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Editorial

This topic is nowadays of trascendental importance in studying the nervous system, due to the preeminence of biochemical investigations concerning the normal and pathological nervous function.

In this way neurological ailments can be investigated from different angles, offering new perspectives to the comprehension of chemical factors, that have adquired such importance that can rule a therapeutic approach, with favourable results. It is evident that one of the investigations that has constituted a real progress in the knowledge of the mechanisms underlying the nervous function is the one concerning the neurotransmitters, *which sheds light in the essential fact of the passing of the nervous influx from one neurone to the next.*

The hystofluorescence, *technique so well applied by the Scandinavian School allows to refer some of the neurotransmitters to definite nervous pathways. This information is of fundamental importance in elaborating a concept about the way in which are accomplished in normal and pathological conditions certain nervous functions connected with specific anatomical system of fibers and nucleous and integrated with complex organic molecules that operate as neurotransmitters.*

These functions are related with motricity, sensibility, reflexes and also with psychological processes. The decrease or excess of the above mentioned molecules, constitute important factors in the genesis of pathological conditions, and may indicate a successful therapeutic resource.

The electrolytes *also participate in the physiology of the nervous system, and their alterations determine clinical pictures. They have an important role in the transmission of the nervous impulses and in the synaptic activity. In the membrane of celular elements (neurons and glia) and at the blood brain and the blood CSF interfaces, the electrolytes have attributes of crucial importance.*

A significative fraction of the metabolic energy is spent by the brain whose finality is the maintenance of the ionic gradients.

Associated to brain hydration and electrolytic disturbances, we can find important and frequent clinical pictures such as cerebral edema installed in various ailments, and the hydrocephalus which has adquired lately a great importance when its vinculation with demential states was placed in evidence.

It must be also considered the new syndroms: the hyposmolarity plasmatic with hyponatremia that may be accompanied with epileptic seizures, confusional states, sometimes coma. The hyperosmolarity with hypernatremia may also engender disturbances of consciousness. The diabetic hyperosmolarity accompanying a great hyperglycemia without acidocytosis, leads to coma.

Concerning the calcium it is important to emphazise that the hypercalcemia it is associated with psychic depression, intelectual deterioration, ataxia, dysartria, electroencephalographic bradyrrithmia.

The hypocalcemia engender intellectual slowness and convulsive crisis. The syndrome Milk-alkali originated by the inmoderate ingestion of milk and alkalines, has been described.

The brain maintains an intense metabolism, consuming from 15 to 20 % of the total oxigen used by the whole organism, being its weight only the 2 % of the weight of the body.

The general metabolic disturbances have very easely repercussion on the brain.

In the anoxia *the brain suffering is evident, generating lesions which if this condition lasts some minutes, are irreversibles.*

The glucose *is the principal source of energy for the brain.*

The hypoglycemia may be manifested by a variety of autonomic features, if important it leads to coma, and if this is not arrested in time, it may produce irrevocable neuronal damage. In the hypoglycemic encephalopathy may be observed focal or generalized epileptic seizures, states of mental confusion, behavior perturbances, focal cerebral syndromes. The infantile hypoglycemia without treatment, originates mental retardation.

Endocrinopathies. *Very frequently they generate in the nervous system changes of varied magnitud and syntomatology. It must be remembered that the glands of internal secretion have a profound influence uppon the metabolism of the nervous system.*

The Hypoparathyroidism *it is accompanied by an important perturbation of the calcium and phosphorus metabolism, that implicate nervous systomatology. Cramps, numbness, spasms carpopedal, convulsions, laryngeal stridor.*

Sometimes it is associated with psychotic ailments. Tetany may appear with its characteristic signs (Chvostek, Trousseau, Erb). It may appear signs of intracraneal hypertension, torticolis, chorea, athetosis, dystonia, parkinsonism. Electroencephalographic changes with bursts of brief duration of waves of frequency from 2 to 5 per second, may be observed.

To the X Rays the skull shows small calcifications distributed uppon the cerebral texture.

The nervous symptomatology in the Hyperthyroidism *shows weakness with hypotonia and sometimes slight atrophy of the muscles with hyporeflexia and a diminution of its electrical excitability. In certain cases consciousness may be affected and even to a stage of coma.*

Psychotic pictures may also appear. In some patients the vertebrae may be damaged, and in a small proportion of cases myeloradicular compressions may be engendered.

Sometimes in Addison Disease, *coma with papilledema and abolished re-*

flexes may be present. In this mechanism hydroelectrolitic changes participate (hyponatremia, hypokalemia), hypotension, hypoglycemia.

In the hyperfunction of the adrenal cortex may be observed the Cushing's disease, *that also may be originated by the administration of corticosteroids.*

Consequently a syndrome of cerebral pseudotumor may appear with cephalalgias, vomits and papilledema. This clinical picture may appear after a brusque interruption of a sterocortico therapy.

The hypothyroidism *it is accompanied with complications in the nervous system as much in the somatic as in the psychic sphere, peripheral neuritis, paralysis of the craneal nerves, myopathy, cephalalgias, confusional states, delirium, depression, hard to control excitability, convulsion, coma, can be observed.*

The hyperthyroidism *shows also important neurological syndromes, tremor, exophtalmos, weakness in converging eyes, scarse blinking, weakness in the muscles of trunk and extremities. In the psychic sphere there is emotional instability, confusional states stupor. Affections can be observed which although they are not originated by the hyperthyroidism, accompany it, like myasthenia. familial periodic paralysis ophtalmoplegia exophtalmica, chronic thyrotoxic myopathy.*

Hypothalamic hypophisary sindromes *by themselves constitute an important chapter of neurology. The hypothalamus is at the anatomical and functional summit of the autonomic nervous and endocrine systems. When it is damaged it may originate metabolic repercusions in other structures of the nervous system and thus it can be observed in the* hiperpituitarism *with overactivity of the basophilic cells in the Cushing syndrome, muscular atrophies, many times so severe that they configurate a progressive muscular distrophy.*

In the child's cerebral gigantism, *Sotas syndrome, possibly originated by an abnormal secretion of the growth hormone, clumsiness, mental retardation, and convulsive seizures, can be observed. In the* Schwartz-Bartter syndrome *generated by an excesive secretion of A. D. H. psychic disorders, lack of awareness, and epileptic fits, may also be observed. This clinical picture was first observed in patients suffering from bronchial cancer, may be due to the fact that cancer segregates an antidiuretic hormone. But the case has also been stablished of a marked secretion of A. D. H. in traumatism of the skull, cerebromeningeal processes, peripheral neuropathies. If there is lesion of the hypothalamus post hypophysary system, it is possible that may exist a lesion on the supraoptic hypophysary axons.*

Concerning the ovarium *we may stress the collateral effects that it has been reported about the use of compounds that contains estrogenic substances used as contraceptives, and that generates migraine, vascular cerebral lesions, optic neuritis arterial hypertension increased intracraneal preassure.*

Hepathic encephalopathy, *may be observed in acute viral hepatitis or chronic cirrosis. It is a frequent methabolic encephalopathy. Two mechanisms participate, one is due to the hepatic insufficency, that generates a lack of the metabolites necessary for the cerebral activity, the other one is the passage of*

a toxic substance from the intestins to the general circulation caused by the shunt porto-cava.

Ammonia is a vell known toxic substance. In this encephalopathy it may be observed extrapyramidal syndromes, confusional states, epileptic manifestations that are less frequent, when the ailment increases may lead to coma and when this happens in the course of an acute hepatitis, its evolution is almost always mortal.

In the hepathic encephalopathy, the EEG is perturbed with some suggestive changes rhythm delta monomorphus, tryphasic slow spikes.

In cirrhoris the encephalopathy may appear originated by a digestive hemorrage or the ingestion of certain drugs such as valium, barbiturics, acetazolamide, morphine.

The uremic encephalopathy *is installed as a consequence of a renal insufficiency and it is accompanied by signs of confusion, a diminution of consciousness, convulsions, flapping tremor, myoclonus.*

That may be caused by the hyperazotemia, acidosis, hyponatremia, hypocalcemia that are present in these clinical pictures.

