minutes before the test in the form of a 45 per cent raspberry spirit diluted 1:1 with water. This small amount of alcohol did not seem to affect the children adversely. The purpose of the measure is to inhibit galactose metabolism and thus to allow the determination not only of the blood glucose levels, but also that of the blood galactose. The oxidisation of aethanol reduces the DPN/DPNH quotient to half the normal. The uridine diphosphogalactose-4-epimerase is the rate limiting factor in the utilisation of galactose and is DPN dependent, whilst DPNH inhibits the enzyme activity so that galactose persists much longer in the blood.

Cellobiose, a disaccharide consisting of two molecules of glucose with the same β1-4 glycosidic bond as lactose, results from hydrolysis of cellu-

lose which is not normally digested in the human gut. Cellobiose, when administered to a human, is of course hydrolysed by lactase. The absorption of cellobiose may be assessed similarly by determining the rise in blood glucose and this then provides a verification of lactase activity. Lactose is, however, split at five times the rate of cellobiose.

The constant association of sucrase and isomaltase deficiencies has made it desirable to check the enzyme activities using both disaccharides for loading tests. Isomaltose is, however, practically unobtainable in adequate quantities and Prader and his team introduced palatinose, an artificial product consisting of glucose and fructose in a β 1-6 glycosidic link similar to that of isomaltose and this can be used as an alternative for the assessment of isomaltose absorption.

Results of the tests should be reproducible—if striking differences are encountered in the same patient they are invalid. An increase of blood glucose of more than 30 g. above fasting level at any point of the tolerance curve may be regarded as evidence of normal disaccharidase activity; values that do not exceed 20 mg. are indicative of impaired absorption. An increase of 20-30 mg. above fasting level is of doubtful significance. The additional control of tolerance tests with equal parts of the constituent monosaccharides is helpful in the elimination of such disturbances as are due to a general reduction of the intestinal absorptive capacity, or to an inhibition of sugar transport systems.

Xylose excretion as well as fat balance studies are further investigations employed in every case, to exclude acquired disaccharidase deficiencies which are by far more common than the hereditary forms.

In the latter, reducing substances are absent from the urine, but may be present in the faeces; the clinitest suggested by Burke *et al.* (1965) as a screening procedure for the presence of reducing sugars in the stools is helpful when positive, but does not rule out a disaccharide malabsorption when negative.

Recently an attempt has been made (Laws & Neale, 1966) to develop the radiological diagnosis of disaccharidase deficiencies. By using a suspension of micropaque barium sulphate with 25 g. of lactose or other sugars according to the suspected enzyme deficiency, the authors recognised characteristic changes. They state that the small intestine appeared distended by dilute contrast medium. The peristalsis was very active and the contrast medium reached the transverse or descending colon within an hour. The haustral pattern was definitely prominent.

At present the assay of disaccharidase activity in mucosal specimens is regarded as the most reliable means for the diagnosis of disaccharidase deficiencies. However, even this method has definite drawbacks and is of limited value. Firstly, it provides information about only a small area of the intestinal mucosa. In conditions where the proximal part is more heavily affected than the distal, as in coeliac disease, the information obtained from

the biopsy specimen may be misleading. Thus, one may be faced with conflicting results of tolerance tests and enzyme assay, namely that disaccharide absorption proceeds even in the seemingly complete absence of disaccharidase activity. Secondly, a variety of agents may temporarily inhibit enzyme activity, so that the resulting disaccharide malabsorption may be only a secondary and somewhat irrelevant sequela of a basic pathological process such as an inflammatory disease affecting the intestinal mucosa. It is also feasible, and in fact already shown to occur, that certain drugs given for a variety of reasons may temporarily interfere with normal enzyme function, thus leading to the assumption that exclusion of the sugar from a patient's diet may cure his disease whilst in reality this may only be achieved by the omission of the noxious drug.

The procedure is not without danger irrespective of the instrument employed, be it the Crosby capsule which is the instrument employed by a number of investigators, or the multipurpose biopsy tube of Brandborg, Rubin & Quinton. It is probably unwise to carry out the biopsy when the infant is in an acute phase of severe diarrhoea. Perforations have been known to occur, and diapedesis of bacteria causing peritonitis have been recorded without any detectable lesion in the intestinal mucosa.

The age at which the procedure can be performed successfully varies with the investigator. We have not attempted it in children under 5 months, but a recent study by Burke and colleagues includes infants aged 2 months. It is a time consuming operation carried out by a team and involves one member of the medical staff, an experienced nurse, and the co-operation of the x-ray department, the pathologist, and biochemist.

The *normal* values for the enzyme activity given by Prader & Auricchio in 1965 relate to the adult. They are expressed in units per g. protein. One unit splits one μ mol. substrate per minute at pH 5·8 and 37° C. Table II. Burke *et al.* (1965) give a different range for children; although the method and units are more or less the same. Table III. Burgess *et al.* (1964) arrive at different results; they define as 1 unit the hydrolysis of

Table II

DISACCHARIDASE ACTIVITIES IN MUCOSA OF JEJUNUM IN THE ADULT
(Prader & Auricchio)

ENZYME ACTIVITY	OPERATIONAL MATERIAL (n EQUALS 15)	BIOPSY MATERIAL (n EQUALS 10)
MALTASE (M)	246 (70-456)	593 (310-1120)
SUCRASE (S)	75 (24-156)	173 (70-325)
ISOMALTASE (I)	74 (27-156)	159 (65-268)
PALTINASE (P)	21 (5-33)	
LACTASE (L)	30 (6-54)	107 (39-258)
CELLOBIASE (C)	6 (1-21)	14 (9-21)

ENZYME ACTIVITIES (MEAN AND RANGE) IN UNITS PER gm PROTEIN
(1 UNIT SPLITS 1μmol SUBSTRATE PER MINUTE AT pH 5.8 AND 37 C)

Table III

NORMAL RANGE OF DISACCHARIDASE ACTIVITY (Burke et al)

	LACTASE	SUCRASE	ISOMALTASE	MALTASE
NO. OF CHILDREN TESTED	17	18	14	21
UNITS OF ACTIVITY				
RANGE	14-132	32-228	31-177	83-615
MEAN	49	95	89	260
UNIT EQUALS μ moles DISACCHARIDE HYDROLISED/MIN/g PROTEIN				

Table IV

DISACCHARDISE LEVEL IN JEJUNAL MUCOSA FROM NORMAL CHILDREN (Burgess et al)

	LACTASE	MALTASE	PALATINASE	SUCRASE
MEAN	8.7	62.6	6.7	17.4
RANGE	6.6-12.4	43.2-88.9		12.4-20.2
ENZYME LEVELS (UNITS) 1 UNIT EQUALS 1 μ mol OF SUBSTRATE SPLIT/gm MUCOSA/MIN				

Table V

NORMAL VALUES FOR INTESTINAL DISACCHARIDASE ACTIVITY (HOLZEL)			
	MALTASE	LACTASE	SUCRASE
RANGE	34-48	4.72-12.4	7.01-20.9
DISACCHARIDASE ACTIVITY IN TWO SIBLINGS RECOVERED FROM LACTASE INTOLERANCE			
	MALTASE	LACTASE	SUCRASE
S.F	34.48	4.72	7.01
N.F.	35.10	6.45	7.48
DISACCHARIDASE ACTIVITY IN THREE CASES OF GIARDIASIS			
	MALTASE	LACTASE	SUCRASE
F.R.	16.49	0.87	4.22
L.B.	17.73	0.46	4.09
A.C.	25.00	1.64	2.80
UNIT μ mol DISACCHARIDE HYDOLYSED/MINUTE/gm/ WET WEIGHT OF TISSUE			

1 micromole of substrate per g./wet mucosa/min. Their figures are therefore much lower. Table IV.

Our values agree closely with those of Burgess as the unit is also expressed as γmole substrate hydrolysed per gm./wet tissue/minute at given pH and temperature. Table V.

Case Report

Two siblings S.F. and N.F. had both suffered from lactose intolerance

and after many months made a good recovery. However the mother was unconvinced that the younger son had completely overcome his intolerance. When the nature of the biopsy was explained to her she insisted that we should carry it out. The values obtained clearly demonstrate that lactose intolerance, even if associated with temporary lactose malabsorption, is not the expression of a hereditary deficient lactase activity. Table V. The mucosal pattern was perfectly normal at this stage.

Disaccharide malabsorption in coeliac disease

For the past four years we have been investigating disaccharide absorption and tolerance in children with gluten induced enteropathy. Our results (Holzel, 1964) show, as those of later studies by Shmerling *et al.*, 1964; Haemmerli *et al.*, 1965, and Arthur *et al.*, 1966, that in the florid phase of the disease when the superficial structure of the intestinal mucosa is grossly disorganised, disaccharide absorption is greatly impaired and that lactose tolerance is more markedly reduced than that of the other disaccharides. During the recovery phase disaccharide absorption improves fairly rapidly. When tested after 8 to 10 weeks this had almost returned to normal with the exception of lactose absorption. A normal rise of the blood sugar level after a lactose load may be obtained only after several months.

Patients with inadequate dietary controls who are somewhat under average in weight and height for their age may have a normal glucose tolerance curve but 'flat' disaccharide absorption tests (Fig. 1). In others, glucose absorption may also remain impaired (Fig. 2). It seems therefore that disaccharide tolerance tests may be an additional useful index of absorptive recovery.

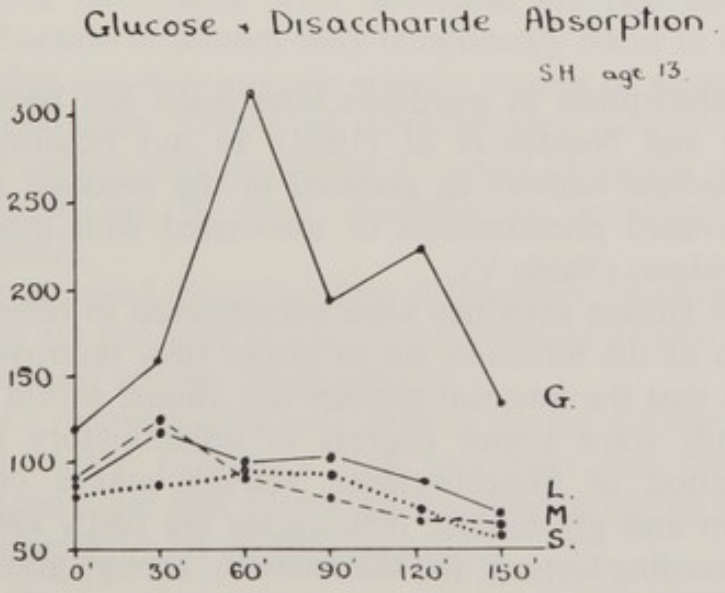

FIG. 1
Incomplete recovery from coeliac disease. Normal glucose but poor disaccharide absorption.

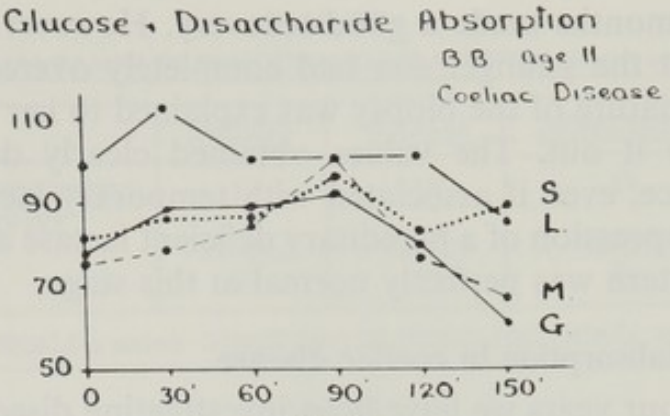

FIG. 2

Impaired disaccharide absorption in a case of coeliac disease with unsatisfactory progress due to poor dietary control.

Intestinal biopsies were carried out in three cases. Assay of enzyme activity agreed very closely with the sugar loading tests. In one case, to be published elsewhere, there was complete absence of lactase activity, and for as long as the baby received milk she suffered episodes of vomiting, diarrhoea, dehydration and metabolic ketosis which can only be described as coeliac crises. A lactose-free and gluten-free diet led to the complete disappearance of the crises and to rapid recovery. The routine introduction, as recently recommended, of a lactose sucrose and gluten-free diet in every case of coeliac disease with impairment of disaccharidase activity in the mucosal biopsy specimen (Arthur *et al.*, 1966) would, in our opinion, place an unnecessarily heavy burden on hospital and home. It is, however, important to remember that in rare instances removal of these disaccharides may hasten the recovery, where a gluten-free diet alone may have been unsuccessful.

Giardiasis

Lactose malabsorption in giardiasis intestinalis has been recorded by Durand (1964) and Nordio *et al.* (1963). In our experience, however, depression of lactase activity as assessed in the mucosal specimen may occur as an isolated phenomenon or associated with inhibition of the other disaccharidases (Table V).

The very low lactase activities were encountered in cases of giardiasis and eradication of the infection led to rather slow improvement. It is of interest to note that the mucosal pattern was almost within normal limits and showed only some minor degrees of inflammatory response. The delayed restoration of disaccharidase activity makes one wonder if giardia infection and glycosidase deficiencies are really causally related. But the long preceding history, and the recovery, though gradual, following treatment in every case is probably convincing evidence. One of our cases regained normal disaccharidase activity only after an interval of six months.

Gastroenteritis

Intolerance to milk feeds following infectious forms of diarrhoea and vomiting has been known for practically half a century or longer; but milk protein and fat and not lactose were generally regarded as the noxious agents. This led Moll & Stransky in 1923 to introduce their pudding as a milk-free diet for the convalescent phase of severe gastroenteritis. For nearly two decades lactose seemed to us the more likely factor in causing a re-exacerbation of the diarrhoea and when Arobon, the carob bean flour preparation became available it was employed as an intermediate stage between the rehydration phase with electrolyte solutions and the introduction of milk feeds.

Two studies (Sunshine & Kretchmer 1964; Burke *et al.*, 1965) have recently provided laboratory support for the clinical and at first purely empirical observations.

A marked reduction of disaccharidase activity was also noted in serious viral infections of the respiratory and intestinal tract. In one, ECHO 14 was isolated from nose and throat swabs as well as from the faeces; in two others only from the stools. The laboratory finding supported strongly the clinical diagnosis of secondary lactose malabsorption.

Decrease in lactase activity was also found in an infant suffering from intestinal milk allergy.

Summary

1. The hereditary disaccharidase deficiencies occur generally as isolated lactase or combined sucrase and isomaltase deficiencies and are relatively uncommon.
2. Lactose malabsorption due to hereditary alactasia or hypolactasia and severe lactose intolerance with lactosuria are two entirely different disorders. Lactase deficiency may temporarily occur in lactose intolerance, but after recovery enzyme activity returns to normal.
3. Acquired secondary defects of disaccharidase activity are common, much more serious and can follow a variety of insults to the intestinal mucosa. These can be viral, bacterial or protozoal, chemical or immunological.
4. Among the acquired disaccharidase deficiencies, impairment of lactase activity is by far the most frequent in childhood as well as in adult life and has the most serious consequences.

   The patient's recovery may well depend on the correct assessment of the disaccharidase deficiency and treatment can be provided by eliminating the sugar that cannot be absorbed or by the introduction of disaccharidases from an exogenous source.

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DISCUSSION OF PRESENTATION BY DR. HOLZEL

Bickel (Marburg). I couldn't understand when you say that the lactose intolerance goes together with very strong lactosuria, whilst malabsorption doesn't seem to show a strong lactosuria. This seems puzzling because both conditions seem to have in common a deficiency of the lactase which leads to absorption of lactose through the intestinal wall. What is the difference between these conditions? Why is it that in one of them so much lactose is absorbed that it can lead to a strong lactosuria, and in the other one there doesn't seem to be so much absorption?

Holzel. The explanation is relatively simple, but I can't prove it. In lactase deficiency there is no damage to the intestinal mucosa and in lactose intolerance there is. I may not have made it quite clear that lactose intolerance may supervene in coeliac disease, as I demonstrated in the one case, when there was already damage of the intestinal mucosa. What injures the intestinal mucosa I do not know, but it is certain that it is the damaged mucosa which allows the passage of lactose, and also other sugars. We found glucose and sucrose with the lactose. Lactose is only the more striking finding in the urine and in the blood and something which one looks for in these cases, but the other disaccharides are also excreted as a result of mucosal injury. Probably the same process which interferes with the absorptive functions of the intestinal mucosa, damages also the tubular epithelium in the kidney, leading to severe aminoaciduria in some cases, and causes liver damage in others.

Bickel (Marburg). So you do not think the lactose itself damages the

intestinal mucosa? I was, of course, reminded of the work of Woolf and Moncrieff on lactosuria and sucrosuria in hiatus hernia with mental damage. Is there mental demage in your case?

Holzel. No. You can find lactosemia in a number of perfectly healthy individuals and protracted lactosemia in the premature baby without any signs of damage to any organ or system.

Black (Sheffield). I still do not quite understand why these children are so ill. Is it the lactose intolerance which causes this? If you give lactose intravenously (which would be a very hazardous thing to do), you can prove that lactose itself isn't damaging. Also, lots of normal people have lactose circulating in the blood without doing them any damage at all. No doubt the symptoms do seem to correlate in these children with the giving of lactose, and they become ill. One simple explanation is that it is because the lactose is in the blood that they do become ill. Is this correct or incorrect?

Holzel. This is correct for as long as the disease is present. One can give, after recovery, any amount of lactose to these children and even obtain a lactosemia, without doing any harm, so there must be something which has altered their response.

Black (Sheffield). But you do not know whether the response to intravenous lactose at that stage is harmful?

Holzel. We do not know. The only thing I can say is that we gave lactose intravenously to one child after that particular phase and she reacted in the same way as a normal child.

Bickel (Marburg). You stressed in your case that the treatment of choice is to give a lactose free diet at an early stage, which to Dr. Black and I suggests that you consider the lactose to be the damaging agent.

Holzel. Yes in so far as it seems to add to whatever lesion there is or to whatever provokes intolerance. In the same way as it does, for instance, in coeliac disease. A child with gluten intolerance but no lactase deficiency became very ill when given 10 g. of lactose. There may be a variety of noxious agents producing damage to the intestinal mucosa and I can think of viral infections very early in life as a cause. We now have evidence that, for instance, Echo 14 can induce lactose intolerance and also lactase deficiency.

Woolf (Oxford). You mentioned the paper by Woolf, Moncrief and Wilkinson on sucrosuria and lactosuria associated with mental deficiency in hiatus hernia. I think here we must separate correlation from causal relationships. These children, I am sure, had the lactosuria and sucrosuria as a result of malabsorption because of some defect in the intestinal mucosa. The mucosa wasn't examined, but it seems very likely on several grounds. They could split some of their lactose and some of their sucrose, but this wasn't an absolutely deadtight division. Now one of them did come to autopsy examination and there were congenital malformations

of the brain which were quite sufficient to explain the severe mental retardation. So I think we have here an example of multiple congenital malformations including the intestinal mucosa. I do not think lactose and sucrose in themselves caused malformation.

Morris (Barrow). I would like to ask Dr. Holzel if the giardia infestation was severe, and the name of the drugs mentioned. Also, in coeliac disease, as there is atrophy of the mucus membrane and a tendency to lactose intolerance, do you get a rise in lactose?

Black (Sheffield). Yes, you do get a rise in lactose in coeliac disease.

Morris (Barrow). So this ties in with the idea of damage to the intestinal mucosa causing lactose to be absorbed?

Holzel. Yes. This has been established for a long time. The giardia infestation was very heavy. Drugs have been reported in adults to have caused damage to the intestinal mucosa.

Visser (Rotterdam). You have mentioned coeliac patients who have a secondary lactase deficiency. Is there any evidence for secondary gluten sensitivity in any of these patients with lactase deficiency? How do you make a differential diagnosis in coeliac disease?

Holzel. We have discussed this before. The mucosal changes which have been accepted as characteristic for coeliac disease are now regarded as non specific, and we have no really pathognomonic tests except the response to gluten. It has been stated that giardia infestation may be complicated by gluten intolerance and that with the cure of the giardia infestation the gluten intolerance disappears. This has also been our experience in one case. It has also been said that in iron deficiency a temporary gluten intolerance may develop, accompanied by mucosal changes, and that with the correction of the iron deficiency, the mucosa will return to normal and the gluten intolerance will clear. How serious this gluten intolerance is I cannot say.

Tempany (Dublin). You mention glucose tolerance test and the xylose tolerance test. Do you have a preference for one or the other of these? Do you find the range of normality in the xylose tolerance test in children rather wide?

Holzel. We have no preference, and we almost always use these two together. The xylose excretion, in our experience, varies greatly with the age of the child. In babies the collection of the urine during the first five hours may present great difficulties. We have extended the test to 12 and 24 hours as has been recommended. We have found in babies, under 6 months, that anything above 15% excretion can be regarded as normal.

Tempany (Dublin). With regard to the glucose tolerance test, the point is sometimes made that one should give extra glucose to these patients a few days before the glucose tolerance test. Is this your custom?

Holzel. When we suspect disaccharide malabsorption—yes.

Clayton (London). The pallor of the lactose intolerance patients has been described. Has this been your experience?
Holzel. Yes, they are pale, but so are many other ill children.
Raine (Birmingham). I would like to confirm Dr. Holzel's remarks about the xylose absorption test. We have been looking at it from the point of view of age dependence and found very much the same as he has.
Hughes (Birmingham). Do you find that in the normal child it is usual to get such enormous rises as from, say 80 to 180 m.g. and above?
Holzel. With glucose, no. A rise up to 50 or 70 m.g. is probably normal.
Hughes (Birmingham). Is a rise of 180 m.g. exceptional?
Holzel. Yes, this is unusual.
Bickel (Marburg). I think that a very important part of treatment is to know more about the sugar composition of the various foods, and I have been very disturbed to find that there are very few detailed data available about their composition. The more we have to deal with various kinds of sugar malabsorption the more we need to know about the exact sugar composition of food. We have tried in our laboratory to solve this problem by chromatographic techniques and we have assayed the most important foods for their sugar content before and after hydrolysis, but this is very difficult work and I wondered if you knew of any further data in the literature.
Holzel. We have analysed various foods and baby foods with regard to galactose, but not disaccharides. The information is included in a little booklet which the Department of Child Health in Manchester has issued.

MONOSACCHARIDE INTOLERANCE

J. A. Black

The nomenclature of the commoner mono- and di-saccharides needs clarification. Sucrose and fructose seem to cause most trouble. Sucrose, a disaccharide consisting of one molecule of fructose and one of glucose, is also known as saccharose and invert sugar; fructose is a monosaccharide, also known as laevulose. Sucrose is of course the sugar in the sugar cane and in the beet root, while fructose mainly occurs in fruit and in honey.

Intolerance to monosaccharides may be due to defective intestinal absorption, or to defective metabolic incorporation. In the first group, the defect is probably in the transport system of the intestinal epithelium, and the chief symptom is diarrhoea; while in the second group the symptoms are mainly those of hypoglycaemia and liver damage although other tissues may also suffer.

When considering the clinical presentation of cases of monosaccharide intolerance one has to bear in mind that the symptoms may appear to be caused by the more commonly occurring disaccharides, whereas the trouble is really due to one or both of the constituent monosaccharides. Thus, lactose, which is split into galactose and glucose will produce symptoms in galactosaemia, and sucrose will cause symptoms in fructose intolerance because it is split into fructose and glucose.

A second point which needs emphasising is that glucose, fructose, galactose and lactose are all reducing substances. All these sugars give a positive reaction to Benedict's test and to 'Clinitest', its contemporary modification. Only glucose gives a positive reaction to 'Clinistix' and other similar test papers based upon the glucose oxidase reaction. Therefore 'Clinistix' should never be used as a screening test for sugars in the urine. Also, if reducing substances are detected by the 'Clinitest' and the presence of glucose is confirmed by 'Clinistix', it should not be assumed, except in obvious conditions like diabetes mellitus, that this is the only sugar present. In galactosaemia, for example, both glucose and galactose may occur in the urine at the same time; but galactose will be missed if it is assumed, because of a positive 'Clinistix' test, that glucose is the only sugar present. When reducing substances are found in the urine in any suspected metabolic disorder, chromatography should be used to identify all the sugars present.

Intestinal monosaccharide intolerance

In these disorders there is apparently a failure of transport across the intestinal epithelium; the sugar then diffuses back into the intestinal lumen and causes an osmotic diarrhoea. Two varieties of defective transport have

been reported. The first was described almost simultaneously in 1962 by Laplane *et al.* and by Lindquist *et al.* and subsequently by Anderson *et al.* (1965). The defect lies in a failure of transport of glucose and galactose across the cell wall. Thus lactose, if given by mouth, will cause diarrhoea, with glucose and galactose in the stool, while glucose and galactose given separately also produce diarrhoea, with the respective monosaccharide in the stool. There is no rise in blood glucose after an oral dose of lactose, galactose, or glucose. Fructose, however, does not cause diarrhoea and produces a slow rise in blood glucose. Infants affected by this condition show symptoms within the first week of life. Anderson *et al.* used a feed consisting of sodium caseinate, butter fat, and fructose (a suitable preparation has recently been produced by Trufood Ltd. as a 'reduced fat fructose formula Galactomin'), solids were introduced in their case at the age of 6 months. On present evidence this condition is probably inherited as a recessive and the defect does not recover.

A second variety of intestinal monosaccharide intolerance was described by Burke & Danks in 1966. Here the symptoms are later in onset, at the age of a few weeks or months, and may follow an infection. Diarrhoea occurs when glucose, galactose or fructose is given. Complete recovery occurs fairly rapidly. Treatment is by a mixture of sodium caseinate and butter fat only. It seems probable that this condition is really due to temporary damage to the intestinal epithelium after an infection.

Defective metabolic incorporation

Three such conditions involving monosaccharides have been described; galactosaemia, fructose intolerance and galactose-fructose intolerance. This last condition described by Dormandy & Porter in 1961, is quite unexplained, but is included for the sake of completeness.

Galactosaemia

The main facts about galactosaemia are well known, but a few points about this condition are not always fully appreciated, and need to be emphasised. In order to do this it is necessary to look at the normal metabolism of galactose (Fig. 1).

Galactose can be used as a source of energy via glucose, or can be converted to liver glycogen. By other pathways galactose may be converted to lactose in lactation, and may contribute towards the formation of more complex molecules like the galactolipids, and polysaccharides such as chrondroitin sulphate. Galactose is a major source of energy only in the breast fed baby and to a lesser extent in the infant fed on cow's milk. A breast fed baby may receive as much as 30 g. of galactose daily, in the form of lactose. An important fact, from the point of view of treatment, is that small amounts of galactose can be synthesised by the body from glucose, so that a completely galactose-free diet will not result in any disturbance of

essential metabolism. This is in contrast to phenylketonuria where small quantities of phenylalanine need to be supplied to allow protein synthesis to continue. The basic abnormality in galactosaemia is the absence of galactose-l-phosphate uridyl transferase. This enzyme is responsible for the conversion of galactose-l-phosphate to glucose-l-phosphate which is then converted to glycogen or to glucose 6-phosphate. In the normal individual this specific uridyl transferase is distributed very widely among the cells of the body, including the red cells. This makes early diagnosis relatively easy, as the enzyme can be estimated in the cord blood of the newborn baby. In the older infant who has received some galactose it is possible to detect excessive amounts of galactose-1-phosphate in the red cells; this reaction may also be used in controlling the effectiveness of diet later on (Schwartz, 1960; Donnell *et al.*, 1963).

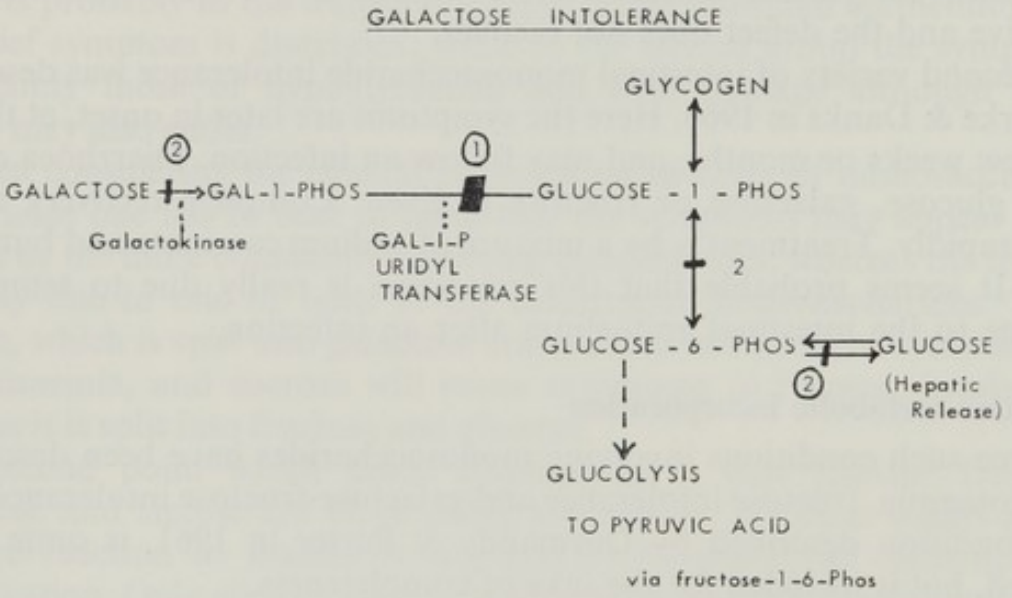

FIG. 1

Major pathways of galactose metabolism
(1) = primary metabolic block in galactosaemia.
(2) = secondary blocks.

The primary block (Fig. 1) results in the accumulation of large amounts of galactose-1-phosphate in those cells which normally metabolise galactose by this path. The accumulation of galactose-1-phosphate in turn inhibits its further formation from galactose by means of galactokinase (this is an irreversible reaction). Galactose then accumulates inside the cell and in the extracellular fluids producing a high level of galactose in the blood, and finally galactosuria. Although the brain does not appear to use galactose very readily under normal circumstances, it does so when the galactose content of the blood is abnormally high. The presence of large quantities of galactose-1-phosphate in the neurone may inhibit its normal metabolism and cause the cerebral damage which usually occurs in untreated cases of galactosaemia. This is a more probable explanation for the brain damage in

galactosaemia than the hypoglycaemia. In fructose intolerance the hypoglycaemia is if anything more severe but the intelligence of even untreated adults is normal.

Other secondary metabolic blocks have also been demonstrated in galactosaemia (Fig. 1). The presence of excessive amounts of galactose-1-phosphate inhibits the conversion of glucose-1-phosphate to glucose-6-phosphate so that a major pathway of glucolysis or glucose utilisation is blocked. It is the inhibition of this pathway which may be responsible for the severe cellular damage which develops in the liver. Another secondary block results from the inhibition of glucose-6-phosphatase, by which pathway the release of hepatic glycogen as glucose normally occurs. Since however the peripheral utilisation of glucose continues unhindered, these blocks in the formation and release of hepatic glycogen mean that the liver cannot respond to a falling blood glucose by releasing more glucose, and therefore the blood glucose continues to fall.

Thus the clinical effects in galactosaemia are hypoglycaemia and hepatocellular damage; less important is renal tubular damage which may be responsible for the amino aciduria which is commonly found in untreated galactosaemia. Lens opacities also occur in such cases and are thought, on the basis of animal experiments, to be associated with the accumulation of the sugar alcohol dulcitol in the cells of the lens (Kinoshita, 1962).

The results of treatment in general are very good if the diagnosis is made early. In spite of the block to the major pathways for the metabolism of galactose, there is evidence that other pathways for the utilisation of galactose exist and the effectiveness of these alternative pathways may explain the differing opinions as to how completely galactose must be removed from the diet. A further point of interest is the possibility of intrauterine damage occurring. Hsia & Walker (1961) pointed out that infants with galactosaemia have a lower than average birth weight, and in some cases cataracts and cirrhosis have been detected at birth.

Fructose intolerance

The second monosaccharide intolerance in the group of conditions with defective metabolic incorporation is fructose intolerance, in which the ingestion of fructose or of sucrose, which is broken down to fructose and glucose, causes nausea, vomiting, abdominal pain and severe hypoglycaemia: liver damage of variable degree occurs in the untreated case.

Fructose is a monosaccharide giving a positive reducing reaction to 'Clinitest' or other modifications of Benedict's reagents. Fructose is found in fruit and in honey, but the main dietary source of fructose is sucrose. The average daily intake of fructose by an adult is about 70 g., and an infant on cow's milk with sucrose added may receive about 20 g. daily. The absorption of fructose from the intestine is somewhat slower than that of glucose, but its uptake by the tissues is quicker. In untreated diabetes

mellitus fructose is used normally and its metabolism is unaffected by the presence or absence of insulin. The breakdown of ethyl alcohol appears to be accelerated by the presence of fructose (Tygstrup *et al.*, 1965).

The normal metabolic disposal of fructose (Fig. 2) occurs mainly in the liver and to a much lesser extent in the intestine and kidney. There is also a special pathway by which fructose is used by adipose tissue in preference to glucose (Froesch & Ginsberg, 1962). In the first stage of utilisation by the liver fructose is converted to fructose-1-phosphate by the specific enzyme

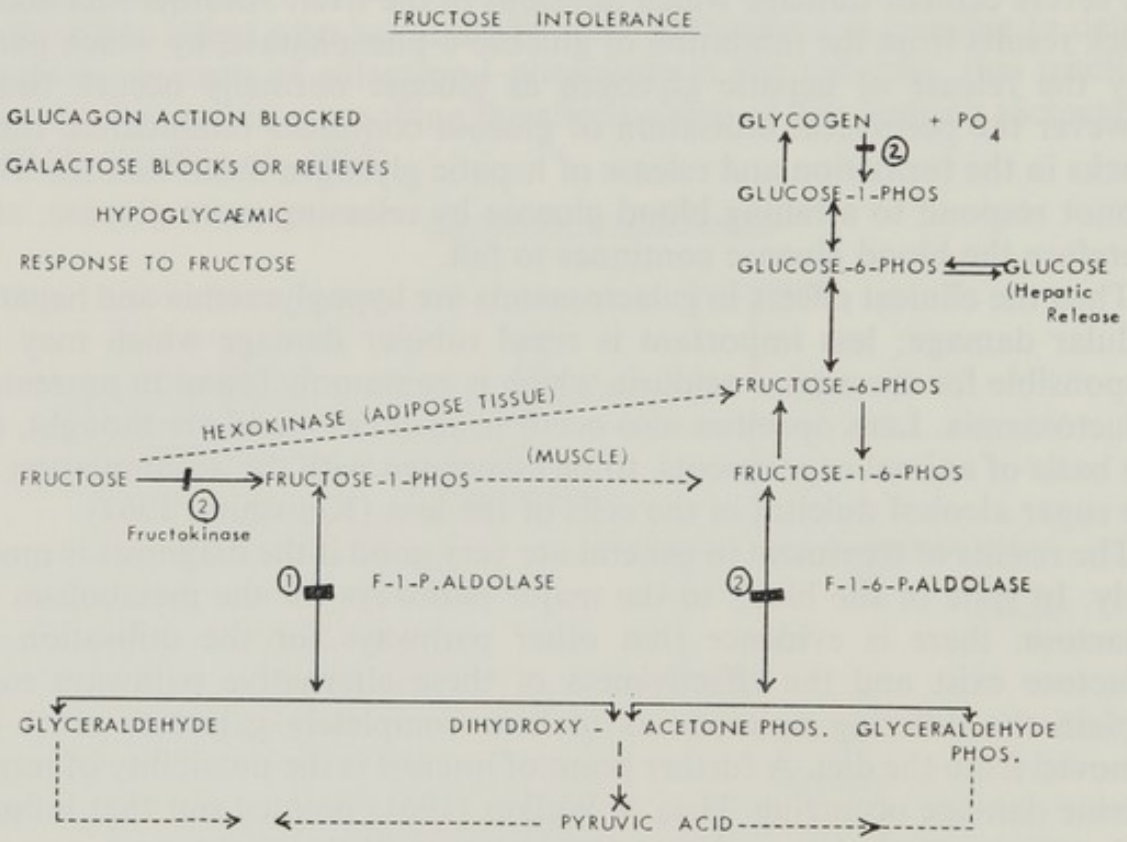

FIG. 2

Major pathways of fructose metabolism.
(1) = primary metabolic block in fructose intolerance.
(2) = secondary blocks.

fructokinase (Fig. 2). It should be mentioned here that there is an entirely benign and symptomless condition, known as essential fructosuria (with fructosaemia) in which hepatic fructokinase is absent (Schapira *et al.*, 1961-62). The fructose-1-phosphate is then broken down by the enzyme fructose-l-phosphate aldolase into dihydroxyacetone and glyceraldehyde which are partly converted to pyruvic acid and partly reconstituted to form fructose-1-6-phosphate by means of fructose-1-6-phosphate aldolase. The fructose diphosphate is then available for conversion to liver glycogen or to glucose. Both aldolases occur mainly in the liver though small amounts may be present in the small intestine and kidney.

The influence of fructose on the metabolism of the intact brain is neglig-

ible, as fructose does not cross the blood-brain barrier (Park *et al.*, 1957), although brain slices can use fructose. Fructose will not therefore relieve hypoglycaemia direct, but will do so by virtue of its rapid conversion to glucose. Hypertonic solutions of fructose may relieve cerebral symptoms in a number of conditions if there is associated cerebral oedema.

The effects of an oral or intravenous dose of fructose in the normal individual are as follows: a moderate rise in blood fructose (the peak value is less than 20 mg. per cent), a fairly rapid rise in blood glucose, and a transient fall in the blood inorganic phosphorus.