Encephalopathies due to deficiencies. *We must recall the encephalopathy of Gayet-Wernicke by* lack of vitamin B_1. *Convulsive crisis and confusional states due to* deficiency of vitamin B6.

In the alcoholics the lack of vitamin PP *generates a dangerous encephalo*pathy that is evidenced clinically by hypertonia confusion and coma.

Deficiency of Vitamin B12 *it is observed in Biermer's pernicious anemia.*

It damages principally the spinal cord. Psychic disturbances may be present a diminution of memory, changes in character, intellectual slow down, confusion, paranoid manifestations. Concerning the neurological aspects, sometimes a polineuritic picture may develop. The optic neuritis is the usual cause of the onset of visual impairment in the Biermer's anemia.

Paraneoplastic neurological syndromes. *They may appear with varied clinical pictures, in certain cases muscular ailments may develop in other peripheric neuropathies, it may be perturbations in the neuro-muscular transmission (Myasthenia, Syndrome of Lambert Eaton) originating in the central nervous system, paraneoplasic encephalomyelitis, myelopathies, brain stem syndromes, paraneoplasic cerebellar degeneration, paraneoplasic metabolic encephalopathies. These last ones may be determined by different mechanisms, hypoglycemia, calcipenia, hyponatremia (syndrome of Schwartz-Bartter), dysglobulinemias.*

Biochemical changes may provoke degenerative lesion on the nervous system, possibly due to the uptake of the cancerous cells of a metabolite used by the nervous tissue or by the antimetabolic action upon the nervous system of a substance generated by cancer.

The lesions may show a great inflamatory character of viral origin or by an inmunitary reaction linked to an antigenic afinity between the cancerous cells and certain nervous cells.

The alcoholism *generates disturbances in the nervous system promoted principally by metabolic perturbances, this gives place to amultiple pathology without aiming to cover all of its aspects, we will mention only some of its most important clinical pictures.*

Acute alcoholic intoxication. *The drunkennes. Consequences of* abstinence, *oniric confusional states, deliriums tremens, and alcoholic epilepsy.*

Nutritional alterations, *encephalopathy of Gayet Wernicke, syndrome of Korsakoff, polyneuritis by lack of vitamin* B_1. *Optic neuritis that may be associated with lack of vitamine* B_6, *and panthotenic acid. Other lesions may be observed such as: alcoholic cerebellar atrophy, encephalopathy of Marchiafava Bignami, the alchol acts directly like a toxic, myelinolisis central pontine (this is originated by dismetabolic factors, that may be seen in non alcoholic patients suffering from diabetic coma, hydroelectric disturbances, chronic renal insuficiency, etc.). The alcoholic's cerebellar atrophy, may appear in patients that generally have gross nutritional disturbances and vitaminic insufficiency.*

The study of diseases due to inborn metabolic defects *is nowadays on the spot-light. Since its complexity attract investigators of several disciplines. They generate clinical pictures in which neuropsychiatric manifestations are just one of its aspects, since there are frequently other organic structures involved which demand special investigations.*

Progress in biological sciences has made it possible to differentiate, and to group the different diseases implicated, and at the same time the chance to detect them are bigger. This has increased the interest for this important subject. Biochemical studies in these molecular ailments have contributed in a great meassure to the comprehension of some normal metabolic processes and have allowed in a certain way to apply adecuate therapeutic indications. Actually, family groups may be advised from the genetic point of view. Very sensitive tests can be used for diagnosis of homozygotes using washed leucocyte preparations on cultured skin fibroblasts, Healthy heterozygous carriers can be detected.

Now in this way, genetic counseling to control pregnancies risking lipid storage diseases, is on use throughout the world.

Generally this kind of illness can be clinically detected early in infancy, and they infer an appreciable damage on the development of the nervous system, originating the onset of serious neuropsychiatric symptoms, epilepsy mental retardation, pyramidal and extrapyramidal syndromes.

We will make a brief enumeration of the different metabolic disturbances that are implicated in this group of diseases. They are metabolic disorders: a) of the aminoacids, b) of the lipids, c) of the mucopolysaccharides, d) of the porphyrins, e) of the carbohydrates, f) metalic.

Lipid storage diseases are originated by deficiencies of catabolic enzymes necessary for the metabolism of the lipids that accumulate.

An important advance in the care of these disorders has been obtained for Gaucher and Fabry diseases. In the Gaucher disease there is deficiency of glucocerebrosidase and in Fabry disease there is of ceramidetrihexosidase. These

substances have been isolated of the human placental tissue and adminestered to patients with these ailments and a beneficial effect has been observed.

Mucopolysaccharidoses have also enzymatic lesions which in the majority of its disorders have been identified. Two methods have been used for the diagnosis of mucopolysaccharidoses, one is the measurement of the amount and size .of acid mucopolysaccharides in urine or cerebrospinal fluid; the other method employed consists in detecting in the fluid element of cultured skin fibroblasts, substances disolved in it that control and prevent the accumulation of radioactive sulfur labeled mucopolysaccarides.

These deficiencies in lipid storage diseases and mucopolysaccaridoses are referred to catabolic enzymes. Now it is also considered that there exist deficiencies of synthetic enzymes that can produce impairment of the Central Nervous System.

Many of the degenerative heredo-familial diseases of the nervous system may find its explanation in the study of its biochemical perturbations.

In this brief review we have casted an appreciative look on the most striking advances on this topic.

We want to thank very much the outstanding group of investigators that have pooled their effort in this issue to advance the frontier of knowledge in this important topic of modern neurology.

It is with great satisfaction that we announce that two very distinguished representative of American and French Neurology, such as Prof. Benjamin Boshes and Prof. Michel Bonduelle have been incorporated to our Editorial Board.

No doubt that their dynamic and brilliant personalities will greatly benefit our publication.

Dr. VICTOR SORIANO.

Recent Developments in Parkinson's Disease And Huntington's Chorea

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INTRODUCTION

Since the early 1960's, when a defect in dopamine metabolism [3, 39] was discovered in Parkinson's disease, and when Levodopa therapy was first introduced [2, 22], there has been a considerable upsurge of interest in the study of catecholamine metabolism in the brain and in the investigation of the possibility that other chronic degenerative diseases of the nervous system may be of biochemical origin. Such studies have resulted particularly in the better understanding of Parkinson's disease and Huntington's chorea, as examplified by a number of recent monographs, symposia and reviews [19, 21, 30, 34, 36, 57, 62, 63, 65, 70, 71, 83, 84, 39].

It would be presumptious of us to attempt coverage of all these aspects in this short paper. We will thus concentrate on the newer findings which may lead to important steps in the near future, and on the still unresolved problems. We have chosen to limit our remarks to these two illnesses mainly because our laboratory has been involved in clinical and fundamental research into these problems but this should not be interpreted as meaning that there has not been any progress in the understanding of other basal ganglia disorders in these last few years. On the contrary many investigators are making significant contributions to the unraveling of the pathophysiology of other extrapyramidal diseases, such as hepatolenticular degeneration, Gilles de la Tourette's disease and "tardive" dyskinesia.

I — PARKINSON'S DISEASE

(a) *Etiological research*

Most anatomists and physiologists consider Parkinson's disease as the result of a specific and localized defect in the dopaminergic nigro-striatal pathway [79]. However this tends to minimize the importance of the many neurovegetative symptoms known to be present in this illness: constipation, impotence, hypersalivation, hypersudation, seborrheic dermatitis, urinary incontinence, orthostatic hypotension [1, 52]. These symptoms are observed in nearly 70 % of patients according to Spiegel and collaborators [87] and to Porter et al.[80]. It is difficult to assign all of them to secondary manifestations of nigro-striatal lesions. At the Third International Symposium on Parkinson's Disease in Edinburgh [43], we reviewed the evidence in favour of the concept that Parkinson's disease was accompanied by a generalized defect in dopamine metabolism [5]. Considerable new biochemical and anatomical evidence has since been gathered to strenghten this hypothesis.