Fructose intolerance is a recessively determined condition which was first described by Chambers & Pratt in 1956 but the details of the metabolic defect were worked out by Froesch & Prader *et al.* (1957) in Zürich. They showed that fructose intolerance is due to the absence of specific liver enzyme fructose-1-phosphate aldolase (Fig. 2). This primary block results therefore in an accumulation of fructose-1-phosphate in the liver cell (Milhaud, 1964; Rossier *et al.*, 1966) and a secondary build-up of fructose in the cell, and subsequently in the extracellular fluids. The level of fructose in the blood rises, and when it exceeds the renal threshold of 10-20 mg. per cent fructosuria results. As in galactosaemia, secondary blocks develop (Fig. 2); fructose-l-phosphate inhibits fructose-1-6-P. aldolase, thus preventing the formation of fructose-1-6-phosphate from pyruvic and lactic acid. Another block occurs in the release of glycogen from the liver by means of the enzyme phosphorylase; this effect may be due to the immobilisation of available phosphate as fructose-1-phosphate, thus preventing the phosphylation which is necessary for the conversion of glycogen into glucose-1-phosphate. Evidence for this particular metabolic block is provided by the fact that hypoglycaemia produced by a dose of fructose in fructose intolerance cannot be relieved by glucagon which acts specifically upon this particular conversion of liver glycogen to glucose: when the effects of fructose have worn off the response to glucagon is restored (Cornblath *et al.* 1963). It has been suggested that phosphoglucomutase, converting glucose-1-phosphate to glucose-6-phosphate is also inhibited, but this seems unlikely as the fructose-induced hypoglycaemia can be relieved by galactose (Cornblath *et al.*, 1963), which must pass through glucose-1-phosphate and glucose-6-phosphate (Fig. 1) before it can be released as glucose.

Thus, when fructose is given in this condition there is, as in galactosaemia, a hypoglycaemia resulting from a failure of release of liver glycogen, associated with an unimpaired peripheral utilisation of glucose. The prolonged lowering of the blood inorganic phosphorus which also occurs is thought to be due to the removal of available phosphate as fructose-1-phosphate. The facts given so far do not explain the severe liver cell damage which occurs in the untreated case of fructose intolerance, but it is possible that the intrinsic metabolism of the liver cell itself is damaged

by removal of phosphate normally available for the formation of ATP (Levin *et al.*, 1963).

The question whether fructose-1-phosphate aldolase is absent from the intestine and kidney in fructose intolerance cannot be clearly answered as yet: the results of intestinal biopsy studies are conflicting. If fructose is given orally in fructose intolerance gastro-intestinal symptoms, such as nausea, vomiting and abdominal pain occur, but these are absent or less marked when the same dose is given intravenously. This suggests that the fructose may cause a transient upset in intestinal function. Similarly, aminoaciduria is common in untreated fructose intolerance, probably indicating some renal tubular damage. Nevertheless it is only the liver which suffers severe damage; but in contrast to galactosaemia the intelligence is always normal, and cataracts do not develop.

The clinical picture in fructose intolerance is rather diverse. The affected infant thrives normally when breast fed, but when cow's milk with sucrose is given, or when mixed feeding is started in a breast fed child, symptoms develop consisting of vomiting, refusal of feeds and failure to gain weight. In young infants the vomiting may be so severe as to suggest pyloric stenosis. In others, severe neonatal jaundice with hepatomegaly may occur and a diagnosis of 'neonatal hepatitis' may be made. In one of our cases this appeared to be confirmed by a liver biopsy examined by orthodox histological methods. However, when the infant returned home and began to thrive it was found that the mother was substituting glucose for sucrose in the feeds. She did this because the elder brother had had similar symptoms in infancy and she had been told by a Health Visitor that he could not tolerate cane sugar: this was in 1954, two years before the first description of fructose intolerance in the medical literature. The diagnosis was subsequently confirmed in both brother and sister.

In later childhood there is a marked aversion to sweet things, and a dislike of school meals (which one has to eat). Hepatomegaly may be considerable and a diagnosis of glycogen storage disease may be considered. Occasionally the condition may present with hypoglycaemic fits. It must be emphasized that fructose is only transiently present in the urine in these cases and its absence cannot be considered as evidence against the diagnosis.

Confirmation of the diagnosis may be made by a fructose tolerance test, or by a liver biopsy. The oral test is done using a dose of 0·5 g. of fructose per kg. body weight. This is likely to cause nausea and vomiting, and an intravenous test using half this dosage scale is preferable. The results of fructose tolerance tests are usually quite clear-cut if adequate amounts of fructose are given. The blood fructose level rises up to or above 20 mg. per cent: the blood inorganic phosphorus shows a sustained fall and the blood glucose falls rapidly to hypoglycaemic levels (Fig. 3). This test has its disadvantages: the hypoglycaemia may be so profound that intravenous glucose is required: or symptoms of hepatic damage may occur shortly

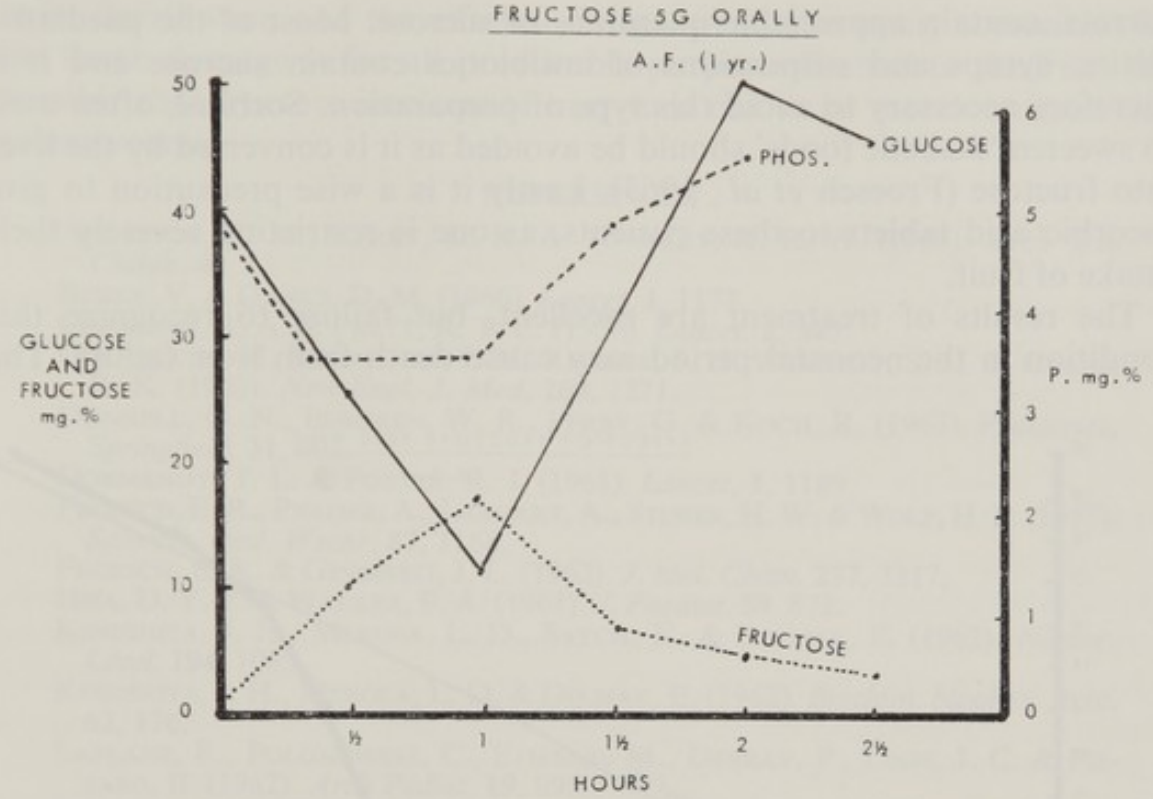

FIG. 3
A typical fructose tolerance curve in a child with fructose intolerance.

after the test. One 8 year old girl developed jaundice and hepatomegaly after an oral tolerance test and became ill for three weeks with very high serum transaminase levels; she was jaundiced for a few days at the beginning of this period. An alternative method of confirmation of diagnosis is by liver biopsy. Orthodox histology is unhelpful; in one of our cases the changes suggested neonatal hepatitis and another showed severe fatty change. If liver biopsy is to be helpful it is necessary to arrange for an assay of the hepatic fructose aldolases to be done on the fresh specimen or on deep frozen tissue. Both fructose-1-phosphate aldolase and fructose-1-6-P-aldolase are reduced, but the monophosphate aldolase is reduced to a much greater extent than the diphosphate aldolase. Using appropriate methods, the normal ratio of the two aldolases is 1:1, but in fructose intolerance the ratio is 1:6 or more. In one of our cases (the infant thought to have neonatal hepatitis) we were able to demonstrate this reduction of fructose-1-phosphate aldolase after the specimen had been in the deep freeze for two years: suitable control material frozen over the same period showed normal levels of both aldolases.

Treatment consists of the exclusion of sucrose and fructose from the diet. The older patients have usually done this fairly completely before diagnosis, but even these self-selected diets usually contain some sucrose or fructose which must be eliminated if liver damage is to be prevented or allowed to recover. It is not always appreciated that most root vegetables, such as

carrots, contain appreciable quantities of sucrose. Most of the paediatric elixirs, syrups and suspensions of antibiotics contain sucrose and it is therefore necessary to avoid this type of preparation. Sorbitol, often used to sweeten 'diabetic foods' should be avoided as it is converted by the liver into fructose (Froesch *et al.*, 1963). Lastly it is a wise precaution to give ascorbic acid tablets to these patients, as one is restricting severely their intake of fruit.

The results of treatment are excellent, but failure to recognize this condition in the neonatal period may cause death from liver failure. The

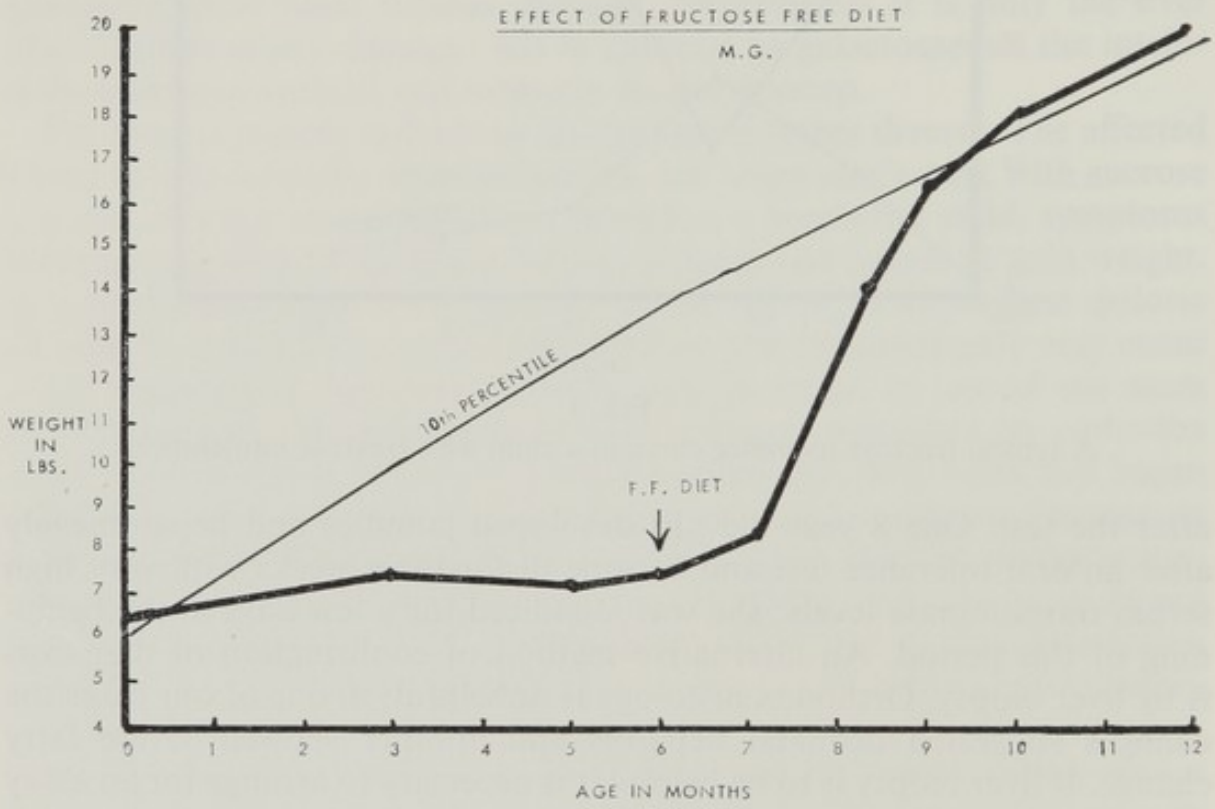

FIG. 4
A weight chart showing the effect of a fructose-free diet.

weight chart shown in Figure 4 shows a very good response in an infant who had failed to gain weight for five months. His sister, whose biopsy showed the fatty changes, had been diagnosed as having glycogen storage disease; this misled us for some time until we were able to examine the original records and the sections from her liver biopsy.

CONCLUSION

Galactosaemia and fructose intolerance make an interesting comparison. In galactosaemia there is a widespread enzyme deficiency which makes the diagnosis on the readily available red cell quite simple: but the fact that the enzyme defect is so widespread means that many tissues are likely to be damaged. In fructose intolerance the enzyme defect is much more limited

in its distribution and the affected tissues are not so easily accessible; but the tissue damage is for all practicable purposes limited to the liver. It is possible that fructose intolerance may turn out to be more common than galactosaemia.

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DISCUSSION ON PRESENTATION BY DR. BLACK

Barry (Cork). I would like to ask some questions on fructosuria. I do not think you mentioned pain as a symptom. A patient of mine had abdominal pains for a long time and she had a very abnormal fructose tolerance test. I think this is an important symptom.

With regard to results, I started this child on a low fructose diet about twelve months ago and I expected her height and weight to shoot up, but nothing of the sort has happened. She rose from just under the 10th percentile to just above the 10th percentile for height and weight and has stuck there. I thought there might be something wrong with the diet, but I have not found anything so far.

Finally, I would like to ask about galactosemia. I had a galactosemic child, who didn't do well at first, but when he settled down to the lactose

free diet he did fairly well. However, he persistently produced a pentose in his urine—xylulose. I wonder, can this be related to his galactosemic state?

Black. With regard to abdominal pain, this is certainly true. You get pain from fructose given orally, but I don't think you get much from giving fructose intravenously. With regard to the poor growth, the progress of my patients has been reasonably normal, but if you do not make a fairly early diagnosis there may be some liver damage which you cannot reverse. The answer to the question of diet inadequacy is to see if there is any fructose in the child's urine on the normal diet at home.

To the last question, I do not know.

Bickel (Marburg). We have never found xylulose in the urine in galactosemia. However, xylulosuria is not so extremely rare that these two errors may not occur together by chance.

Gitzelmann (Zurich). It is very important at what stage you examine the patient. For instance, some workers have found that there may be three different pools of galactitol in galactosemic patients. They found this by injecting radioactive labelled galactose and assaying for the biological half life. From their kinetic data they conclude that there must be two or possibly more pools of galactitol. It is quite possible that this is dependent on the state of nutrition and the state of galactose load these patients are in when you examine the urine.

Raine (Birmingham). An audience such as this provides a valuable opportunity to get collective experience on the importance of galactose in galactose containing trisaccharides of soya in treating galactosemics. The Manchester workers raised this question, and then we understood that Professor Prader found that in practice this did not matter. Dr. Fenton studied the patients in Newcastle by following galactose-1-phosphate levels in the blood, and his patients did not do at all well on a soya bean preparation. We have followed a patient in Birmingham using galactose-1-phosphate levels and he is thriving on a soya containing feed.

Holzel (Manchester). Dr. Gitzelmann has shown that there are galactosidases in the human which will split the galactose components in soya beans but that these are not in the intestinal mucosa. In my experience, whenever we have tried a number of children on soya bean preparations, at least half of them had diarrhoea, but we do not know what upset them.

Gitzelman (Zurich). There is no galactosidase. Fructosidase does not attack the molecule as shown by loading an adult with fructose intolerance with 40 gms. raffinose, when we couldn't demonstrate any drop in phosphate. Also in a 5 or 6 year old galactosemic we showed that there was no rise in galactose-1-phosphate if we substituted his diet for a long time with large amounts of the sugars in soya.

It is quite a different point with babies. If babies have diarrhoea the sugars are subjected to bacterial hydrolysis already in the small intestine

and then the baby can be damaged by the free galactose in the small intestine where it is rapidly absorbed. I think this actually happened to these babies, and this is the reason why some develop diarrhoea and do poorly on soya, all you have to do is to discontinue the soya diet for one day, give dextromaltose solution, and they will do well the next day on soya again. This is our experience.

In another experiment with two babies I gave alternately soya for 2 weeks, Moll's pudding for 2 more weeks and so on, and we found no change in the galactose-1-phosphate levels. As it is well known, and as Schwarz showed, there is also some galactose in Moll's pudding. In all proteins there are galactolipids and we do not know anything about their availability for absorption.

It is possible that the small intestine becomes settled with bacteria at a time when diarrhoea occurs and since the small intestine is capable of absorbing free galactose very rapidly, in contrast to the colon, this may constitute a danger to the child.

Bickel (Marburg). It is important to stress the diagnostic danger of only using glucose oxidase test papers. I know of at least two galactosemic children who have died because the house physician or technical assistant has thought that testing for sugar means using Test Tape or Clinistix. When the tests were negative they reported no sugar present and this was taken to mean the exclusion of galactosaemia. There is much to be said for getting a proper Benedict's test as a routine instead of using more specific test methods straight away.

I also wanted to ask if you had any experience with the chromatographic demonstration of sugar excretion in stools. One thinks of mellituria and of the importance of paper chromatography to discover the mellituria, and by analogy one would like to use paper chromatography for the detection of various kinds of faecal sugars. Unfortunately, the intestinal bacteria are an intervening factor so that one should work on sterile animals to see what the bacteria are really doing to the sugars in the lower intestine. Have you or Dr. Holzel used paper chromatography to any extent to discover the selective disturbances of sugar absorption?

Black. We have to some extent. We have also used a screening test at the same time and I think we have discovered where the screening test seemed to go wrong. The screening test consisted of examining the stool with clinitest. What we had done wrong was to use the solid part of the stool. When we came to test the loose stool in a urine bag we started to get positive results, but if you just scrape the stool off and suspend it you don't. I can't say anything about paper chromatography; we have done it, but I am not prepared to say anything at this stage.

Holzel (Manchester). We have done a great deal of stool chromatography, but I do not think it has been of great help because one can find a certain amount of sugar in perfectly healthy babies. It may be significant where you

have glucose, galactose and fructose in the faeces, but not even then does one know if this is due to bacterial hydrolysis or not. It really doesn't help very much.

Bickel (Marburg). This is not quite my experience. We have done some work on the mellitorrhoea of normal infants. You do find traces of various sugars in their stools, but these amounts are very small and in the few cases I have seen of monosaccharide and disaccharide malabsorption there is a much stronger increase of the sugars, so I think that the method is useful.

Holzel (Manchester). I am sorry. I didn't wish to give the impression that it is useless.

Woolf (Oxford). I would like to re-inforce the remarks about the dangers of the use of clinistix. There is a second danger. Galactosemic babies excrete an excess of glucose in their urine; if you get reduction with Benedict's and then do a clinistix you get a positive result and decide it is glucosuria, but it could be glucose plus galactose secondary to galactosemia. It is now possible to test for galactose by galactose oxidase. We have found several galactosurics in our routine screening, but not as yet any actual galactosemics.

GALACTOKINASE DEFICIENCY
A NEW INBORN ERROR OF METABOLISM

RICHARD GITZELMANN

The patient

GALACTOKINASE deficiency was not recognized in mammals until recently (Gitzelmann, 1965). The first recorded case is an adult male, now 43 years of age. He had juvenile cataracts and was operated on more than one occasion before the age of 9 years. At that time he was found to have diabetes and transferred from the Ophthalmology Department of the University of Zurich to the Paediatric Department. It was then recognized that he excreted sugar in his urine mainly after ingestion of milk. A chemical investigation proved that this sugar was galactose. This rare observation of a galactose diabetes was reported by Fanconi in 1933.

Today, the patient is blind as a result of recurring cataracts. His intelligence appears to be within the normal range. He drinks up to 2 litres of milk daily without discomfort. He has granted permission to collect blood and urine specimens at his home. After milk ingestion, he has hypergalactosaemia. He excretes large amounts of galactose, together with some glucose. His erythrocyte galactose-1-phosphate uridyltransferase is normal.

Galactokinase deficiency was suspected and diagnosis was established by incubating intact red blood cells with ^{14}C-labelled galactose (Robinson, 1963) and by subsequent separation of the reaction products by paper chromatography; no phosphorylated products from galactose were found. In addition, whole blood haemolysate from the patient formed only insignificant amounts of $^{14}CO_2$ when incubated with galactose-1-^{14}C (Weinberg, 1961).

Family history

Two sisters of the patient also had juvenile cataracts and were operated upon at a preschool age. One of the two sisters, now aged 64, was located. Her red cells also lacked galactokinase activity, and her urine also contained galactose. Moreover, all of her 4 living children and one grandchild had markedly reduced red cell galactokinase activity. One brother of the two patients and some of his children have low erythrocyte galactokinase activity. Haemolysates of a group of over 80 healthy persons served as controls. From the findings in this family one might conclude that galactokinase deficiency is inherited as an autosomal recessive trait.

Pathogenesis of cataracts

Attention was focused on the pathogenesis of the juvenile cataracts. Galactose cataracts are known to occur in untreated galactosaemic humans

as well as in young rats poisoned with galactose. The possible pathogenic role of galactose-1-phosphate accumulation is well recognized (Sidbury, 1961). Galactitol, another galactose intermediate, has been discovered in brains, plasma and urine of galactosaemic patients and in galactose-fed rats; it has also been demonstrated recently in the lens of a galactosaemic infant, post mortem (Gitzelmann, Curtius & Schneller, 1967). Thus, a number of urine specimens of the first patient were examined by gas chromatographic procedures and found to contain surprisingly high concentrations of galactitol (Gitzelmann, Curtius & Müller, 1966). It is at present unknown in which tissues this galactitol was produced. Accumulation of galactitol (formed in the lens by an aldose reductase; van Heyningen, 1959) is thought to possibly be the first step in the genesis of galactose cataracts (Kinoshita, 1963), and it is presumed to have caused the cataracts in these two galactokinase deficient patients.

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DISCUSSION OF PRESENTATION BY DR. GITZELMANN

Holzel (Manchester). Did you look for galactitol in the brain of the baby you investigated?

Gitzelmann. No.

Woolf (Oxford). Is there any explanation for the glucose that the patient excreted (the one with the galactokinase deficiency)? Was there any other evidence of renal tubular dysfunction, such as aminoaciduria?

Gitzelmann. We know that glucose and galactose compete for reabsorption and this may be reason for it. We also know that the proximal tubules are damaged in the galactosemic so there may also be a pathological mechanism.

Holzel (Manchester). I didn't understand why galactitol is excreted in large amounts in the urine.

Gitzelmann. I don't know either. I don't know where it is produced. I have an idea that it could be in the kidney, but I can't prove it.

Woolf (Oxford). Have you examined this patient's urine for amino acids?

Gitzelmann. Yes, with normal results.

Bickel (Marburg). One fascinating aspect of your case with the galactokinase block, is that it may help us to see a little more clearly what really damages the brain and the lens in galactosaemia. If your case has no brain damage then it can't be galactose. It was suggested a long time ago that the damaging agent may be galactose-1-phosphate.

HISTOCHEMISTRY OF THE INTRINSIC NERVES OF THE RECTUM AND COLON

ETHEL FINCH, JOHN L. EMERY and JAMES LISTER

Introduction

THE enzymes of the large intestine which are the subject of my paper differ from those discussed by previous speakers in not being associated with a metabolic disease, but with the metabolism of the neurohormones or chemical transmitters of the autonomic nervous system. The autonomic nerves are defined as the motor nerves of the sympathetic and parasympathetic systems. The chemical transmitters released by stimulation of these nerves are noradrenaline and acetylcholine, and the relevant enzymes concerned with their metabolism, monoamine oxidase and cholinesterases.

Our primary aim was to investigate the distribution of these enzymes in the spastic or functionally abnormal zone of the colon in patients with Hirschsprung's disease, or congenital megacolon.

In this disease there is a history of constipation from birth with delayed passage of meconium and consequent signs of acute intestinal obstruction.

Rectosigmoidectomy is the recognised treatment for such patients, but this is not without risk to the neonate. A preventative measure would be a preferable course, but, before this becomes a possibility, a better understanding of functional relationship between smooth muscle and innervation of the colon and rectum is required. Further study suggested investigations into the origin and development of the intrinsic nerve plexuses of the intestinal tract and its neural regulation.

Innervation

The relationship between the intrinsic innervation and extrinsic innervation to the colon is not fully understood, but it is generally accepted that the extrinsic nerves are postganglionic sympathetic nerves from the inferior mesenteric ganglion, and preganglionic fibres from the vagus and sacral parasympathetic nerves. The sympathetic nerves enter the intestinal wall in company with branches of the large blood vessels and are thought to run directly to innervate blood vessels and smooth muscle cells in the walls of the colon. The vagus is thought to innervate the colon as far as the middle of the transverse colon, whilst the distal end is innervated by nerve fibres from the sacral division of the parasympathetics.

The intrinsic nerves consist of nerve cells in groups or ganglions connected by non-myelinated fibres of both intrinsic and extrinsic origin, and

* This research was supported by a grant from the Endowment Fund of the United Sheffield Hospitals.

forming plexuses. Fibres from the ganglion cells pass to the involuntary muscle layers of the gut wall.

Auerbach's plexus, between the longitudinal and circular muscle, is the most prominent plexus, and with Meissner's plexus in the submucosa and Henle's in the inner circular muscle, constitute the three main plexuses. Another plexus, or structural element in the innervation of the gut is the rich network of interstitial cells first described by Cajal in 1892. This consists of small, spindle-shaped cells most heavily concentrated in the areas round the ganglion cells of the intrinsic plexuses and the innermost region of the circular muscle. Their ramifying beaded processes contact each other and run between cells of adjacent muscle fibres.

It is not known whether they are a primitive neural network or connective tissue. It has been suggested that their function may include the spread of impulses from postganglionic autonomic fibres to muscle fibres by liberation of chemical transmitters. They may initiate myogenic contractions, or integrate them.

In the light of more recent knowledge, they may well be part of the adrenergic nervous system.

Chemical transmitters

The discovery of the chemical transmitters acetylcholine and noradrenaline has led to the assumption that the effects of the parasympathetic system are brought about by the release of acetylcholine, and that of the sympathetic system by noradrenaline. In view of the direct effect of these two substances on movement of intestinal muscle, it seems probable that the fibres having an excitatory effect are cholinergic and those having an inhibitory one, are adrenergic.

Since the fugitive action of acetylcholine was attributed to its destruction by enzymes, the cholinesterases, much work has been done on their distribution throughout the animal species. It has been shown that in the animal body, there are at least two types of cholinesterase which differ in their substrate specificity and response to inhibitors. Alles & Hawes (1940) compared the specificity of cholinesterases of human erythrocytes and serum on the hydrolysis of acetylcholine and related choline esters.

They found that one type is highly specific for acetylcholine and closely related cholinesters; the other is capable of hydrolysing both choline and aliphatic esters. They came to be known as 'true' or specific cholinesterases occurring in erythrocytes, and pseudo, or non-specific cholinesterases occurring in serum. This substrate specificity has become the means of identifying and studying their distribution in animal tissue.

The earlier work on distribution of these enzymes was based on chemical and pharmacological studies using acetyl beta methyl choline and benzoyl choline as substrates. In 1949, a histochemical approach was made to the problem by Koelle & Friedenwald. After investigating numerous substrates

they found that acetylthiocholine iodide and butyryl-thiocholine iodide were the substrates most suitable for the localisation of these enzymes in tissue sections, as a visible reaction product was produced at the site of enzyme activity.

The thiocholine method depends upon the enzymic hydrolysis of the carboxylic acid esters of thiocholine sulphate in the presence of a cupric ion complex, followed by the precipitation of the liberated thiocholine sulphate as a copper mercaptide. The site of deposition of this copper thiocholine was made more conspicuous by treatment with dilute ammonium sulphide and precipitation as brown copper sulphide (Holmstedt, 1957).

In the absence of a specific substrate for true cholinesterase, the method is made specific for this enzyme by using acetylthiocholine iodide as substrate in the presence of an inhibitor for the pseudoenzyme which can also hydrolyse the acetyl derivative. Butyrylthiocholine appears to be a specific substrate for the pseudo enzyme.

By the use of these substrates, Koelle (1950, 1951) studied the distribution of cholinesterases in the tissues of the cat and rabbit. His results have been used as a basis for comparison of similar studies in human tissue.

Of interest to our human studies were the findings in the ileum of the cat of true and pseudocholinesterase activity in the ganglionic plexuses. The true or specific cholinesterase appeared in nerve fibres and ganglion cells only, whilst the pseudo or non-specific was present in and around the ganglion cells of Auerbach's & Meissner's plexuses and in association with the muscle fibres of the longitudinal muscle, muscularis mucosae, and to a less extent in the circular muscle where it was in highest concentration in the inner layer. It was also present in the controversial interstitial cell plexuses of the myenteric and submucous plexuses.

The association of adrenalin with the sympathetic nervous system was known many years before that of acetylcholine with the parasympathetic nerves. However, it is only comparatively recently that the synthesis and metabolism of adrenaline and associated catecholamines has been worked out although many gaps in our knowledge still exist. It is known that adrenaline and noradrenaline are synthesised in the adrenal medulla where they are stored in the chromaffin granules until liberated.

During the last 10 or 12 years, the major metabolites of catecholamines have been identified and their metabolic pathway elucidated. With appropriate stimulation, adrenaline and noradrenaline are discharged into the blood stream mainly from the adrenal medulla. They act as hormones at distant organs where they are taken up by the tissues at or near sympathetic nerve endings. Here, some of the noradrenaline is stored as granules until released again. Experiment has shown that there exist in the animal body, two bound pools of noradrenaline, one which is easily released, the other more firmly bound. The former can be released by sympathomimetic

amines such as tyramine, whilst the latter requires nerve stimulation to release it. It has been suggested that the easily released noradrenaline is metabolised by orthomethylation and the more tightly bound by deamination by monoamine oxidase (MAO) in the nerve fibre.

The exact pathway by which amines are oxidised in plant and animal tissues is not fully understood, but an aldehyde is thought to be the first reaction product. This formed the basis for the earlier histochemical localisation of monoamine oxidase by the production of a coloured compound using Schiff's reagents and tyramine as substrate.

In 1957, Glenner, Burtner & Brown published a method using tryptamine as a substrate and an easily reduceable tetrazolium salt as hydrogen acceptor. The enzyme is located by the precipitation of blue diformazan pigment at the sites of activity. It appears that the indole carbonyl compound resulting from the reaction of MAO on tryptamine is necessary for the dye reduction.

$$\text{Tryptamine} \xrightarrow{\text{MAO}} \text{indoxyl 3 acetic acid} \xrightarrow{\text{oxid}^{n}}$$

$$\text{tetrazole} \xrightarrow{H_2} \text{diformazan.}$$

Using the tetrazolium salt technique Glenner *et al.*, showed that sites of monoamine oxidase occurred in autonomic ganglia and fibres, mucosal epithelium of the duodenum and in certain zones of the renal tubules.

Koelle & Valk (1954) found MAO in varying concentrations in all types of peripheral nerves and in moderately high concentration in the smooth muscle of arteries and the muscularis mucosae. It was virtually absent from the external muscle of the ileum, but present in ganglion cells in varying concentrations. This ubiquitous distribution in all nerves was thought to suggest its participation in amine metabolism common to all types of nerves. Sections of spinal cord and many other sites containing thick trunks of myelinated nerves showed an intense reaction of MAO. This is interesting in the light of more recent work by Tewari & Bourne (1963) on monoamine oxidase distribution in the cerebellum of the rat using the tetrazolium technique. They showed that, in the white matter, both afferent and efferent fibres possess intense MAO activity associated with the myelin sheath. It appears that the neurokeratin network of the myelin sheath is a centre of great metabolic activity, non-specific cholinesterase being one of the enzymes active there. It was found that the true cholinesterase is located in the axon of nerve fibres.

This lends support to the theory that whilst true or specific cholinesterase is undoubtedly concerned in neural transmission, non-specific cholinesterase is associated with metabolism of myelin or other phospholipids and may affect the permeability of lipid membranes to electrolytes also concerned in nerve transmission.

Previous studies

Prior to the early 1950's, observations on the nerve plexuses in the colon in congenital megacolon were made on routine histological staining. Following the development of histochemical enzyme techniques the first paper on the histochemical localisation of cholinesterases in congenital megacolon was published in 1953 by Kamiyo, Hiatt & Koelle. They also studied the effects of acetylcholine and anticholinesterases on the motility of smooth muscle fibres in this disease compared with a control.

They confirmed the absence of ganglion cells in Auerbach's plexus in the affected zone and the presence in their place of large bundles of non-myelinated nerve fibres with strong, true cholinesterase activity. These they classified as cholinergic and postganglionic. From this, they postulated an alternative theory to the 'agenesis' theory of Bodian & Carter (1951), and suggested that ganglion cells were present, but in a situation outside the colon, whilst their postganglionic fibres extended to their normal terminations.

In 1960, a second paper, by Adams, Marples & Trounce studied the amount and distribution of cholinesterases in achalasia of the cardia and Hirschsprung's disease. In the former condition, there is histological evidence that the ganglion cells have disappeared in the dilated oesophagus which showed no nerve fibres and no cholinesterase activity. This portion of the oesophagus was very sensitive to acetylcholine, supporting Cannon's law of the hypersensitivity of denervated structures (Cannon & Rosenblueth, 1949). The aganglionic segment of the colon was not abnormally sensitive to acetylcholine.

Histochemistry once again revealed normal or increased cholinesterase activity associated with the large nerve fibres replacing the ganglion cells in the spastic colon. The differences in sensitivity between the dilated aganglionic oesophagus and the contracted aganglionic colon to acetylcholine was due to the absence of cholinesterase in the abnormal oesophagus and its presence in the abnormal colon.

A third histochemical study of eleven cases of congenital megacolon by Niemi, Kouvalainen & Hjelt (1961), using both location of cholinesterase and monoamine oxidase amongst other enzymes, again confirmed the absence of ganglion cells by routine stains and monoamine oxidase in the spastic area, but detected small ganglionic areas by cholinesterase activity.

From this, they suggested that enzymic activity might be a more useful tool than routine stains for locating intrinsic ganglion cells.

The conflicting evidence for presence or absence of ganglion cells, pre- or postganglionic, sympathetic or parasympathetic nerve fibres and their origin, led us to undertake a study into the development in intramural nerve plexuses of the intestinal tract and the enzymes regulating neural control in both congenital megacolon and control subjects.

Table I. *Summary of the hypotheses put forward by previous workers as to the developmental abnormality of the affected zone in congenital megacolor.*

Absence of ganglion cells caused by:—
1. Agenesis of ganglion cells.
2. Ganglion cells situated outside the colon, possibly more centrally.
3. Degeneration of cells originally present.

Presence of bundles of non-myelinated fibres may be:—
1. Postganglionic sympathetic fibres.
2. Postganglionic parasympathetic fibres from more centrally situated intrinsic ganglia.
3. Pre-ganglionic parasympathetic fibres seeking 'lost' ganglion cells.

Present study—Methods

During the last two years we have carried out histochemical studies on the distribution of cholinesterases in the colons of 29 patients with congenital megacolon and a control group of children with no known lesion of neural elements. The ages ranged from one day to adult life. From the majority of patients with congenital megacolon, we received specimens of both 'spastic' and 'hypertrophic' areas. In a few we received either the 'normal' or 'abnormal' tissue.

Table II. *Age range and number of specimens studied.*

Age	*Congenital megacolon*	*Controls*
1st week	6	8
2nd week	2	3
2-4 weeks	2	2
4-12 weeks	2	3
3-6 months	0	4
6-12 months	7	2
12-24 months	11	3
2-11 years	5	9
Adult	2	—
Total	37	34

The majority of control specimens were obtained from postmortem material removed within 12 hours of death, but best results were obtained from biopsy material. Cholinesterases, however, seem remarkably stable even after death.

The usual procedure with all specimens was to pin them out as flat as possible on cork with the muscle layers upwards. Slight stretching was usual before pinning down. The tissue was then frozen in liquid nitrogen and stored in plastic containers in the deep freeze —20° C. For enzyme studies, blocks of tissue were taken from the abnormal and normal colon in

Table III. *Solutions for Cholinesterases.*

Gomori Stock Media for Cholinesterase	
Copper Sulphate $\cdot 5H_2O$	0·3 g.
Glycine	0·375 g.
Magnesium Chloride $\cdot 6H_2O$	1·0 g.
Maleic Acid	1·75 g.
N. Caustic Soda	30 ml.
40% Anhydrous Sodium Sulphate	170 ml.
Koelle Substrates	
Acetylthiocholine Iodide = AcThCh	
Butyrylthiocholine Iodide = BuThCh	
Pearse Inhibitors	
1·5 BIS-4-Trimethylammonium-Phenyl Pentan-3-One. = 62 C 47 10^{-5}M	
Tetraiso-Propyl Phosphoramide = Iso OMPA 10^{-6}M.	

cases of megacolon and from approximately 2, 6 and 12 cm. from the anus from the control patients. Longitudinal and transverse sections were cut at 10μ with a freezing microtome (cryostat) at —20° C. and dried on coverslips in the air. Sections were then transferred to the respective incubation media.

Other sections were stained with haematoxylin-eosin, Unna-Pappenheim for ribonucleic acid and Holmes' silver stain for ganglion cells and nerve fibres. For cholinesterases, acetylthiocholine iodide and butryl-thiocholine iodide were used for location of true and pseudo cholinesterases respectively (Table III). As the acetylthiocholine is hydrolysed by both enzymes, a specific inhibitor for the pseudo enzyme was incorporated into the substrate to make it specific for the true cholinesterase (Table IV).