(1) *Biochemical evidence*

As will be seen below, the involvement of dopamine is not the only biochemical defect observed in the brain of parkinsonian subjects. Serotonin noradrenaline, gamma-aminobutyric acid (GABA) and acetylcholine are also involved to variable degrees. Most of the indirect studies of dopamine

metabolism concentrate on CSF and/or urine values of dopamine and homovanillic acid (H.V.A.). Thus many authors since Guldberg et al. in 1967 [49] have shown that CSF, H.V.A. concentrations are considerably reduced in Parkinson's disease (for review see Barbeau 1972 ref. 14). It is now becoming evident that the values of H.V.A. are decreased in proportion to the degree of akinesia observed in the patient [73, 74, 81]. However, before interpreting single values, one should be very conscious of the fact that H.V.A. concentrations vary with age [25, 48] having a tendency to increase in the CSF with advancing years. Therapy with phenothiazines and anticholinergic drugs can also modify the base values [26, 76].

The lowering of H.V.A. concentration in the CSF is unfortunately not pathognomonic of Parkinson's disease. It is also present in many forms of progressive dementia, with or without signs of Parkinsonism. For example it has been reported in senile and presenile dementias [46, 47] and recently in the Parkinson - Dementia - A.L.S. complex of Guam [28]. Finally there is evidence of low CSF, H.V.A. in temporal lobe epilepsy [20,74]. This latter observation may eventually be of importance because it has been shown that dopamine can protect experimental animals by elevating the convulsive threshold [23, 50]. It can also be considered in the light of recent studies on the effect of ouabain seizures upon ATP'ase activity and brain monoamines [37, 38].

Although H.V.A. is the main metabolite of dopamine, it reflects mainly extraneuronal metabolism of the amine. It would be of greater interest to know in detail what happens to DOPAC [85] and to the methoxy derivatives. Recent detailed studies of dopamine metabolism by Goodall and Alton [45] would suggest that the true picture will only emerge when a multitude of other metabolites are also investigated. However even such complete panoramic view may not be sufficient. Turnover rates and dynamic components should be known. In this light we had carried out studies with tritiated dopamine which indicated that the turnover from dopamine to H.V.A. was much more rapid in parkinsonian patients [4]. These findings were compatible with those of Goodall and Alton [44] with C14-dopamine who demonstrated a decreased synthesis of noradrenaline under load conditions. From these observations we postulated [5] that Parkinson's disease was associated with a defect in binding or retention of dopamine in cells where it is normally accumulated. We have since pursued the study of this phenomenon with the help of the platelet model, as described by Boullin and O'Brien [24]. It now appears that dopamine is taken up by these cells in a normal fashion but that, in untreated parkinsonians, dopamine is not as well retained as in control subjects (ref. 24, and unpublished results from our laboratory). If these observations are confirmed, they would have major import on the demonstration that the defect in Parkinson's disease is generalized, for it is difficult to conceive how the platelets would be under the direct influence of the central nervous system.

The basic defect responsible for the weakened binding or retention of dopamine has not yet been identified. Recent studies in our laboratory [16] have demonstrated that a diet low in magnesium given to young female dogs will result in specific decreases in the concentration of dopamine in the striatum. Similar changes in dopamine fluorescence have been shown in the C-cells of the thyroid gland in magnesium deficient animals by Stachura and Pearse [88]. If these observations are confirmed, it may be worthwhile in Parkinson's disease to search for a generalized defect of magnesium metabolism or for a generalized metabolic change secondarily affecting magnesium concentrations. Studies along these lines are continuing.

(II) *Anatomical evidence*

There are also a number of anatomical arguments in favour of a generalized defect in Parkinson's disease. Lewy bodies have been found in almost all areas of the central and peripheral nervous systems where catecholamines are present. They have been identified in the substantia nigra, the dorsal nucleus of the vagus, the locus coeruleus, the median eminence of the hypothalamus, sympathetic ganglia, C-cells of the

thyroid, adrenal medulla, and sympathetic endings in mesenteric and renal vascular beds [52, 53]. The sphingomyelin content of these bodies has been characterized by Jager [53]. Recently the same author was able to identify abnormal sphingomyelin accumulations in the adrenal gland of parkinsonian patients [54].

(b) *Therapeutic research*

The use of Levodopa in the akinetic-rigid form of the illness is now recognized as the treatment of choice [6, 7] despite a number of disagreable side-effects of central or peripheral origin [10]. In recent years the peripheral side-effects of nausea, vomiting, cardiac arrhythmias and, to a lesser degree, orthostatic hypotension, have been minimized with the use of peripheral dopa-decarboxylase inhibitors (D.C.I.) [9, 15]. Two such compounds are now being tested: Ro 4-4602 (for review ref. 18) and MK-486 [72]. This combination treatment has improved the ease of handling of Levodopa, permitting much lower dosage of the precursor and shorter induction phases of the therapy. To date there has been no clinical toxicity of any significance detected after nearly 4 years of experimental human use of these compounds.

Unfortunately the combination of Levodopa and a D.C.I. has not modified the incidence of induced abnormal involuntary movements (A.I.M.) or the frequency and severity of the oscillations in performance noted with long term Levodopa therapy (see below. Neither has this combination improved the efficacy of Levodopa upon the symptom tremor in Parkinson's disease. Because of these limitations the search for still other approaches goes on.

One of the logical avenues explored was the use of analogues of DOPA which could act upon the striatal dopamine receptors. Apomorphine is such a substance, and had been used many years ago in the treatment of Parkinson's disease by Schwab [82]. It has recently received experimental attention in the U.S.A. [35] and France [31]. There is no doubt that apomorphine can act as an antiparkinson drug, but for the moment the side-effects of nausea and pre-renal azotemia seem to preclude any long term therapy with this agent. Newer derivatives are being prepared and will shortly undergo preliminary clinical trials.

It should be noted that a clinical paradox is observed when apomorphine is used as opposed to Levodopa. This latter drug is most effective against akinesia and secondarily against rigidity. Tremor, when helped, is reduced only after some months of treatment and rarely disappears. Presumably [51] Levodopa acts through its conversion to dopamine and through stimulation of dopaminergic receptors in the striatum. On the other hand apomorphine, which is known as a specific dopamine receptor agonist [90], is most effective against tremor, then rigidity and only partially against akinesia. Trivastal (Servier, S.A.), an analogue of apomorphine, possesses the same clinical properties [32]: again tremor is the symptom best improved. This observation may force us to reevaluate the postulated mechanism of action of Levodopa through the predominent stimulation of dopamine receptors.

In recent months we have been investigating the human physiology and pharmacology of a peptide whose structure is thought to be that of the MSH release-inhibitory factor [68]: L-prolyl-L-Leucyl-glycine-amide (L-Pro-L-Leu-Gly-NH_2). This substance has been shown to potentiate L-DOPA in animal tests [77] and to counteract oxotremorine induced tremor [78]. In preliminary clinical trials [58], we demonstrated that L-Pro-L-Leu-Gly-NH_2 can improve mainly the symptoms of rigidity and tremor and appears to decrease the incidence and amplitude of Levodopa-induced A.I.M. Controlled clinical trials are now underway. Only the results of these will permit any assessment of therapeutic usefulness in Parkinson's disease and other movement disorders and should precede any discussion of the mode of action of this substance.

(c) *Study of the long term side-effects*

As more experience is gained with Levodopa therapy, it is becoming evident that two major problems still face the experimental neurologist: the presence of induced-

abnormal involuntary movements [10] and the development of a late appearing oscillation in performance. The latter phenomenon, sometimes called the "on and off effect" is becoming more frequent in our practice and has therefore received considerable attention both in the laboratory and in the clinic. We have recently described four types of oscillations in performance [1,2]. They are listed in *Table 1*. The main point to remember is that the different types are actually phases of the same continuous inexorable process. All patients receiving high daily doses of Levodopa eventually enter this cycle. At the end they become dependent on the presence of A.I.M. for a kinetic response. Otherwise the patients assume a new form of hypokinetic, astasic, bradykinesia which is different, but often more incapacitating, from that present before Levodopa therapy. It is not yet certain whether this phenomenon is secondary to Levodopa or is the unmasking of a relentless progression of the underlying illness to stages not previously observed. However in favour of the former explanation would be the fact that our experience with a gradual, slow, reduction in total daily dosage of Levodopa in most of these subjects has permitted the reversal by stages of the clinical picture (from phase 4 to phase 3 or even 2 in certain patients - unpublished results). In a search for mechanisms to explain the "on and off" effect we have been drawn to the fact that Levodopa can produce many changes in hormonal balances. It is known, for example, that Levodopa increases growth hormone release [27], inhibits prolactin secretion [42] and in certain conditions will also influence T.R.H. [86], ACTH [92] and glucose metabolism [91]. In previous studies we had shown [8, 11] that the striatum, and particularly the striatal dopamine receptors, form an important relay station in the feedback integration of hormonal and autonomic functions. It is thus conceivable that secondary hormonal modifications of the responsiveness of striatal receptors is responsible for the pathogenesis of the oscillations in performance. Studies at the molecular biochemistry level are in progress in conjunction with clinical experiments investigating the results of hormonal approaches to this problem.