Table IV. *Enzyme localisation scheme for cholinesterases.*

Substrate	*Inhibitor*	*Enzyme*
AcThCh	None	Total AChE
AcThCh	Iso OMPA	True ChE
AcThCh	Iso OMPA + 62 C 47	Negative
BuThCh	None	NS ChE
BuThCh	Iso OMPA	Negative

Hydrolysis of the substrates releases the thioanalogues of acetyl and butyrylcholine. They react with copper sulphate in the media to produce mercaptides which are precipitated at the site of enzyme activity. After washing well, immersion of the sections in dilute ammonium sulphide replaces the mercaptide by a dark brown precipitate of copper sulphide.

Cholinesterase + substrate ⟶ Thiocholine derivative ⟶
⟶ Copper thiocholine sulphate + Ammonium sulphide ⟶
⟶ Copper sulphide.

Diffusion of the enzymes is prevented by the presence of approximately 28 per cent sodium sulphate in the substrate and working at an acid pH about 6·0. Counterstaining of the sections with 1 per cent light green makes a better contrast of enzyme with background tissue.

The method of Glenner, Burtner & Brown with some modifications, was used for monoamine oxidase localisation (Table V). Incorporation of 20

Table V. *Tetrazolium method for monoamine oxidase.*

Incubation Medium	
Tryptamine Hydrochloride	25 mg./ml.
Nitro-Blue Tetrazolium	5 mg./ml.
0·2 M Phosphate Buffer pH 7·6	Equal parts
40% Anhydrous Sodium Sulphate	
Pre-Incubation Solution	
Equal parts—0 2 M Phosphate Buffer 40% Sodium Sulphate	
Inhibitor	
Iso-Nicotinyl-2-Isopropyl Hydrazine = Marsilid 10^{2}–M.	

per cent sodium sulphate and a more dilute solution of the tetrazolium salt made the reaction more reliable and prevented diffusion. Incubation for at least six hours, or overnight, gave a more definite location of enzyme distribution.

Present study—Results

All staining and enzyme techniques showed ganglionated intrinsic plexuses in all the sections of both control and proximal or 'normal' zones of the colon in congenital megacolon in all age groups. In the rectal areas from both groups nerve fibres were often present in the more distal sections. Nerve fibres were very rarely seen in sections taken more than 1 cm. proximal from the internal anal sphincter. Sections containing some striated muscle of the sphincter usually contained nerve fibres.

True and specific cholinesterase occurred in all ganglion cells of all plexuses and in occasional small nerve fibres in association with them or running into the muscle layers. Non-specific cholinesterase occurred most strongly in association with ganglia of the intramural plexuses. A diffuse reaction was present in the longitudinal muscle, inner circular muscle and muscularis mucosae. A weaker reaction occurred in the rest of the circular muscle (Figs. 1, 3 and 5).

In the older age group, i.e. over one month, the distal spastic zone of colon appeared lacking in ganglion cells in all plexuses, their place was taken by bundles of nerve fibres which ramified through the muscle layers in an indiscriminate manner (Figs. 2, 4 and 6). These fibres were strongly positive for acetylcholinesterase (Fig. 2) and weak or negative for butyryl-cholinesterase (Fig. 4). The longitudinal muscle showed reactions varying

from very strong in places to weak or negative in others. In most sections, the muscularis mucosae was also strongly positive whilst the inner circular muscle was usually negative for the pseudoenzyme.

The young age group of congenital megacolon from birth to 4 weeks showed a rather different picture from that of the older children (Figs. 7 and 8). Although in most cases some nerve fibres were present in the plexus areas and were strongly positive for acetylcholinesterase, nearly every patient showed some butyrylcholinesterase of normal intensity in small scattered zones in Auerbach's plexus and/or in the longitudinal muscle layer. This presence of the pseudoenzyme in the plexus area suggests the presence of ganglion cells. Koelle (1955) claimed that the presence of butyrylcholinesterase was a unique feature of the ganglionic plexuses of the intestinal tract, especially Auerbach's plexus.

The distribution of monoamine oxidase appears very widespread (Figs. 5 and 6). In the tissue from the control patients and the 'normal' zone of congenital megacolon there appears to be a fine network of activity in a regular pattern throughout all muscle layers and a more diffuse network in the submucosa.

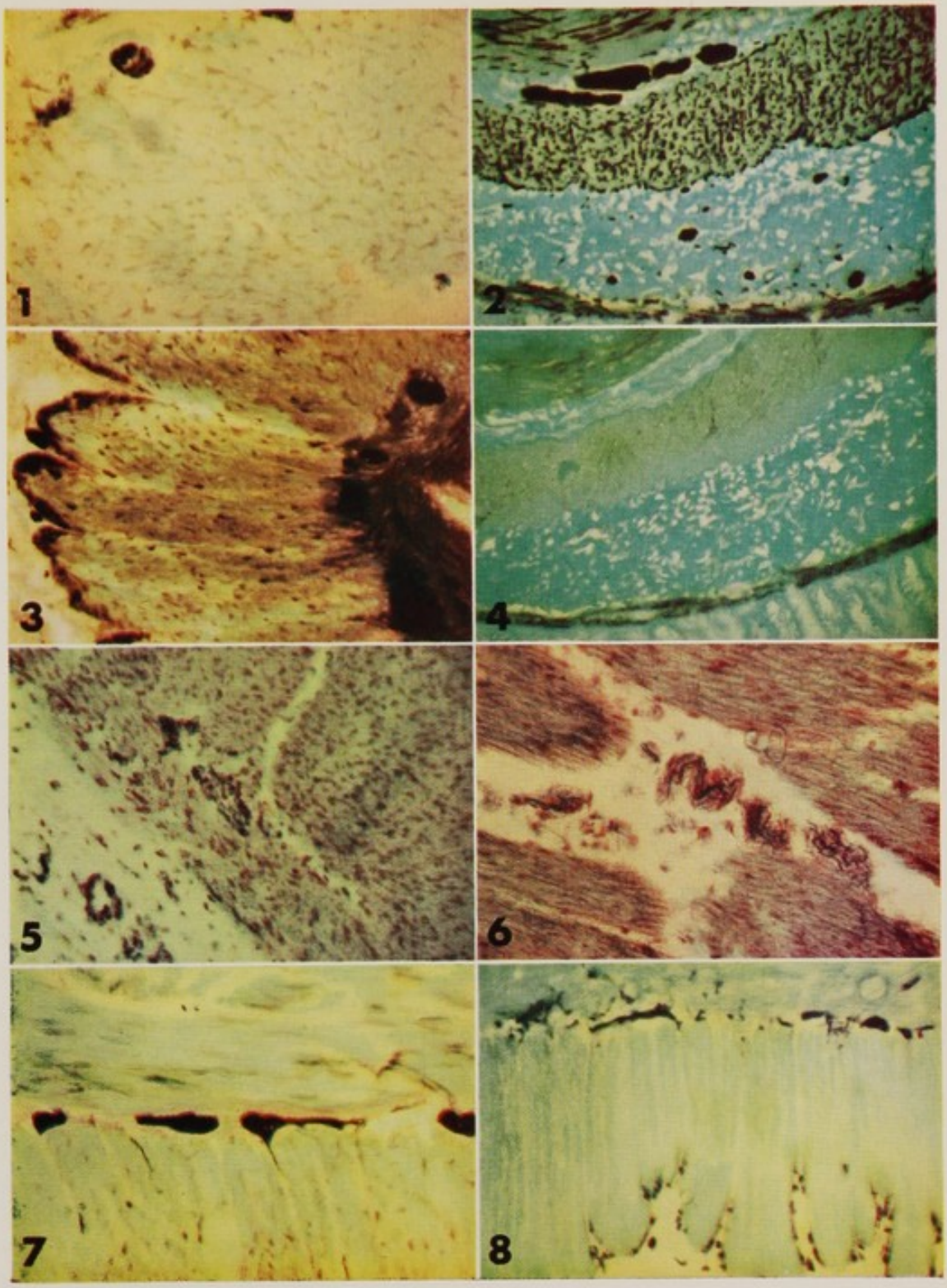
Ganglion cells are well defined by coarse granular staining throughout the cytoplasm and in the adjacent tissue of the plexuses.

In many control sections a strongly positive zone of monoamine oxidase activity occurred in the inner circular muscle, the distribution resembling that of butyrylcholinesterase activity.

The spastic distal colon of older megacolon patients also showed a diffuse network of activity, but not in a regular pattern. In the muscle layers there occurred patchy larger areas of activity and occasional positive nerve fibres. In the larger fibres of Auerbach's plexus there was often a characteristic pattern of very strong activity suggesting myelinated areas which resemble those of the extrinsic nerves.

Following the finding of this widespread distribution of MAO, it is of interest to note that recent work on intestinal adrenergic innervation (Norberg, 1964) using the fluorescent histochemical technique already mentioned, has shown that, apart from the vasomotor fibres most adrenergic nerves terminate in the enteric plexuses as a fine network of thin, highly fluorescent fibres. They are arranged in a 'basket-like' shape around the ganglion cells which are themselves non-fluorescent. Similar fine fibres were seen in the submucous plexus, whilst bundles of fluorescent and non-fluorescent axons were found in the submucous and subserous layers. Fluorescent fibres were scarce in the muscle layers, except in the colon where an occasional fibre appeared to terminate on smooth muscle cells. This is in contradiction to the classical view (Hill, 1927) of direct sympathetic innervation of smooth muscle which is based on morphological data.

In the light of this new knowledge of adrenergic nerves terminating



Figs. 1, 3, and 5 are taken from normal controls aged respectively, 3 yrs., 3 yrs. and 10 months.

Figs. 2, 4 and 6 are taken from the spastic segment in cases of congenital megacolon aged respectively 9 months, 9 months and 10 months

Figs. 7 and 8 are taken from different portions of the colon in a 4 week old child with congenital megacolon.

Figs. 1 and 2, stain, acetylcholinesterase. Increased activity in all areas of the spastic sement. Figs. 3 and 4, stain, butyrylcholinesterase. Decreased activity in Auerbach's plexus, increased activity in muscularis mucosae in spastic segment. Figs. 5 and 6, stain, monoamine oxidase. Strong activity in muscle layers and in nerve fibres in Auerbach's plexus in spastic segment. Figs. 7 and 8, stain, acetylcholinesterase. Activity in nerve fibres and small ganglion cells in Auerbach's plexus and inner circular muscle in spastic segment.

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around the parasympathetic enteric ganglia, it seems that their inhibitory effect on muscle is exerted by an indirect effect on the enteric neurones. It is suggested that inhibition of the colon involves three neurones in a chain; one preganglionic and cholinergic with its cell body in the spinal cord and terminating in a prevertebral ganglion; one postganglionic and adrenergic with its cell body in a prevertebral ganglion and terminating in the intramural ganglia of the gastro-intestinal tract. The final link is the postganglionic parasympathetic neurone in the intestinal wall.

The ganglion cells of the intramural plexuses appear to be links in both adrenergic and cholinergic innervation of the smooth muscle of the intestines.

Discussion

The use of enzyme histochemistry for localisation of ganglionic plexuses of the intestinal tract gives a more definite picture than that of routine histological stains, especially in the very young infant.

In all the young patients with congenital megacolon, the activity of both acetyl and butyrylcholinesterases signified the presence of small ganglionic plexuses.

The older patients appeared devoid of ganglion cells in the spastic zone. Their place was taken by numerous bundles of nerve fibres which ran into the muscle layers in an apparently haphazard manner. More fibres were visible in the tissues from the older children.

There is a possibility that absence of ganglion cells in the plexus with which some of the extrinsic nerves make contact may simulate the sectioning through of a nerve with consequent proliferation of nerve fibres from the proximal end. Sawyer (1946) sectioned guinea pig sciatic nerve with the result that numerous non-myelinated fibres grew from the proximal severed end. These fibres had three to four times the true cholinesterase activity as that of the control non-severed nerve.

In many instances it was possible to identify the nerves in the plexus areas as continuations of the extrinsic nerves in the mesentery. They all had intense true cholinesterase activity with weak or absent pseudoenzyme activity. The presence of 'myelinated' areas with strong monoamine oxidase activity suggested that some of the nerves were adrenergic. That the adrenergic and cholinergic nerves were often the same nerve could be seen from reactions on serial sections.

It has been known for some time that, although acetylcholine is regarded as the transmitter of parasympathetic nerves, stimulation of many sympathetic nerves liberates acetylcholine as well as noradrenaline.

In 1959, Burn & Rand put forward a new hypothesis to explain this phenomenon. They suggested that noradrenaline is not directly released by a nerve impulse, but acetylcholine is first liberated. If the nerve impulse is weak and infrequent, only a little acetylcholine is released which is

rapidly destroyed by cholinesterases. On the other hand, if the impulse is strong and frequent enough acetylcholine is liberated to release noradrenaline before cholinesterases stop the reaction. This hypothesis explains many anomalies of nerve stimulation, but has still to be generally accepted.

In conclusion, it appears that enzyme histochemistry for cholinesterases together with fluorimetric localisation of catecholamines should shed more light on the balance of the two types of innervation in animal tissues. We still need more knowledge of development of intrinsic plexuses and stimulation of extrinsic nerve growth to elucidate the abnormality of the spastic colon in congenital megacolon.

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Discussion

The editors regret that the recording of the discussion was technically unsatisfactory.

GARGOYLISM: BIOCHEMICAL ASPECTS

J. R. BAKER

Urinary excretion of acidic glycosaminoglycans

AMONG the known mucopolysaccharidoses it is only in gargoylism that the urinary excretion of acidic glycosaminoglycans is much in excess of the normal level (10-14 times the normal level according to Berry, 1965). Gargoylism includes that group of hereditary mucopolysaccharidoses characterised by excessive urinary excretion of dermatan sulphate and/or heparan sulphate. In this group are Hurler's, Hunter's, Sanfilippo's and Scheie's syndromes.

Since Dorfman & Lorincz (1957) demonstrated the presence of excessive amounts of acidic glycosaminoglycans in the urine of gargoylism patients, methods of detection of urinary acidic glycosaminoglycans have been favoured as diagnostic aids. Dorfman (1958) introduced a method whereby urinary acidic glycosaminoglycans produce a white turbidity upon addition of bovine serum albumin at an acid pH. Although it is reported that false positive tests have occurred only rarely, the method lacks specificity.

A well-known and analytically useful property of acidic glycosaminoglycans is their precipitability by cationic detergents—the one most used is cetyl pyridinium chloride. Manley & Hawksworth (1966) have given details of a screening method for Hurler's syndrome based upon this property. Under their conditions, normal urines give a turbidity with an optical density of $<0{\cdot}5$, whereas urine from Hurler's patients show values between $0{\cdot}62$ and $0{\cdot}85$. Although from results so far obtained no overlap occurs between normal and pathologic values, this assay does not reflect their gross difference in urinary acidic glycosaminoglycan levels.

Compared with the methods quoted, paper spot tests tend to be simpler and quicker. Berry (1959) introduced one such method which depends upon the staining of urinary acidic glycosaminoglycans by toluidine blue. With this reagent, a polymer bearing periodic anionic groups (e.g. acidic glycosaminoglycan, nucleic acid) will give a metachromatic reaction. Levels of acidic glycosaminoglycans in the urine of gargoylism patients are sufficient to give a positive result, but normal urinary levels do not. Alcian blue has been used likewise. It does not give a metachromatic reaction, but is simply bound, so that, on paper, the presence of acidic glycosaminoglycan is indicated by retention of blue dye on an otherwise white to pale blue background. The use of Alcian blue makes possible the differential staining of acidic glycosaminoglycans, although not as usually employed in acetic acid. Alcian blue bears only one type of cationic group and its staining of various polyanions may be abolished by particular

Table I. *Critical Salt Concentrations for staining of acidic glycosaminoglycans with Alcian blue.*

	$MgCl_2$
Hyaluronic acid	0·1
DNA	0·2
Heparan sulphate	0·3
Chondroitin sulphate	0·5
Heparin	0·6
Keratan sulphate	0·8

Staining of 2-3 μl. spots of each polyanion on Whatman No. 50 paper was carried out as described by Scott, Dorling & Quintarelli (1964).

concentrations of inorganic salts (e.g. $MgCl_2$) in the staining medium. Scott & Dorling (1965) have developed this technique of differential staining of acidic glycosaminoglycans for histochemical purposes. Table I lists the minimum concentrations of $MgCl_2$ required to abolish the Alcian blue staining of the known acidic glycosaminoglycans. To use Alcian blue in 0·2M $MgCl_2$ would appear to offer the advantage of abolishing possible staining by small amounts of soluble nucleic acid and sialic acid-containing mucins which may be present in the urine, whilst retaining the staining of heparan sulphate and the chondroitin sulphates. In practice, another advantage of including $MgCl_2$ became apparent. Background colour was cleared more rapidly and completely. The use of No. 50 rather than No. 1 paper is not important. No. 50 simply has greater wet stregth and finer texture. A possible disadvantage is loss of staining by hyaluronic acid.

Twenty-four normal adult urines screened in this fashion were completely negative. The urines of six hospitalised children similarly examined showed one with slight retention of stain, which would not be confused with the staining intensity of the urines of established gargoylism patients of which five were available for testing. The method looks useful for screening purposes, although many more normal urines, particularly from children, must be checked.

An interesting possibility is the recognition of Sanfilippo's syndrome by a simple paper spot test. Clearly, urine from such a patient, which would contain only heparan sulphate, should show +ve staining with Alcian blue in 0·2M $MgCl_2$ but not in 0·4M $MgCl_2$. This possibility has not been checked owing to the lack of a suitable patient.

Methods for quantitatively estimating urinary acidic glycosaminoglycans are generally unsatisfactory. Yet such methods are important for the recognition of possible correlations with severity of symptoms, nutritional factors and in the future to monitor the effects of potential therapeutic agents. One method (DiFerrante & Rich, 1956) depends upon the direct addition of cetyl trimethyl ammonium bromide to the urine, and deter-

mination of acidic glycosaminoglycans via the uronic acid content of the precipitated complex. As the solubilities of chondroitin sulphate complexes are thought to be in the region of 1 mg./100 ml. (Scott, 1958) underestimates of urinary acidic glycosaminoglycans would be expected by this method. Particularly if normal urinary levels are to be determined, some concentration of the urine is essential prior to precipitation with cetyl pyridinium chloride. Then, desalting also becomes necessary. Commonly, dialysis has been employed for desalting, although the molecular size of some urinary acidic glycosaminoglycans is sufficiently small to allow some leakage. Sephadex gel filtration provides a useful alternative.