The second side-effect of importance on long term Levodopa therapy is the transformation of the A.I.M. from simple stereotyped phenomena to more complex choreic and dystonic dyskinesias [10]. The investigation of this phenomenon has led to the realization that there exist within the brain a complex interplay of neurotransmitter balances [8, 11] and that modifying the concentration of one neurotransmitter (*i.e.* dopamine) may result in clinical changes

TABLE 1

OSCILLATIONS IN PERFORMANCE DURING LEVODOPA THERAPY

1 — Type One (first stage) oscillations
- a) Diurnal (circadian) rhythm of akinesia
- b) Essentially a deficiency syndrome
- c) No abnormal movements
- d) Corrected by modifications in treatment schedule

2 — Type Two (second stage) oscillations
- a) Presence of stereotyped dyskinesias with their own temporal pattern
- b) Persistence of independent oscillations in akinesia
- c) Correction more difficult without loosing efficacy

3 — Type Three (third stage) oscillations
- a) Sudden akinetic "crises", usually pre-dyskinesias
- b) "Free" periods related, but not yet dependent on presence of dyskinesias
- c) Slight decrease in overall effectiveness of Levodopa therapy with more severe dyskinesias

4 — Type Four (fourth stage) oscillations
- a) Presence of periods of "akinesia paradoxica"
- b) "On and off" phenomena (rapid onset and termination of both akinesia and abnormal movements
- c) Obligatory dependence of "free" periods on the presence of abnormal movements.

through the effect of many others (serotonin, histamine, acetylcholine, GABA, ... etc.). Moreover basic changes in protein chemistry as nucleic acid composition of brain cells are produced by long term Levodopa administration [93]. Which of these modifications is involved in the pathogenesis of choreic or dystonic movements is still completely unknown.

One useful side-effect of these observations has been the renewed interest in the biochemistry of diseases such as Huntington's chorea.

II — HUNTINGTON'S CHOREA

The eventual therapy and genetic prediction of Huntington's chorea will depend on the accurate identification of the primary biochemical defect of this autosomal dominant disease. As recently as 1966 and 1968, two reviews by Myrianthopoulos [67] and Bruyn [29] failed to identify any specific biochemical defect in Huntington's chorea. However, at the Centennial Symposium held in March 1972 in Columbus, Ohio, we had the occasion to take a second look at this problem [17] and to indicate that a number of promising avenues had suddently appeared, mainly as a fall-off from the observations in Parkinson's disease with Levodopa therapy. There has been further progress even since that recent Symposium [13] and now we can state with confidence that we stand at the threshold of important developments.

Early studies relating to catecholamine metabolism in Huntington's chorea were on the whole negative, except for an indication of decreased concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and increased output of noradrenaline in the urine [60]. More recent reports [17] indicate that these may be a shift in metabolism towards methylated derivatives of dopamine rather than through the usual DOPAC pathway. Simultaneously noradrenaline synthesis appears increased as evidenced by enhanced activity of dopamine-β-hydroxylase [61].

There is a considerable wealth of information from human and animal pharmacological studies which indicates that the metabolism of cerebral monoamines must be involved in the pathophysiology of the choreic syndrome. These studies illustrate that the abnormal movements of Huntington's chorea are improved by a variety of agents, such as reserpine, tetrabenazine, alpha-methyl-paratyrosine, alpha-methyl-dopa, phenothiazines and butyrophenones, all of which act by interferring with normal dopamine metabolism in the basal ganglia, either by depleting the amine, by substituting for it or by blocking the specific receptors on which it acts [17]. Conversely Levodopa will make choreic symptoms worse and has even been used as an experimental predictive test [60]. This would seem to indicate increased concentration, or at least increased utilization, of dopamine within the extrapyramidal centers of the brain.

However, early studies by Ehringer and Hornykiewicz [39] had shown that striatal dopamine concentrations were normal, with slightly decreased H.V.A. values. More detailed recent regional determinations by Bernheimer and Hornykiewicz and by Ando and Barbeau (unpublished) now indicate that caudate dopamine and H.V.A. concentrations are significantly decreased, but that putamen and substantia nigra values are within normal limits. This is true in both the classical adult form and in the juvenile cases. This specific distribution is different from the findings in Parkinson's disease where the putamen and substantia nigra are specifically involved [39]. These observations may be of great importance and may indicate that one should not search for a defect in the nigrostriatal dopamine pathway, but rather in as yet unchartered pathways perhaps from the centre median of the thalamus[64] or the hippocampal complex.

The next chapter of recent importance is the emphasis placed on the state of basal ganglia receptor responsiveness in different disorders of the extrapyramidal system. It is becoming more and more evident that absolute concentrations of amines may have little true significance. Turnover rates and active concentrations of the neurotransmitters at the receptor site are one side of the picture, the state of responsiveness of that receptor being the other. Feedback neuronal and hormonal mechanisms are in-

creasingly recognized [8] as fundamental factors which should be monitored in each individual disease condition. We feel that Huntington's chorea should be considered as a state of dopaminergic dominance whereas Parkinson's disease is a state of cholinergic dominance (Fig. 1).

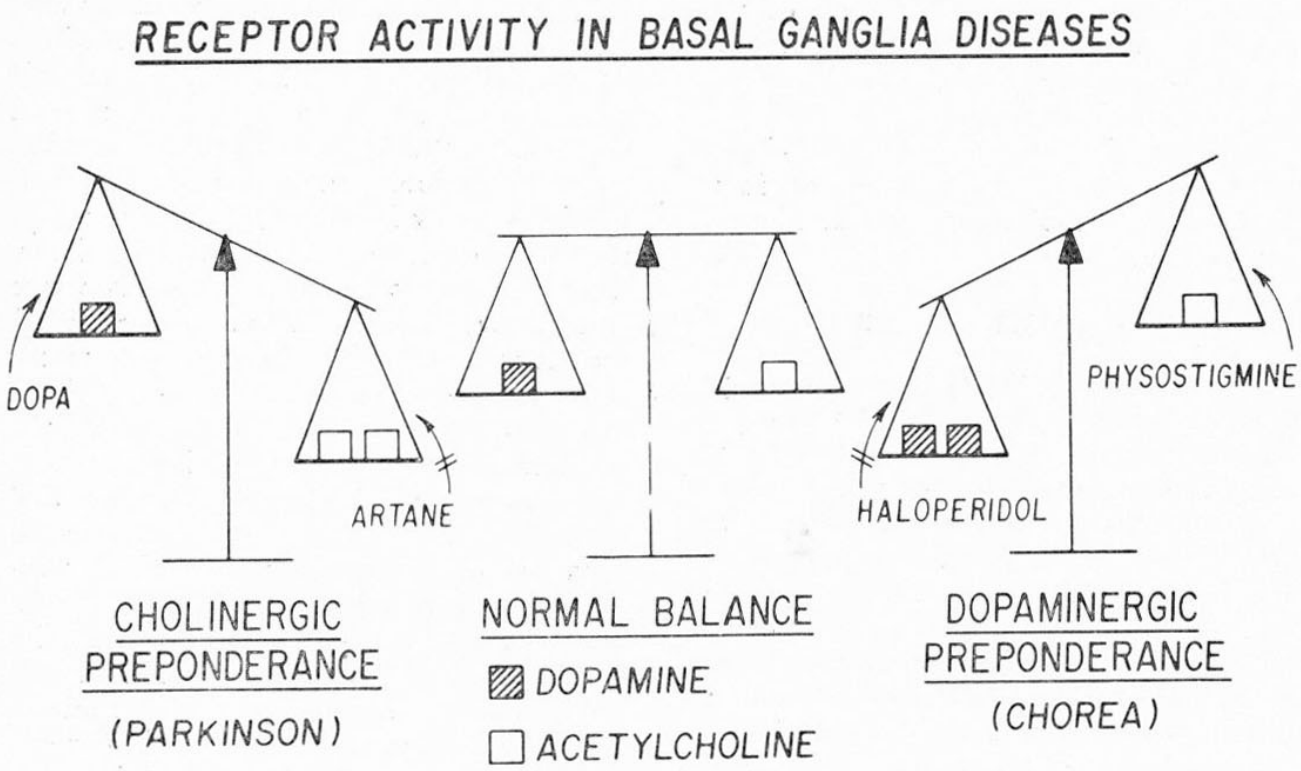

Fig. 1. — *"Amine balance" concept in extrapyramidal disorders.*

Recent developments regarding the possible role of a new putative neurotransmitter system in the basal ganglia should now be considered. The demonstration of the neurotransmitter role of gamma-amino-butyric acid (GABA) was accomplished in simple nervous systems such as that of the lobster, before it was established in the brain. It has now been shown that GABA is released from mammalian brain surface during inhibitory activity or after stimulation of subcortical regions [55, 56]. Generally, iontophoretic applications of GABA have revealed this substance to have inhibitory properties, especially in the spinal cord and cerebellum [69].

The distribution of GABA in the central nervous system and of the enzyme responsible for its synthesis, glutamic acid decarboxylase (GAD) have recently been studied with new methods [33, 40]. High levels of GABA and GAD have been noted in structures associated with the globus pallidus and substantia nigra [40, 41]. Lesions to the region of the globus pallidus in cats cause a decrease in GAD in the substantia nigra, but not in the caudate nucleus. Lesions in the caudate, on the other hand, cause decreases in GABA concentrations in the substantia nigra and in the globus pallidus. Recently some evidence has been obtained to show that the inhibitory influence from the caudate to the substantia nigra is mediated by GABA [41]. Thus it is likely that a GABA-ergic pathway exists between the caudate nucleus and the substantia nigra, on the one hand, and the globus pallidus on the other [59]. This is illustrated in *Fig. 2.* This pathway is probably operating in balance or feedback fashion with the nigrostriatal dopaminergic fibers. From such studies we postulated [17] that in Huntington's chorea the GABA-ergic pathway may be deficient, this facilitating abnormal movements. This was demonstrated by the elegant studies of Perry and collaborators [75]

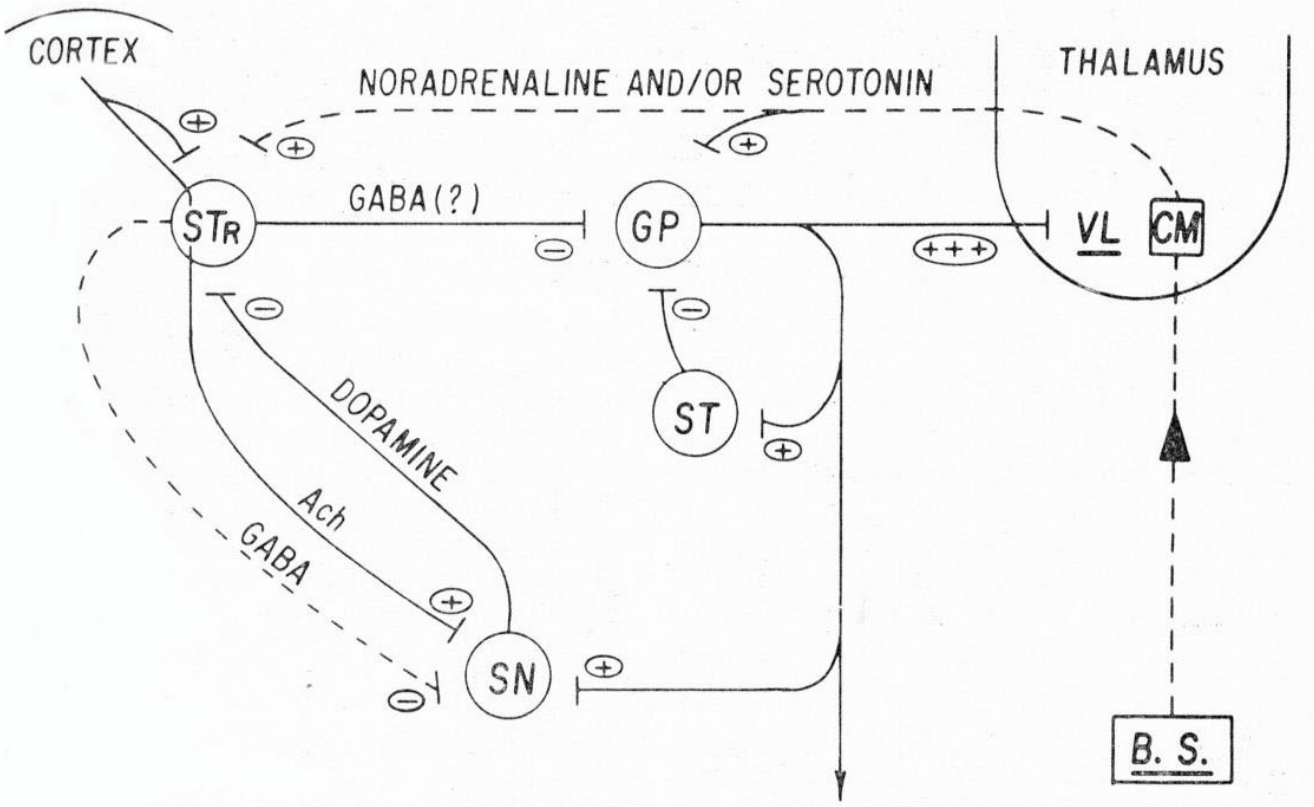

Fig. 2. — *Monoamine pathways in the basal ganglia.*

and since confirmed by us in adult and juvenile cases (Ando and Barbeau - unpublished results).

From such studies, a therapeutic approach immediately suggests itself, on the model of the "replacement" therapy with Levodopa in Parkinson's disease. GABA crosses the blood-brain barrier with difficulty, but a number of GABA analogues are now being tested. Initial results (Barbeau - unpublished data) with one of these analogues justify further control studies.

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SUMMARY

We nave reviewed recent developments in neurochemistry which relate to the pathophysiology of Parkinson's disease and Huntington's chorea. We have focussed on anatomical and biochemical arguments in favour of generalized defect in the mechanism of dopamine binding in Parkinson's disease and shown that certain new findings may permit the linking of magnesium metabolism to this generalized defect. In Huntington's chorea we demonstrated a localized deficiency in caudate dopamine and probably also a defect in the newly identified stio-nigral GABA-ergic pathway. New therapeutic approaches derived from these observations are outlined.

RESUMEN

Hemos revisado los recientes avances concernientes a la fisiopatología de la enfermedad de Parkinson y la corea de Huntington. Sobre bases anatómicas y bioquímicas, hemos insistido en favor de una alteración generalizada en el mecanismo de la conjugación o enlace de la dopamina en la enfermedad de Parkinson. Estudios recientes nos hacen pensar en una posible relación entre este defecto generalizado y el metabolismo del magnesio. Con respecto a la corea degenerativa hereditaria, hemos demostrado que existe una deficiencia de dopamina, localizada en el núcleo caudado y probablemente una alteración del sistema GABA-ergico estrio-nigral. Estas observaciones nos hacen pensar en nuevos aspectos terapéuticos.