A satisfactory procedure for quantitative estimation is outlined in Table II. The initial treatment of the urine with NaOH is aimed at ensuring the release of acidic glycosaminoglycans from insoluble material in the urine. Acidic glycosaminoglycan-protein bonds which are known to be alkali-labile, will be disrupted by this treatment. Sephadex G25, which excludes macromolecules of molecular weight $>5{,}000$, in a column ($50 \times 2{\cdot}5$ cm.) completely desalts 100 ml. of urine (Table III). The 'macromolecular peak' appears in an eluant volume of 100-220 ml. More than

Table II

DESALTING AND CONCENTRATION OF URINE USING SEPHADEX G25 PRIOR TO ASSAY OF ACIDIC GLYCOSAMINOGLYCANS AS URONIC ACID

1. 100 ml. OF URINE + 5 ml. OF 10M NaCH. LEFT AT 4° OVERNIGHT. CENTRIFUGED (5,000 x g FOR 15 MINS).
2. 100 ml. OF SUPERNATANT ON COLUMN (50 x 2.5 cm.) OF SEPHADEX G25. 'MACROMOLECULAR' FRACTION COLLECTED AND LYOPHILISED. TAKEN UP IN 10 ml. OF H_2O.
3. A SUITABLE ALIQUOT WAS TAKEN FOR URONIC ACID ASSAY.

	mg. URONIC ACID /L OF URINE
NORMAL	6.3 (AVERAGE OF 18 DETERMINATIONS)
KNOWN HURLER'S SYNDROME	76.8
SUSPECTED HURLER'S SYNDROME	17.2
MARFAN'S SYNDROME	14.4

Table III

	ELUANT VOLUME ml.
'MACROMOLECULAR PEAK'	100 - 220
PIGMENTS (FOLLOWED BY, BUT OVERLAPPING WITH NaOH AND OTHER SMALL IONS)	230 -

230 ml. of eluant water are required before pigments, overlapping with NaOH and other small ions, appear.

The concentrated 'macromolecular peak' is then in a suitable form both for quantitative and further qualitative examination. To illustrate the method, a few urinary acidic glycosaminoglycan levels have been estimated as uronic acid (Gregory, 1960) (Table II). In a more comprehensive study it would be useful to determine the effect of age on normal values, and presumably the spread of values would be reduced by taking samples from 24 hour urines. Large amounts of other sugars interfere in the carbazole estimation of uronic acid; so in some cases CPC precipitation prior to estimation may be necessary.

For qualitative examination of urinary acidic glycosaminoglycans, I have used a thin layer chromatographic method (Wusteman, Lloyd & Dodgson, 1966). It allows separation of all seven well authenticated acidic glycosaminoglycans, is rapid, and only very small amounts of samples are required. Thus, the urinary acidic glycosaminoglycans of two typical Hurler's syndrome patients were shown to be predominantly dermatan sulphate with some heparan sulphate. The urinary acidic glycosaminoglycans from three brothers with a mild form of gargoylism was dermatan sulphate alone. This finding taken in conjunction with other symptoms described by Steen (1959), establish their condition as the Scheie syndrome.

The metabolic defect in gargoylism

A constant feature is the storage and excretion of unusually large quantities of dermatan sulphate and/or heparan sulphate. Therefore it is reasonable to postulate that a defect in some enzymic reaction leading to the overproduction or underdegradation of these two acidic glycosaminoglycans is responsible for the condition. Even this very general statement leaves one important question unanswered. How can one altered enzyme, produced by one defective gene, cause a disturbance in the metabolism of two molecules which are chemically quite distinct?

The study of human metabolic disorders often has to await the description of satisfactory assay methods applicable to blood and urine specimens. Two enzymes involved in acidic glycosaminoglycan metabolism, serum acidic glycosaminoglycan sulphotransferase and serum hyaluronidase, are assayable. Adams (1964) has recently published a method for assaying the former; so the possibility that Hurler's patients possess an inactive dermatan sulphate sulphotransferase was investigated. Details of the assay conditions employed and the results obtained are given in Table IV. The radioactivity reported as incorporated into dermatan sulphate from PAP^{35}S was shown to be inseparable from dermatan sulphate upon electrophoresis. Incorporation of ^{35}S into dermatan sulphate catalysed by both a control serum and the Hurler's serum was very low, and certainly there is no indication of over-incorporation in the latter case.

Table IV

THE ENZYMIC TRANSFER OF SULPHATE FROM PAP^{35}S TO DERMATAN SULPHATE BY A SERUM SULPHOTRANSFERASE

INCUBATION MIXTURE (TOTAL VOLUME, 0.15ml.) CONTAINS	0.10M PHOSPHATE BUFFER (pH7.0) 0.09M NaF 0.05ml. SERUM 80μg. DERMATAN SULPHATE 100,000cpm PAP^{35}S
INCUBATION TIME 1.5 hr.	
SERUM FROM:	CPM INCORPORATED INTO DERMATAN SULPHATE
NORMAL	265
HURLER'S SYNDROME PATIENT	237

The substrate specificity of serum hyaluronidase has not been described, but if it is an enzyme which has leaked into the circulation from a number of tissue sites, activity against dermatan sulphate might be expected to be present. Using the sensitive toluidine blue assay method of Bowness (1965) no enzyme activity could be demonstrated even in normal serum with dermatan sulphate as substrate. As reported by Bowness, the serum enzyme was active in degrading chondroitin sulphate. Thus, there was no chance of demonstrating a reduced level of a dermatan sulphate-degrading enzyme in the serum of Hurler's syndrome patients.

It has not escaped the notice of some investigators that the dermatan sulphate which is stored in many organs and excreted by Hurler's syndrome patients is associated with very little protein. This fact has led to speculation, particularly by Dorfman (1964), that an enzyme defect in linkage of acidic glycosaminoglycan to protein is involved. As the nature of the protein-acidic glycosaminoglycan bond is the subject of much current investigation (e.g. Lindahl & Roden, 1966), it should soon become feasible to investigate this possibility.

Austin and others (1964) have reported elevated levels of brain lysosomal aryl sulphatase B from gargoylism patients. This finding has led Gordis 1966) tentatively to classify the defect in gargoylism as 'excess lysosomal hydrolase', but it is difficult to understand how this particular enzyme excess can be related to the presence of excessive amounts of dermatan and heparan sulphates. Possibly this finding may be explained in terms of a more generalised lysosome dysfunction—as has been postulated in lupus erythmatosus (Weissmann & Thomas, 1962) and Gaucher's disease (Bean & Janoff, 1965).

To make studies of this condition less haphazard, more information on the structure and normal metabolism of acidic glycosaminoglycans,

particularly dermatan and heparan sulphates, is required. Studies of the factors controlling differentiation of those connective tissue cells which are responsible for the synthesis and breakdown of acidic glycosaminoglycans are likely to be most fruitful.

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DISCUSSION

The editors regret that the recording of the discussion was technically unsatisfactory.

GARGOYLISM: CLINICAL ASPECTS

VICTORIA P. COFFEY

CLINICAL FINDINGS

THIS syndrome is one of a group of inborn errors of metabolism in which two of the sulphated mucopolysaccharides are excreted in excess in the urine: the two sulphated mucopolysaccharides or acid glycosaminoglycans, to give them their correct name—involved in this condition are:—

(1) Dermatan sulphate—previously called chondroitin sulphate B
(2) Heparan sulphate.

Since these two glycosaminoglycans are resistant to hyaluronidase their differentiation from the mucopolysaccharides of normal urine is a simple matter.

The syndrome was first diagnosed by Hurler in 1917, although, according to Henderson, it was initially recognised and recorded by Thompson of Edinburgh in the early 1900's. Thompson reported his findings in three siblings suffering from the condition later to carry Hurler's name. Since the publication of the first confirmed case in 1917 many others have been recorded from time to time in the medical literature until close on 250 cases have now been reported. I would like to add another three cases to the literature.

Taking gargoylism as meaning Hurler's syndrome, in the strict sense of the word, the condition is an autosomal recessive inherited disease and, unlike Hunter's Syndrome, which it so closely resembles, both sexes may be equally affected.

Clinical findings in a typical case

The patient appears normal at birth and it is often because of macroglossia or failure to thrive in the first few weeks of life that routine investigations reveal the condition.

1. The skull is usually doliocephalic in shape but may be oxycephalic or even hydrocephalic with the sella turcica elongated, and in a very young infant this abnormal skull circumference is often associated with unduly prominent scalp veins.

2. The eyes, which are set well apart, have wide slit lids and corneal opacities are found in about 70-90 per cent of the cases.

3. The bridge of the nose is flat or saddle shaped with very wide nares and chronic rhinitis is an almost constant finding.

4. Macroglossia, which is more in the nature of a protruding tongue, than true macroglossia, is a typical finding, and this, associated with thick coarse lips, is the reason why the condition is so frequently mistaken for cretinism, especially in the early stages.

5. There may be hypertrophy of the bony alveolar ridge and of the overlying gums. The teeth are slow in developing and when they do they are widely spaced and small.

6. The neck is short with narrow shoulders giving the appearance of the head just sitting on the trunk. This is accentuated by the fact that the patient finds great difficulty in turning the head since rotation is very limited. The ears are big and placed in a low position.

7. The chest shows a bulging of the manubrium sternum with splaying of the ribs—probably caused by the hepatosplenomegaly—and the lower ribs show an unusual club-shaped deformity, causing a diagnosis of rickets to be made in some early cases.

8. The abdomen is prominent and a protruding umbilicus or umbilical hernia is a constant finding. The liver and spleen are enlarged, often to a marked degree, and the external genitalia, although normal, are infantile, and in the female menstruation does not occur. Frequently an inguinal or scrotal hernia is found.

9. It is in the skeleton that the most typical findings are recorded; The long bones are short and heavy (the changes being more marked in the upper than in the lower limbs), the predominant changes being in the diaphyses; the hands which are broader than they are long are held in a clawlike position, the fourth and fifth fingers being incurved, resulting in a rather typical 'claw hand'; the metacarpal bones are bottle shaped and the basal phalanges cylindrical; the radius and ulna are short and thick and radiologically often end in a V-shaped angulation. The spinal column shows what has been described as a 'gibbus' or cat-like deformity, the angulation being caused by shortening of the vertebral bodies in the sagittal direction; the spinous processes point downwards and the first and second lumbar vertebrae are small and displaced backwards. Limitation of extension of the joints is a marked feature of this condition and is probably caused by changes in the tendons and ligaments. This inability to fully extend the joints results in flexion of the hips, knee and elbow joints and the child will often walk on his toes to compensate for this deformity.

10. Mental retardation in Hurler's syndrome is always an accompanying feature although the degree of retardation is in no way associated with the severity of the condition. It is, however, likely to be progressive.

11. Thickening or grooving of the skin especially over the thorax and upper arms is a common and often marked finding, and sometimes the body is covered with a fine hair-like growth.

12. No consistent pathological findings have been recorded in the endocrine system even though the deposition of mucopolysaccharides or glycosaminoclycans is found in many tissues.

13. Cardiomegaly and cardiac murmurs of both systolic and diastolic

types are very common findings and death from cardiac complications (i.e. angina and coronary heart disease) is frequently reported.

Diagnostic Tests in Hurler's Disease

A. Biochemical

Full details of the biochemical estimations to be carried out in the investigation of this syndrome have been given by the previous speaker; the 'spot test' or 'colour test' carried out on paper and the location with toluidine blue or more recently Alcian Blue have been discussed and explained so I will proceed to the other means of diagnosis. I would, add however, that hyaluronidase resistant staphlococci are frequently found in the urine of cases of Hurler's Syndrome.

B. Haematological investigations

Examination of peripheral blood in suspected cases for metachromatic bodies or 'Reilly Bodies' is a routine procedure. These inclusions are found in the polymorphonuclear leukocytes and lymphocytes. Muir thinks that examination for inclusions in lymphocytes is more useful diagnostically and he considers that they are a constant finding in some of the patients. They are larger than the ordinary granules and are easily demonstrated, using special staining of either Toluidine blue or the Wright Giemsa technique. These inclusion bodies have been shown to consist of acid mucopolysaccharides. Cells from bone marrow have been shown by Jermain to consist of similar inclusions. Vacuolations in the lymphocytes is another fairly constant finding in cases of Hurler's syndrome.

C. Tissue biopsy

1. *Skin Biopsy.* A biopsy is taken from the thickened area of the skin on the back of the neck or shoulder or from the fingers; it is fixed by putting it into a solution of 10 per cent. formalin and absolute alcohol to preserve the mucopolysaccharide—it is then stained, preferably with Toluidine Blue, and examined under dark field illumination for the presence of mucopolysaccharide material.

2. *Conjunctival and Corneal Biopsy.* Scheie describes the examination of conjunctival and corneal biopsies for the presence of MPS material; it is a difficult and specialised procedure and is not a routine diagnostic test for Hurler's syndrome. It is used in the diagnosis of atypical or forme fruste types of MPS.

D. Skin inflammatory test

In this test an abrased area of skin is covered with a cover slip for about 24 hours following which the cover slip is dried in the air and the cells of the exudate are examined when a large number of monocytes containing

basophilic metachromatic granules in their cytoplasm are found in cases of Hurler's Syndrome.

Differential Diagnosis of Hurler's Syndrome

The two main conditions from which a differential diagnosis of Hurler's syndrome must be made are (A) Cretinism and (B) The other MPS group of diseases.

A. Cretinism and pseudohypothyroidism

It is understandable that stunted growth, macroglossia, thickening of the skin, shortness of the extremities and the appearance of the hands form a clinical picture that on superficial examination may make these two conditions resemble each other, but the differential diagnosis is easily made by

Table I

DIFFERENTIAL DIAGNOSIS OF MUCOPOLYSACCHARIDOSES
CLINICAL FINDINGS

CLINICAL FINDINGS	MPS 1 HURLER'S SYNDROME	MPS 2 HUNTER'S SYNDROME	MPS 3 SAN FILIPPOS	MPS 4 MORQUIO-BRAILSFORD SYNDROME	MPS 5 SHIES SYNDROME	MPS 6 NON-CLASSIFIED GROUP
SKELETAL CHANGES	+	+	-	+	- +	
MENTAL RETARD-ATION	+	-	+	-	USUALLY REPORTED HIGH	
EYE OPACITIES	+	-	+		PRE-DOMINANT FINDINGS	
DEAFNESS	-	+	-		-	
ENLARGE-MENT OF VISCERA	+	-	+	+	-	
HAND DEFORMITIES	+	-	-	+	+	
CARDIAC	+	-			- +	
GARGOYLE FACES	+	-	-	-		
RETARDED GROWTH	+	+	+	+	SHORT IN STATURE	
REILLY BODIES IN LYMPHOCYTES	+	-	-	+ -	-	

P.B.I. levels; x-ray of the skeleton, clinical findings of enlargement of the liver and spleen; cloudiness of the cornea and finally by biochemical investigation of the urine as described by the previous speaker.

B. Other group of MPS

It is from this group of metabolic defects that the main differential diagnosis lies, and the following two tables give the main points to be considered in the diagnosis of each of them (Tables I and II).

It is, however, by the combination of clinical and biochemical findings that the final cause for this inborn error of metabolism will be found, and a very brief review of this condition shows that 80 per cent of the glycosaminoglycans excreted in the urine of these children is dermatan sulphate and 20 per cent is heparan sulphate.

According to Meyer, dermatan sulphate and heparan sulphate are clinically unrelated, and the genetic defect involves only dermatan (the overproduction of the heparan being a secondary phenomena) it is dermatan sulphate that is the responsible factor. Dermatan sulphate is normally present in skin, tendons, and blood vessels and has been isolated from cartilage and bones only in cases of gargoylism; it is associated with coarse collagen fibres, and, to quote Wright, 'it is a cementing material of connective tissue'; in this very broad sense the excessive excretion of the dermatan S. can be associated with the clinical findings, but the exact biochemical disturbance that initiates the mucopolysaccharide upset is as yet unconfirmed. Dr. Baker discussed the possible basic defect which could

Table II

DIFFERENTIAL DIAGNOSIS OF MUCOPOLYSACCHARIDOSES
BIOCHEMICAL

McKUSICK'S CLASSIFICATION OF MUCOPOLYSACCHARIDOSES	SYNDROME	MODE OF INHERITANCE	GLYCOSAMINOGLYCANS OR MUCOPOLYSACCHARIDES FOUND IN URINE
MPS 1	HURLER'S	AUTOSOMAL RECESSIVE	1) DERMATAN SULPHATE 80% 11) HEPARAN SULPHATE 20%
MPS 2	HUNTER'S	SEX LINKED RECESSIVE	1) DERMATAN SULPAHTE 55% 11) HEPARAN SULPHATE 45%
MPS 3	SAN FILIPPO	AUTOSOMAL RECESSIVE	EXCESSIVE SECRETION OF HEPARAN SULPHATE ONLY
MPS 4	MORQUIO BRAILSFORD	AUTOSOMAL RECESSIVE	LARGE AMOUNTS OF KERATAN SULPHATE
MPS 5	SHIES OR ADULT FORM	AUTOSOMAL RECESSIVE	LARGE AMOUNTS OF DERMATAN SULPHATE
MPS 6	UNCLASSIFIED GROUP i.e. MARFANS ETC.		MORQUIO ULLRICH SYNDROME

be responsible for Hurler's syndrome and has given us some logical reasons for his opinions but it is only an opinion as yet, and we are hoping to get a lead from some of the scientists present here this afternoon.

Treatment

Not knowing the actual cause of the condition, treatment either as a preventive or remedial measure is not possible. The only treatment available is that of orthopaedic surgery which simply prevents the skeletal deformities getting worse. A good deal more work needs to be carried out in the biochemical field of this syndrome before a final answer is obtained.

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DISCUSSION

The editors regret that the recording of the discussion was technically unsatisfactory.

LIST OF EXHIBITS

1. Congenital Intrinsic Factor Deficiency.
   A potential Cause of Brain Damage.
   Brian McNichol and Brian Egan.

2. Renal Tubular Phosphate Reabsorption in Childhood.
   (a) Vitamin D resistant rickets.
   (b) Occult malabsorption.
   (c) Renal tubular acidosis.
   S. Dundon.

3. Fructosaemia.
   R. G. Barry.

4. Metabolic Disorders associated with Mental Handicap.
   B. Stokes.

5. Investigation of Autistic Children.
   N. Healy, I. Ascher, and T. Ware.

6. Maple Syrup Urine Disease.
   M. Curtin and J. M. O'Callaghan.

7. Urine Chromatography on Patients in St. Vincents.
   J. Cooney, V. Coffey, P. T. Moore, and N. Martin.

8. Facio-Scapulo-Humeral Muscular Dystrophy in a Family Associated with a Basic Aminoaciduria.
   N. A. J. Carson, L. J. Hurwitz, I. V. Allen, T. E. Fannin and D. W. Neill.

FACIO—SCAPULO—HUMERAL MUSCULAR DYSTROPHY IN A FAMILY ASSOCIATED WITH A BASIC AMINOACIDURIA*

NINA A. J. CARSON, L. J. HURWITZ, INGRID V. ALLEN, T. E. FANNIN and D. W. NEILL

Age of Onset	=	2nd-4th decade.
Muscles Involved and the Degree of Weakness	=	Symmetrical involvement of facial and shoulder girdle. Severe in a few. Slight to moderate pelvic girdle weakness occurring at a later stage (Figs. 2, 3 and 4).
Rate of Progression	=	Slow. I_2, II_3 had periods of more rapid progression (Fig. 1).
Electromyography	=	Myopathic pattern (Fig. 14, Table I).
Serum Enzymes	=	Mild elevation in creatine phosphokinase in II_5, II_1, II_2, II_3. Aldolase at upper limits of normal (Table I).
Electron Microscopy	=	Fibre degeneration, Mitochondrial changes (Figs. 11, 12 and 13).
Inheritance	=	Autosomal dominant.