RÉSUMÉ

Nous avons passé en revue les développements récents concernant la pathophysiologie de la maladie de Parkinson et de la chorée de Huntington. Nous avons insisté sur les arguments anatomiques et biochimiques en faveur d'une atteinte généralisée du mécanisme de liaison de la dopamine dans la maladie de Parkinson et montré certaines voies nouvelles qui permettront peut-être de relier le métabolisme du magnésium à ce défaut généralisé. En ce qui concerne la chorée dégénérative héréditaire, nous avons démontré qu'il existe une déficience en dopamine localisée au noyau caudé et probablement une atteinte du système GABA-ergique strio-nigral. De nouvelles approches thérapeutiques découlant de ces observations sont entrevues.

ZUSAMMENFASSUNG

Wir haben neue Gesichtspunkte hinsichtlich der Pathophysiologie der Parkinson'schen Krankheit und der Chorea Huntington besprochen. Besonderen Wert haben wir auf die anatomischen und biochemischen Argumente gelegt, die für eine allgemeine Beeinträchtigigung des Mittler-Mechanismus durch Dopamin sprechen. Wir haben neue Wege gezeigt die es eventuell ermöglichen, den Magnesium Metabolismus mit diesem generellen Defekt in Verbindung zu bringen. Hinsichtlich der erblichen debenerativen Chorea haben wir gezeigt, dass hier im nucleus caudatus ein lokaler Mangel an Dopamin vorliegt und dass wahrscheinlich das GABA-ergische strionigrale System beeinträchtigt ist. Neue Möglichkeiten der Behandlung, die sich aus diesen Beobachtungen ergeben, werden besprochen.

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Neurological diseases as reflections of general metabolic disturbances

(Wilson's disease, Refsum's disease and metachromatic leukodystrophy) by

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INTRODUCTION

A number of conditions described in text books of neurology have been regarded as essentially disorders of the nervous system, central or peripheral. Instances of such include hepatolenticular degeneration described by Wilson [55], where specific pathological lesions were found in the brain associated with cirrhosis of the liver. Refsum [42] recorded his findings in patients where there was involvement of the peripheral nerves together with the other symptomatology of the condition that now bears his name. The German and British neurological literature is full of clinical and histological descriptions of a condition now called metachromatic leukodystrophy. Within the past decade or two it has been realised, mainly as a result of biochemical investigation, that in all three conditions there is a generalized metabolic disturbance, which is not limited to the brain and nerves but can be demonstrated in many differing tissues and body fluids.

It is the purpose of this paper to illustrate the widespread metabolic abnormality of these three conditions by personal observations and from reports in recent literature on the subject.

Hepatolenticular Degeneration.

The clinical features of this condition are given in many neurological text books but can briefly be said to consist of disturbances of movement with tremor and rigidity, of liver and renal changes as well in a proportion of patients of some intellectual impairment and of joint abnormalities. Apart from young children all show a Kayser-Fleischer ring. Most of the clinical features were recognised by the earlier writers (Wilson [55], Hall [26], Lüthy [34], Denny-Brown [17]). The condition was generally recognised as being hereditory, the familial nature being remarked upon by Wilson, and accounting for between 55 - 74 % of all patients, the remainder being sporadic cases.

It has now been generally accepted that the condition is due to an abnormality in copper metabolism as a result of studies by a number of workers culminating in a conclusive finding in 1948, when it was suggested that the disease was the result of an inborn error of metabolism (Cumings [8]). Since that time there have been many attempts to understand the nature of th biochemical defect but without final success.

Although details are available in many places it is desirable to record briefly the major findings, the better to comment on more recent studies which attempt to elucidate the cause. The sex and age ratio remain very similar to that already recorded (Cumings, 1968a) namely about 3 males to 2 females with the major incidence in the second and third decades of life, and this includes 106 personal cases as well as those of Strickland *et al* [50].

The condition has long been accepted as inherited as an autosomal recessive trait (Bearn, 1953, 1960) and a recent study of

patients from Taiwan as well as Great Britain has confirmed this (Strickland *et al*[50]). These last authors considered that there were some unusual features between the two groups, in that females showed an earlier age of onset as well as of death than for males.

There was moreover a greater number of affected females in the Chinese group, but the reverse was found in the British patients. A further feature was that when more than one member of a family was affected it was more common for the affected subjects to be of the same sex.

It has been generally accepted that at death the liver is almost invariably abnormal, for in the literature there are only 4 patients where some form of cirrhosis was not present (Cumings [9]). Recently it has been found that Wilson's disease occurring in young children presents with signs which are almost invariably hepatic and not neurological (Saas-Kortsak *et al* [45]). While this clinical difference is not so noticable in more adult patients, yet as it has a bearing on the fact of the whole body and not merely the brain being affected, it is of interest to record the findings in some cases seen recently. Among 50 Chinese subjects (Strickland *et al* [50]) 13 showed hepatic signs and 25 neurological features as compared with 6 and 9 for the British group of 39 cases, while in a personal series the numbers were 24 and 50 respectively for all patients over 10 years of age. These figures indicate the high frequency of liver disturbance found clinically at some stage of the disease.

Kayser-Fleischer zones of pigmentation almost always are present in affected adults but in children under 10 years or so of age they may not be found (Cumings [16], Strickland *et al* [50]). Renal tubular acidosis is present in a few cases and is often correctable but is a further indication of the widespread pathology of the disease.

Biochemistry

Although there are few additional aspects concerned with the major biochemical features which have been recorded recently, Table 1 shows the abnormalities that are commonly present.

TABLE 1

Abnormal biochemical features in Wilsons disease

	Normal	Wilsons Disease
Brain copper in mg/100 g dry tissue ..	2 — 6	15 — 50
Liver " " ..	up to 10	20 — 150
Kidney " " ..	up to 3	Up to 130
Serum copper in µg/100 ml	85 — 115	40 — 60
Urinary copper in µg/day	up to 30	100+
Serum Caeruloplasmin in mg/100 ml.	20 — 40	up to 20
Bile Copper in µg/100 ml.	up to 205	Reduced
	126 ± 68	43 ± 6

It is accepted that there is a deposition of copper in the tissues generally and that there is an increased urinary excretion. Serum levels of copper and of caeruloplasmin are both definitely reduced except in the very occasional patient, while levels of copper in the bile are usually at the lower end of normal (Walshe [53], Frommer [24]).

Recent Advances

Many of the studies which have been conducted over the past few years relate to copper transport, the nature of caeruloplasmin, the question of biliary excretion of copper and the nature of copper complexes in bile, and these will be discussed in greater detail for they have a bearing on extracerebral metabolic activity.

Copper transport
Normal

Copper is taken in from the diet and in the stomach converted to Cu^{2+} by acid in that organ. It is absorbed from the stomach and small intestine, transported to the liver bound to serum albumin (Bearn and Kunkel [7]) concentrated in hepatocytes where a small proportion combines with apocaeruloplasmin to form caeruloplasmin. Part of the liver copper is excreted in the bile, a small proportion passes to the various organs and some is excreted in the urine. Much of the detail concerning these facts has been established by the use of isotopes ^{64}Cu and ^{67}Cu (see Walshe [53], for a full discussion). Frommer [24] in a recent study was able to show that the normal rate of biliary excretion of copper using duodenal intubation was of the order of 164 ± 0.8 μg for every 20 mins of the test period.

Wilson's Disease

Most of the definitive observations have been made using isotopes of copper. Initially Earl, Moulton and Selverstone [19] and Bearn & Kunkel [6], were able to show differences in the binding of copper to the various proteins in blood plasma. Since that time Scheinberg, Walshe and their colleagues have demonstrated considerable differences not only in transporting the blood but also relating to the deposition of copper in the liver, kidneys and other organs. Osborn and Walshe [39] showed that in the normal liver some 90 % of intravenously injected radioactive copper was present at 10 hours falling to 50 % after 70 hours as compared with only 25 % at 10 hours and 37 % at 50 hours in a case of Wilson's disease. Sternlieb and his colleagues [49] have investigated both *in vivo* and *in vitro* the fate of caeruloplasmin labelled with isotope to determine any special properties concerning caeruloplasmin and its copper binding (see Walshe [53]). It would appear that in Wilson's disease copper is not used normally in the build up of caeruloplasmin, nor excreted normally by the bile. The liver thus fails to remove copper from the blood which is then deposited in the various organs, or excreted in the urine. Frommer [24] has shown that the bile from cases of Wilson's disease not only contains less copper but the actual rate of excretion of copper is reduced by about half. Wintrobe and others originally stated hat in view of a positive copper balance there was over absorption of copper from the gut but Frommer's results suggest that the positive balance, if it exists and there is some doubt, is due to the diminished ability of the liver to secrete copper with the bile.