Unusual features

1. High penetrance in the second generation.
2. 'Pseudohypertrophy'.
3. Biopsy findings show enlarged muscle fibres with little or no degenerative changes except in II_3, who showed typical features of progressive muscular dystrophy, i.e. fibre degeneration and atrophy with increased connective tissue and fatty infiltration (Figs. 8, 9 and 10).
4. Aminoaciduria—Increase in basic amino acids, lysine and ornithine, with variable arginine and cystine excretion, associated with normal blood values of these amino acids. There is dominant inheritance with apparent independent segregation from the muscular dystrophy (Figs. 5 and 7).

   Load tests of the above amino acids indicate poor intestinal absorption in one patient tested with the aminoaciduria (Fig. 6).

   Renal clearance values in two members with the aminoaciduria show defective renal reabsorption of lysine and ornithine.

* This exhibit has now been published in full elsewhere (*Brain* (1967) **90**, 799) and figs. 1, 5 and 6 and Table 1 are reproduced in both publications by agreement of the authors, editors and publishers.

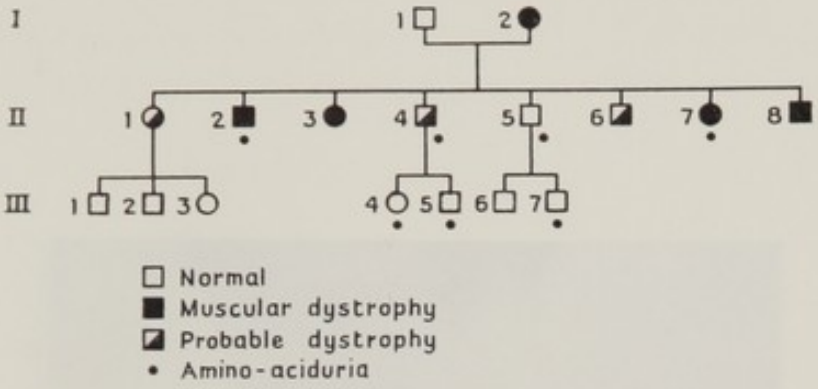

FIG. 1

FIG. 2

Patient II_3. Note hunched forward position of the Scapula and the suggestion of increased bulk of deltoid muscles.

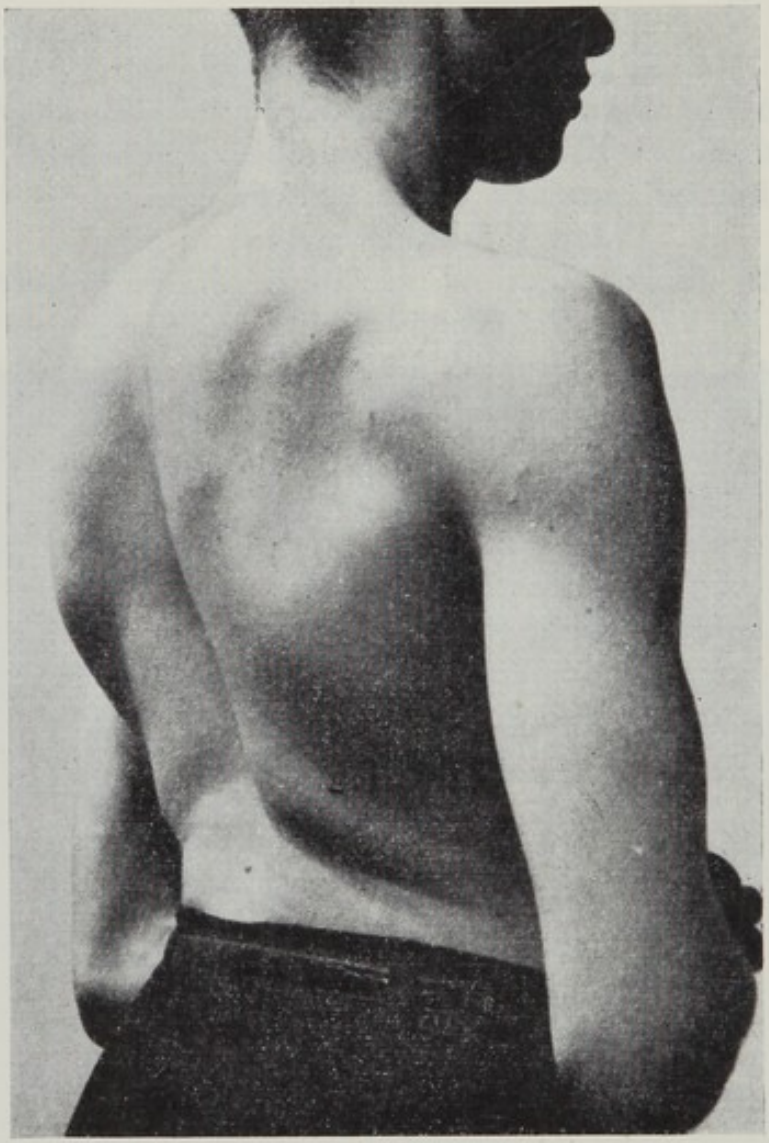

FIG. 3

Patient II_6. No symptoms, but slight clinical evidence of shoulder girdle weakness.

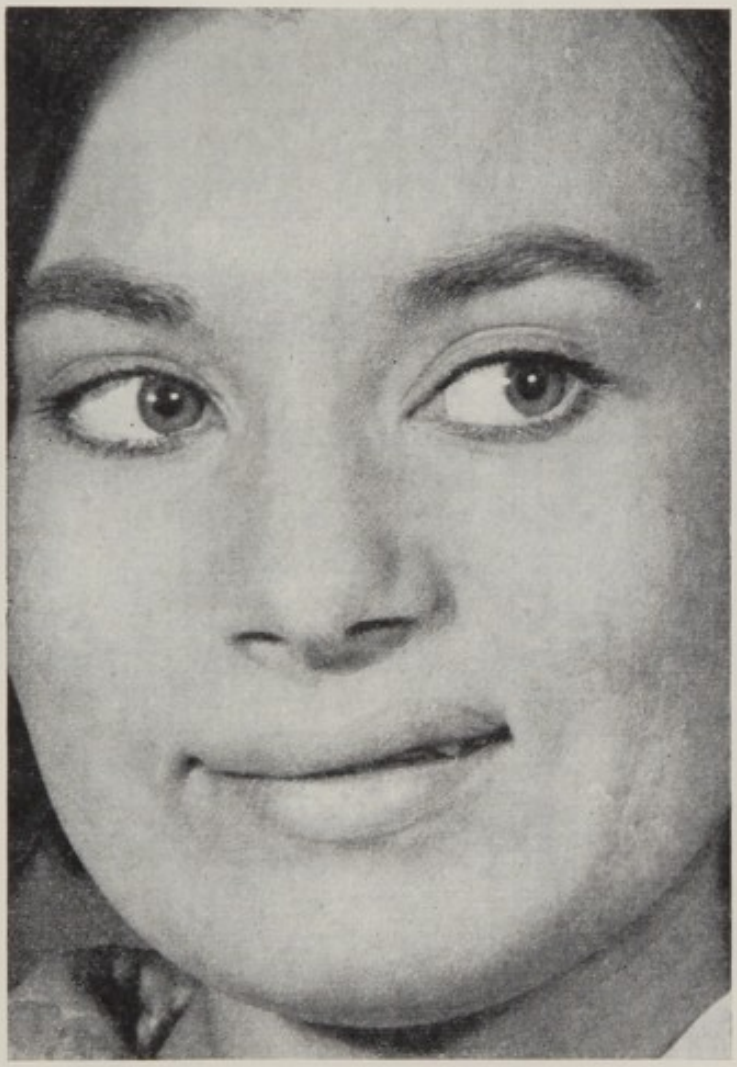

FIG. 4

Patient II_3. Transverse smile and absence of wrinkling on forehead.

Table I. *Electromyography and serum enzymes.*

Patient	*Sex and age*	*Dystrophy*	*Maximum effort pattern*		*Mean duration action potential (percentage increase or decrease)*	*Incidence polyphasic potentials (per cent)*	*Creatine phosphokinase (units)*	*Aldolase (units)*
			Interference	*Amplitude*				
II								
1	F 30	±	I	N	+4	8	10·0	10
2*	M 25	+++	M.P.	R	—51	35	5·8	22
3	F 24	+++	I	R	—11	19	12·7	23
4*	M 23	±	I	R	—26	15	1·1	23
5*	M 21	0	I	N	+6	19	7·2	23
6	M 19	±	I	N	+1	20	4·7	19
7*	F 16	++	I	R	—12	6	1·5	24
8	M 13	+	I	R	—5	32	1·1	18

* Abnormal minoaciduria.
0 No clinical evidence of muscular dystrophy.
± No complaint of weakness but signs of dystrophy present.
\+ Mild muscular dystrophy.
++ Moderate muscular dystrophy.
+++ Severe muscular dystrophy.

I Normal interference pattern on maximum effort.
M.P. Mixed interference pattern on maximum effort.
N Normal amplitude of interference pattern.
R Reduced amplitude of interference pattern.
Creatine phosphokinase: Normal range 0·3-4·5 units.
Aldolase: Normal range—Women 6·1-19·2 units: Men 7·2-21·3 units.

ANALYSIS OF AMINOACIDS IN URINE AND PLASMA

Patient	LYSINE			ORNITHINE			ARGININE			CYSTINE		
	Plasma (mg. per 100 ml)	Excretion (ug. per min.)	Clearance (ml. per min.)	Plasma (mg. per 100 ml)	Excretion (ug. per min.)	Clearance (ml. per min.)	Plasma (mg. per 100 ml)	Excretion (ug. per min.)	Clearance (ml. per min.)	Plasma (mg. per 100 ml)	Excretion (ug. per min.)	Clearance (ml. per min.)
I_2	3.4	33	0.98	1.36	7.2	0.55	1.8					
II_3	3.1	44	1.42	1.36	5.3	0.39	2.8					
II_4	2.7	90	3.3	1.9	128	6.6	1.9	19.8	1.0	1.7	10.8	0.6
II_7	1.5	159	10.5	1.3	32	2.4	1.3	5.5	0.4	0.8	Trace	..
Normal Range +	2.1 - 2.4	11 - 45	0.2 - 1.9	0.6 - 1.2			1.0 - 2.4	2.2 - 8.0	0.2 - 0.8	0.2 - 0.8	4.5 - 10	0.7 - 2.9

+ Cusworth and Dent, Biochem., Jl., 3, 550-561, 1960.

FIG. 5

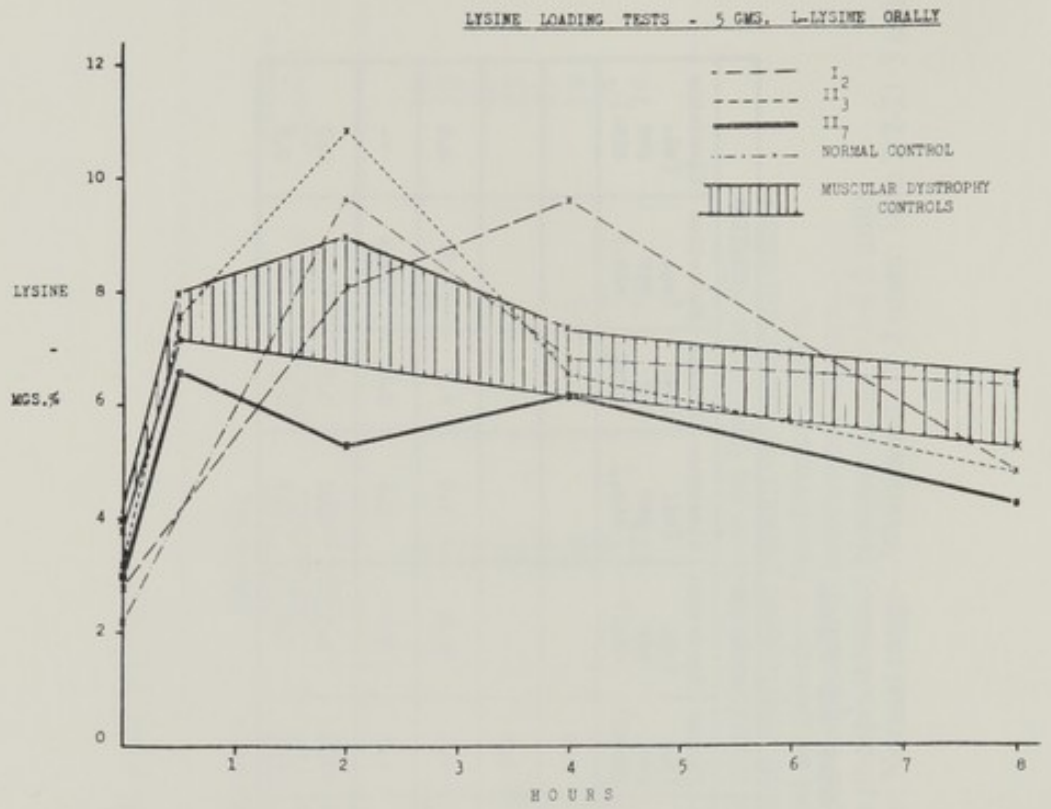

FIG. 6
Graph of Lysine loading tests.

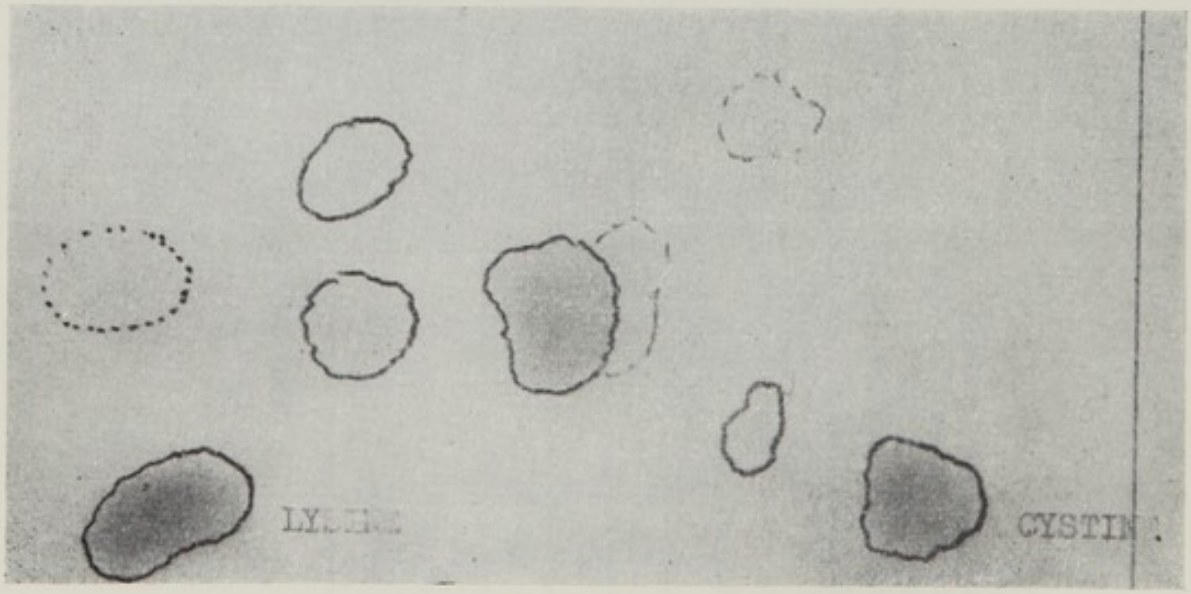

FIG. 7
Urinary amino acid chromatogram

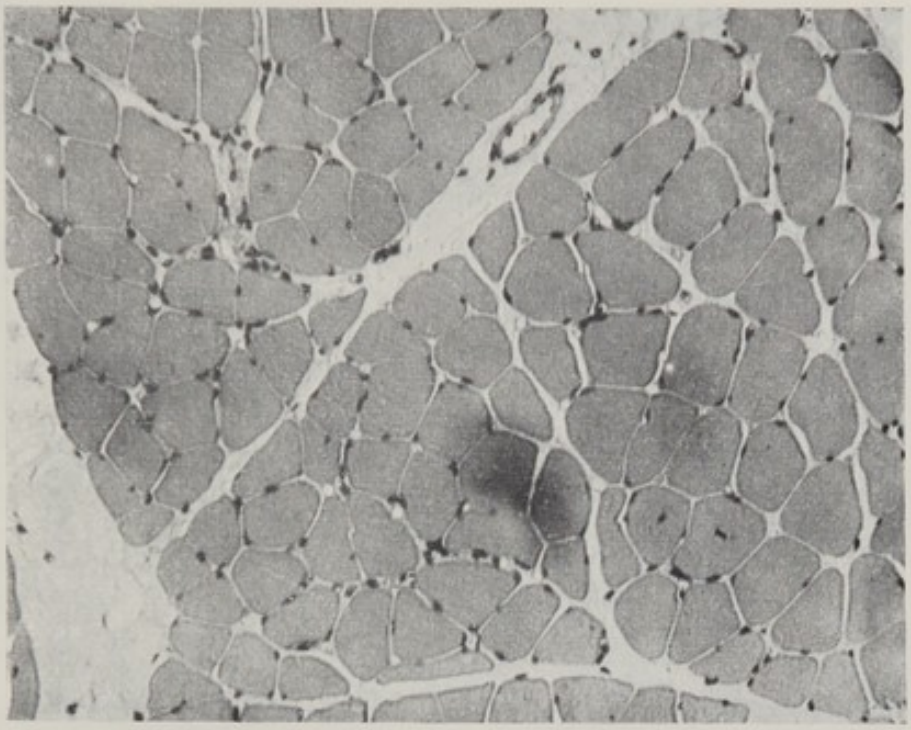

FIG. 8

Light Microscopy

Normal control. (Deltoid). H. & E. × 140. Note the polygonal appearance with peripheral nuclei. Maximum fibre diameter is approximately 65μ.