One further aspect relating to bilary excretion of copper can be mentioned, namely that according to Lewis [33] the copper is not complexed to protein or present as free ionic copper but is associated with taurochenodeoxycholate.

Copper toxicity

When given or taken in large doses copper can give rise to intestinal disturbance in man, while in animals as in sheep it can result in haemolytic jaundice; similarly in man haemolytic anaemia is found in some patients with Wilson's disease (McIntyre *et al* [35]). Certain enzymes may be affected in toxicity from copper, as glucose-6-phosphate dehydrogenase (Fairbanks [23]). Peters and Walshe [40] found that in pigeons copper could result in convulsions and cause death

with an associated inhibition of oxygen uptake, which Peters, Shorthouse and Walshe [41] suggested later was the result of an action of the metal mediated through inhibition of membrane ATPase. It has been suggested that the effect is basically lysosomal in origin.

Caeruloplasmin

There have been very many studies on this enzyme ever since Holmberg and Laurell discovered it in 1948. Attempts to determine if it was structurally different in Wilson's disease have been made, but Uriel, Götz and Grabar [51] found no evidence of any abnormality in an inmunological study, nor did Holtzman *et al* [30] more recently.

It has been found possible to prepare an asialo-caeruloplasmin which after suitable isotope labelling can be followed in animal experiments. This substance can be detected in hepatocytes and the rapid uptake appears to be dependent upon the exposure of the terminal galactosyl residue (Morell *et al* [37]). These authors suggest that if in Wilson's disease an abnormal caeruloplasmin, one with an abnormal or one without a galactosyl moiety, was present this might be the explanation for some of the findings in the disease.

Nature of the disease

In addition to the suggestion of Morell *et al* [37] concerning abnormality of the structure of caeruloplasmin there are a few other possibilities which have been mentioned. Originally it was thought that the cause of the condition was a lack of caeruloplasmin but when it was found that occasionally normal enzyme levels were present in the disease (Cumings [12]) this theory was untenable. It was suggested that the apocaeruloplasmin to which copper was combined might be abnormal but apart from the work of Morell *et al* [37] there is little positive evidence. Another possibility was that a further enzyme was required for the combination of copper to apocaeruloplasmin but as yet this is unproven. Frommer [24] has suggested that in the disease there is a block to transport of copper after it has entered the hepatocyte, and prior to incorporation into caeruloplasmin.

Final comments and discussion

There are many other interesting features one of which concerns the diagnosis of the disease in sibs of known cases (Cumings [12]) and another relates to methods of therapy but these are well documented (Walshe [52], Cumings [13]).

From the foregoing it is clearly seen that whereas when Wilson gave his classical description of hepatolenticular degeneration it appeared to be a neurological disorder, it is now clearly a generalized metabolic disturbance of copper metabolism in which some enzyme defect associated most probably with a liver derangement is present. Further the enzyme may be of lysosomal origin connected with the hepatocytes of the liver and is probably unassociated with any abnormality of intestinal absorption. The exact biochemical defect still eludes the scientist as well as the clinician.

Refsum's disease

Refsum [42–43] described a condition in patients showing a peripheral neuropathy, ataxia, deafness, night blindness and a form of retinitis pigmentosa. This appeared to be a typically neurological disorder clinically well described in many neurological text books. Although its morbid anatomical appearances were well documented its basic biochemical abnormalities were not discovered until 1963, when Klenk and Kahlke [32] detected in the blood and some tissues a markedly raised level of a branched-chain fatty acid, known as phytanic acid (3, 7, 11, 15 - tetramethyl hexadecanoic acid). This finding resulted in a series of papers by many workers concerning the biochemical aspects as well as procedures concerning therapy of the disease.

Tissue and body fluids

In the normal subject there is no phytanic acid in the blood serum, while in the brain and other organs only traces are found. The patient with Refsum's disease accumulates

this branch chained acid in many organs and tissues as well as in the blood because as it will be shown later there is a specific enzyme deficiency. Table 2 illustrates the findings which have been seen in some 22 cases personally studied (Cumings [16]).

TABLE 2

Phytanic acid in Refsum's disease

Blood Serum	4.4	—	37.0 %	of total fatty acids.
Cerebrospinal fluid	7.0	—	22.5	"
Brain	Traces	—	2.9	"
Eye - choroid			14.9	"
Liver	12.3	—	34.4	"
Spleen	2.0	—	5.6	"
Heart	22.2	—	25.2	"
Peripheral nerve	3.7	—	5.7	"
Optic nerve	1.2	—	5.4	"

The condition is therefore one with a generalized abnormality even though the brain is minimally involved.

Phytanic Acid. This acid is a normal metabolite originating initially from acetyl and CoA and mevalonic acid. Although the pathway has been studied by a number of workers (see Wilson and Thompson[54], the degradation of phytanic acid was investigated by Eldjarn[21] and by Eldjarn *et al*[22] but the results obtained were not conclusive as to the normal pathway. Steinberg *et al* [47] however demonstrated the fundamental importance of α - oxidation in the normal metabolic chain of events by which phytanic acid was converted to pristanic acid after which β - oxidation took place in a series of steps. The enzyme which results in α - oxidation is known as phytanic acid α - hydroxylase and it has been shown that even skin fibroblasts contain the enzyme. In Refsum's disease this enzyme is greatly reduced or lacking in tissues, in skin fibroblasts and also in amniotic cells while in some heterozygotes there is approximately a 50 % reduction of the enzyme (Herndon *et al* [27–28]).

Therapy. The raised level of phytanic acid and the absence of the enzyme indicated to some workers that it might be worth reducing the intake of precursors of phytanic acid, such as phytol, in the diet (Steinberg *et al* [47], Eldjarn *et al* [22], Steinberg *et al* [48]. Results have been partially successful but some clinical features as deafness and deficient vision have not been improved. Skrbic and Cumings [46] found raised tissue cholesterol, particularly esterified cholesterol, in the tissues of those who died and this may partly account not only for death but also for failure in therapy. The damage these authors found was present not only in nervous tissue but more particularly in the heart and liver, thus stressing the general nature of this disorder.

Metachromatic Leukodystrophy

The clinical and histopathological features have been known in greater or less degree since 1928. The condition is seen mainly in three age groups, late infantile, juvenile and adult (or adolescent) accord-

ing to the age of onset, and there are slight differences of the length of the disease. A total of 38 cases have been seen personally of which 3 have fallen into the adult group (see Cumings [15]). The histological features have been well described by Diezel [18]. Recently the electronmicroscopic appearances (EM) have been described, in which the abnormal lipid which is deposited especially in the white matter has been shown to correspond to a pseudocrystalline structure (Resibois [44]); other inclusions within oligodendroglia and macroglia have been reported by Gregoire *et al* [25].

Biochemistry

Edgar [20] initially demonstrated a raised hexosamine level in the white matter of the brain, and shortly after Austin [2] showed an increase of sulphated cerebroside (sulphatide) to be a characteristic feature of the disease. This has been confirmed by many workers since (see Cumings [15]).

The abnormality has been shown not only to affect the brain but also the kidney and leucocytes. Further the peripheral nerves were shown to be involved first by histological examination and then by means of nerve conduction studies. These findings indicated the wide-spread nature of this condition.

It was early shown that the urine contained a raised content of sulphatide (Cumings [11]) and that in the cells of the urinary sediment metachromatic material was present (Austin [1]).

A typical example of the findings in the brain of one case is illustrated in Table 3.