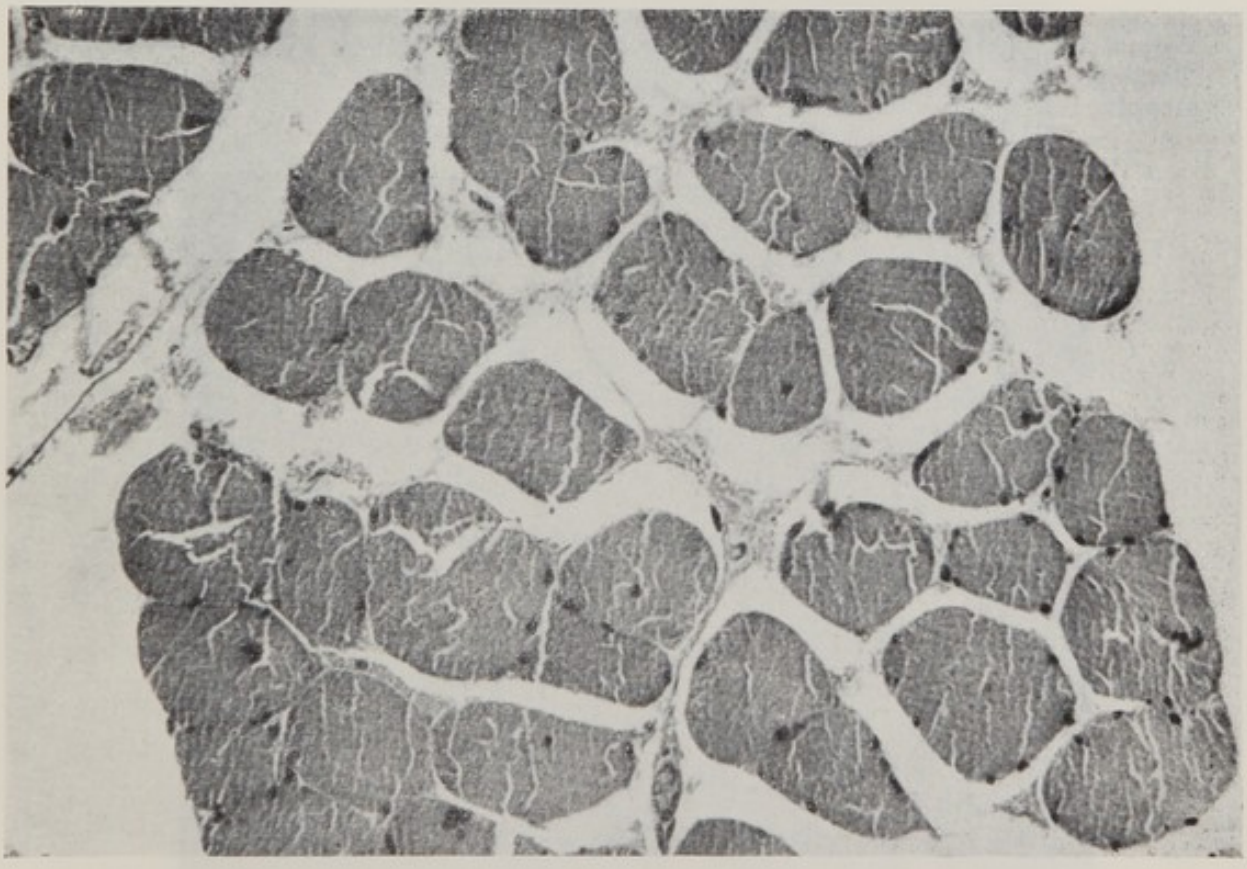

FIG. 9

Light microscopy

Patient I_2. (Deltoid). J. & E. × 210. Rounded, enlarged fibres with occasional central or eccentrically positioned nuclei. Maximum fibre diameter is approximately 110μ. Note the absence of secondary fibrosis and fatty change.

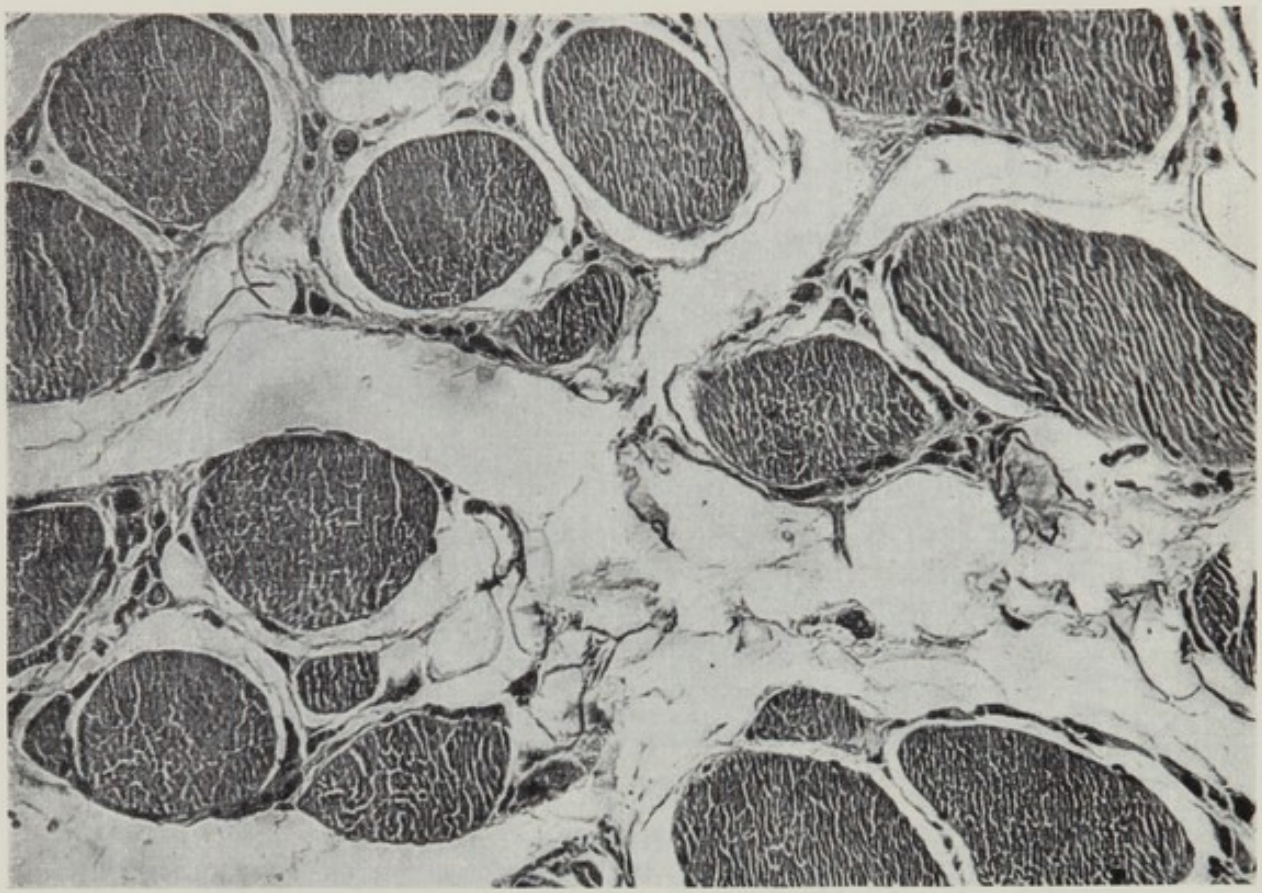

FIG. 10

Light microscopy.

Patient II_3. (Quadriceps). H. & E. × 210. Note the marked variation in fibre diameter with the appearance of very large fibres (up to 140μ) and numerous small atrophic fibres (arrowed) of less than 10 μ. Moderate fibrosis and fatty tissue change.

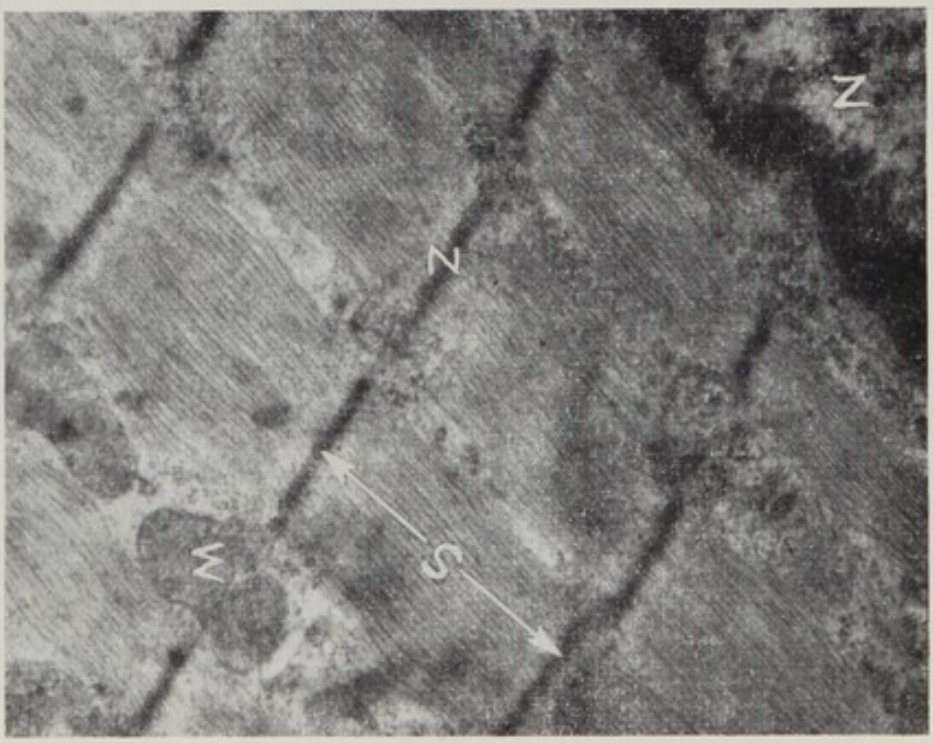

FIG. 11

Electron microscopy

Normal control × 30,000.

(S) — Sarcomere. (M) — Mitochondria. (Z) — Z.Band. (N) — Nucleus.

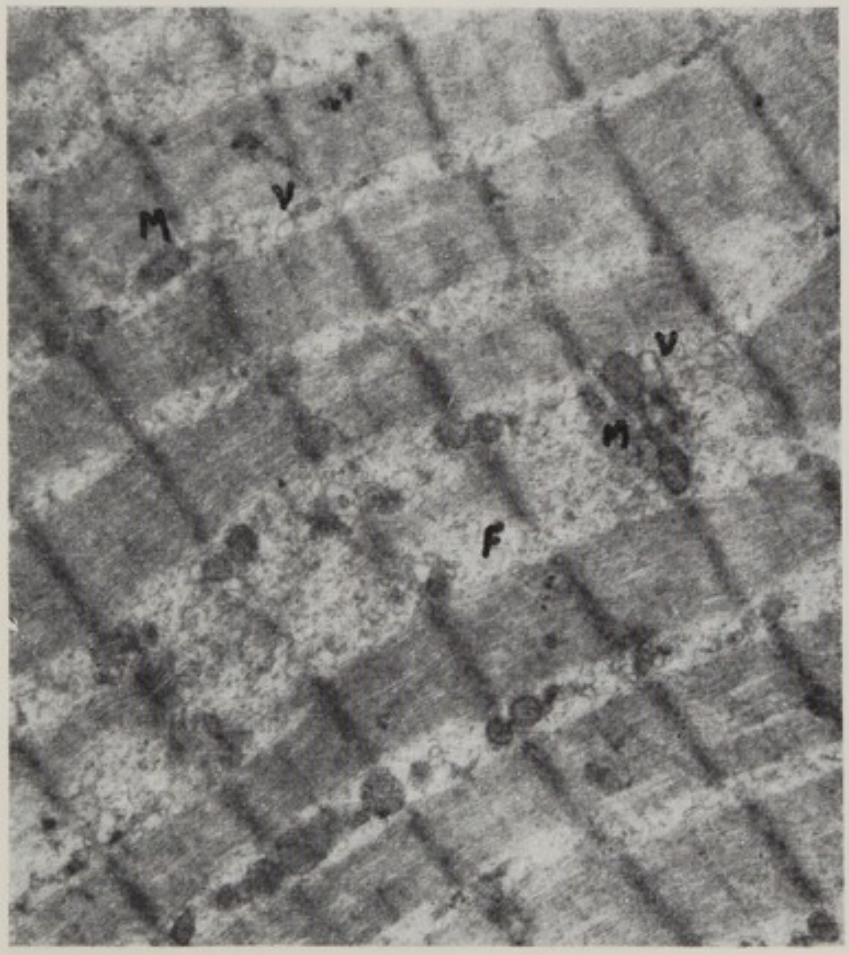

FIG. 12

Electron microscopy

Patient I_2. (Deltoid). × 21,700. Moderate variation in fibril diameter with splitting of myofilaments. Fibril degeneration (F) with enlarged mitochondria (M) and small vesicles (2). Increased interfibrillary granular material.

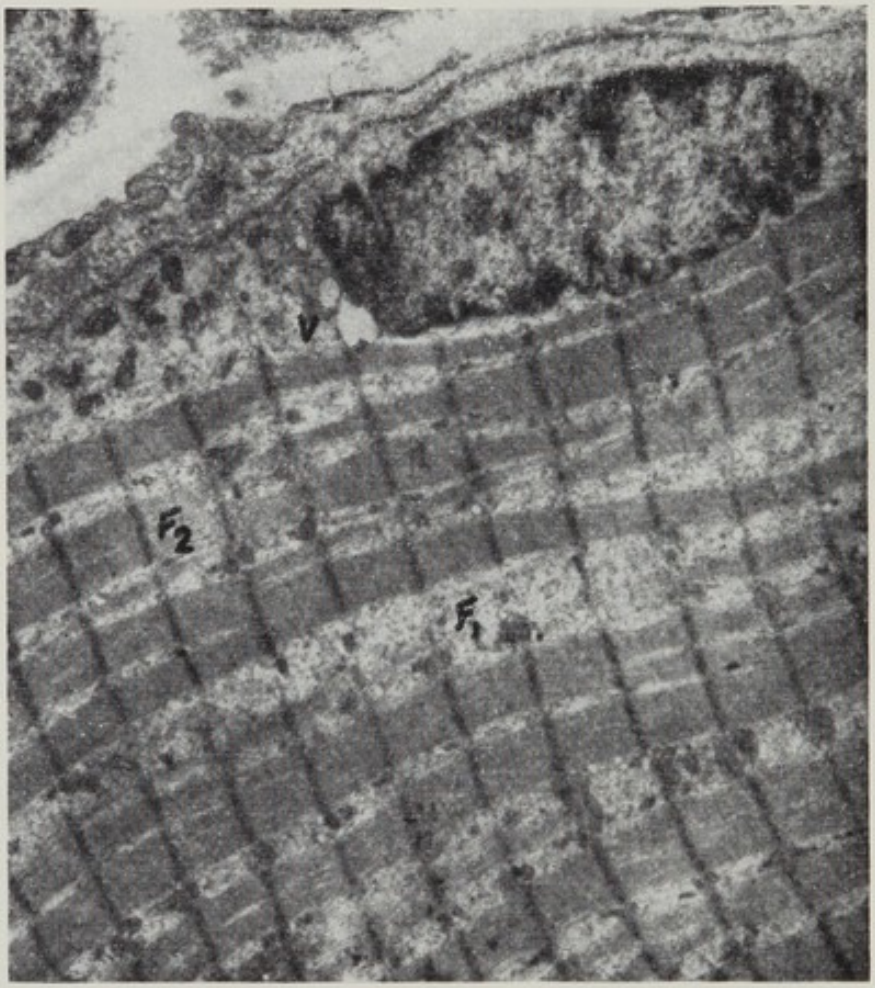

FIG. 13

Electron microscopy

Patient I_2. (Deltoid). × 6,700. Note widespread loss of myofibrils (F.1) and early degeneration (F.2). Splitting of fibrils is prominent. Nuclear indentations are marked. There is increase in subsarcolemmal space with occasional vesicles (V).

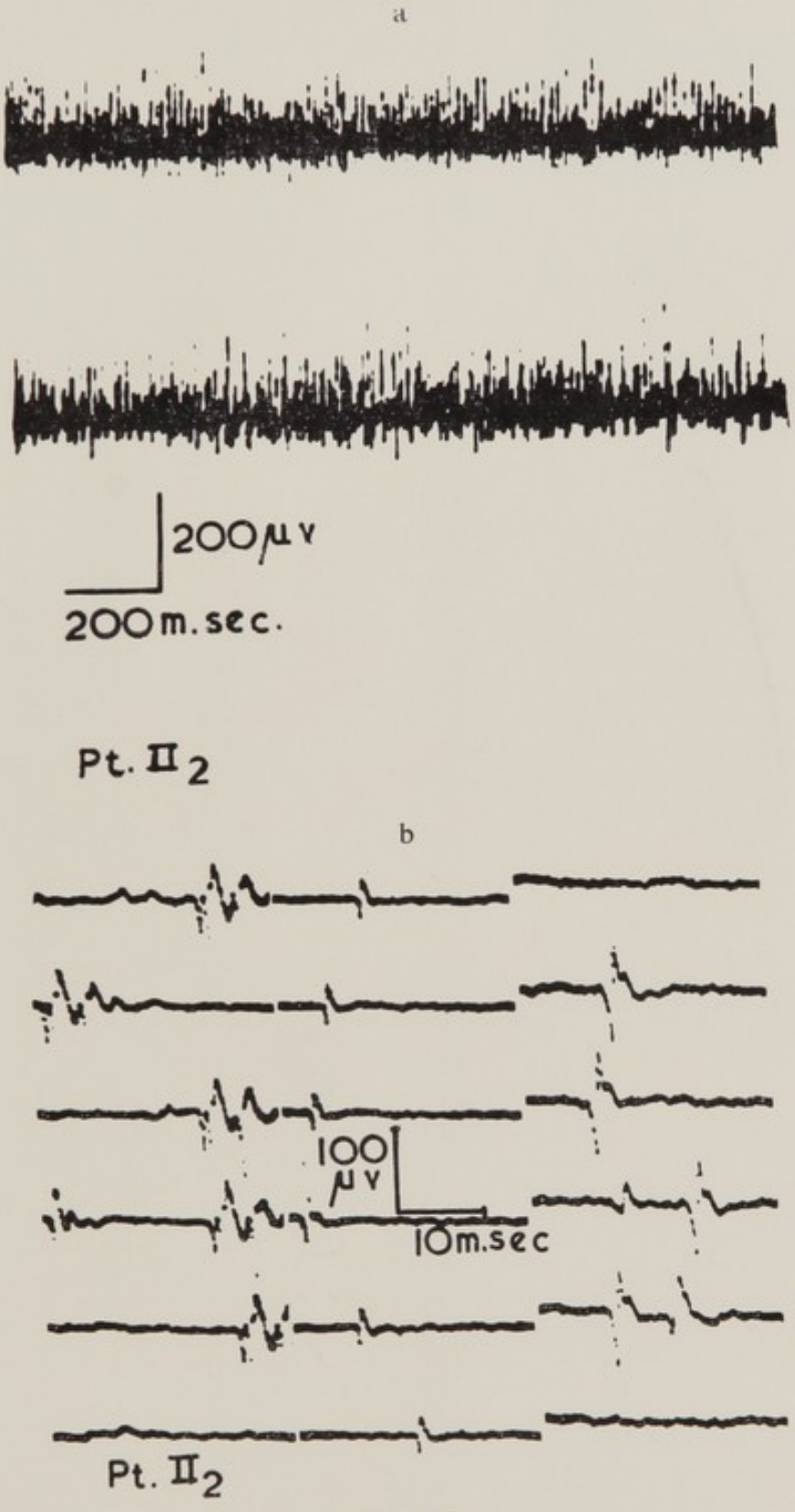

FIG. 14

Electromyography.

(a) Continuous record showing mixed interference pattern of reduced amplitude produced by maximal voluntary effort. (b) Interrupted record showing muscle action potentials of low amplitude and of short duration (mean duration decreased by 51 per cent of normal) and polyphasic potentials.

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