TABLE 3

Cerebral lipids in metachromatic leukodystrophy in a boy of 11 years

Cerebral white matter

	Patient	Normal
Total Phospholipid	22.1	25.0
Total Cholesterol	8.3	13.0
Esterified Cholesterol	0.5	0.3
Total Cerebroside	2.2	13.4
Sulphatide	5.6	1.6
Water %	83.9	67.9

Results in g/100 g dry tissue

Not only can an increase of sulphatide be found in the cerebral white matter but it has been demonstrated that myelin isolated from the brain is abnormal, for sphingomyelin is reduced by more than half, and cerebroside by 90 %. Sulphatides are increased five-fold, so that whereas in normal myelin the cerebroside: sulphatide ratio is 4:1 in metachromatic leukodystrophy myelin it is 1:20 (Cumings [14]).

Enzymic Defect

The condition was assumed to be a genetically determined defect (Cumings [10]) and accordingly a search for an enzyme defect was made by a number of workers, who thought a diminished sulphatase activity would be present. It has been shown that aryl-sulphatase A is defective being very considerably reduced or completely absent is brain tissue (Austin *et al* [3], Mehl and Jatzkewitz [36] and Jatzkewitz and Mehl [31]). The enzyme is normally present in many other tissues, but in patients with metachromatic leukodystrophy it has been shown to be reduced or absent in the kidney, in blood leucocytes and in cultured skin fibroblasts. Nadler and Gerbie [38] have demonstrated a deficiency of the enzyme in cultured amniotic fluid fibroblasts. The condition is not only widespread from a histological approach but the biochemical abnormality of a defective enzyme activity has been shown to affect many areas of the whole body.

Final Comments

These three disorders have been chosen to illustrate that although each was originally considered to be essentially a neurological disease all of them affect many other areas than the nervous system. It is almost certain that other genetically determined diseases will eventually be placed alongside these three, and in fact various other lipid diseases, as the gangliosidoses, can be included in generalized metabolic disturbances.

SUMMARY

A description in biochemical terms is given of three diseases originally considered as essentially neurological in character. It has been shown that in Wilson's disease, Refsum's disease and metachromatic leukodystrophy there is a widespread biochemical abnormality present, consisting of an absence or a reduction of an enzyme, which is normally present in many tissues of the body. Observations in regard to these conditions, chosen as representative of a group of neurological diseases, are drawn from the many patients investigated with these conditions.

RESUMEN

Damos una descripción en términos bioquímicos, de tres enfermedades originalmente consideradas de carácter esencialmente neurológico. Ha sido demostrado que en la enfermedad de Wilson, en la enfermedad de Refsum y en la leucodistrofia metacromática existe una extensa anormalidad bioquímica, consistente en la ausencia o reducción de una enzima normalmente presente en muchos tejidos del cuerpo. Las observaciones referentes a estas condiciones, elegidas como representativas de un grupo de enfermedades neurológicas, han sido obtenidas de muchos pacientes investigados con estas condiciones.

RÉSUMÉ

Une description en termes biochimiques est donnée de trois maladies autrefois considerées de caractère essentiellement neurologique. Il a été démontré que dans la maladie de Wilson, la maladie de Refsum et la leukodystrophie métachromatique il existe une abnormalité biochimique répandue, qui consiste d'une absence ou d'une réduction d'une enzyme qui est normalement présente dans nombreux tissus du corps. Des observations concernant ces conditions, choisies comme représentatives d'un groupe de maladies neurologiques, sont tirées d'examinations de nombreux malades ayant ces maladies.

ZUSAMMENFASSUNG

Eine Beschreibung in bio-chemischen Begriffen von 3 Erkrankungen ist gegeben, welche erwogen wurden als wesentlicher neurologischer Beschaffenheit. Es hat gezeigt, dass bei morbus Wilson, morbus Refsum und metachromatischer Leuko- Dystrophy eine weitverbreitete biochemiche Abnormalitaet vorliegt. Diese besteht aus einer Abwesenheit oder Verminderung eines Enzyms, welches normalerweise in vielen Geweben des Koerpers anwesend ist. Beobachtungen zu diesen Verhaeltnissen, gewaehlt als Vertreter einer Gruppe neurologischer Erkrankungen, wurden gemacht an vielen Patienten, welche untersucht wurden auf diese Verhaeltnisse.

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Studies on the Differential Diagnosis of Muscle Glycogenoses:

The Hexose-monophosphates Contents in Muscles with Lowered Activity of Phosphorylase

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In 1951 McArdle [1] presented a paper dealing with a new syndrome characterized by intolerance to severe exercise and occurrence of muscle stiffness and cramps on exertion. He described the failure to show a rise in venous lactate in response to an ischemic forearm exercise in the syndrome, postulating a defect in one of the enzymes necessary for breakdown of glycogen to lactate. It was not long before the biochemical analyses [2, 3] on muscle biopsies of similar cases revealed that McArdle's syndrome is due to an absence of muscle phosphorylase activity. In 1965 three patients from a single family complaining of easy fatiguability without any neurological abnormalities were extensively studied in our laboratory [4], and it was demonstrated that there was a normal activity of phosphorylase and an almost complete lack of phosphofructokinase in muscle specimens of the patients. Mc Ardle's disease and our disease have ever since been designated as Type V and Type VII glycogenoses [5, 6, 7] respectively (Table 1).

Since continuous vigorous muscular exercise depends exclusively on the breakdown of glycogen via Embden-Meyerhof's glycolytic pathway in muscles and both phosphorylase and phosphofructokinase are key enzymes of this pathway it is no wonder that Type V and Type VII glycogen-storage disease share common clinical symptoms including life-long history of muscle weakness, stiffness and pain produced by exercise, occasional myoglobinuria, lack of rise in venous lactate after ischemic works, and marked glycogen storage in skeletal muscles. Consequently it has become important to establish the several points which could provide a specific diagnosis.

As one of the candidates for these points, we[4] have pointed out the marked accumulation of hexose-monophosphates in muscles lacking phosphofructokinase activity. In this connection changes in glucose-6-phosphate and fructose-6-phosphate concentrations in muscles with McArdle's disease are of special interest. However, no patient with McArdle's disease has been as yet described in our country. Illingworth et al.[8] demonstrated that the total phosphorylase activity of the skeletal muscles of rats maintained on a pyridoxine deficient diet falls markedly after 7 to 8 weeks. Therefore, some biochemical characteristics associated with lowered activity of muscle phosphorylase seem to be analysable on such "experimental McArdle's disease" in rats.

The present study was designed to measure the activities of glycolytic enzymes (phosphorylase, phosphofructokinase, pyruvate kinase) and the concentrations of glycogen and glycolytic intermediates (glucose-6-phosphate, fructose-6-phosphate) in muscles of pyridoxine deficient rats and to compare the fluctuations of these parameters with those in muscles lacking phosphofructokinase activity. Another points

TABLE 1 — **CLASSIFICATION OF CLYCOGENOSES**

Type Brown & Brown	Enzyme Altered	Common Name in Papers	Principal Sites of Involvement	Glycogen Structure
I	Glucose-6-phosphatase	von Gierke's disease	Liver, kidney, thrombocytes	Normal
II	α - 1,4-Glucosidase (active at pH 4)	Pompe's disease	Liver, heart, muscle, leucocytes	Normal
III	Amylo-1,4-glucosidase and oligo-1,4-1,4 glucan transferase (debrancher)	Limit dextrinosis (Forbe's disease)	Liver, leucocytes, (muscle)	Outer chains missing or short
IV	α-1,4-Glucan: α-1,4-glucan 6-glycosyl transferase (bancher)	Amylopectinosis (Andersen's disease)	Liver, leucocytes, spleen (muscle)	Long inner and outer branched chains
V	Glycogen phosphorylase	(McArdle's disease)	Skeletal muscle	Normal
VI	Phosphorylase kinase or phosphorylase	(Hers's disease)	Liver, leucocytes	Normal
VII	Phosphofructokinase	(Tarui's disease)	Skeletal muscle, erythrocytes	Normal

of differential diagnosis on biochemical investigation between Type V and Type VII glycogenosis were also discussed.

Materials and Methods

Animals and Diets: Male wealing rats of Wistar strain were employed throughout. They were fed on a synthetic diet (Table 2) with or without pyridoxine hydrochloride for 3 months. The pyridoxine deficiency was confirmed by the estimation of total pyridoxal phosphate content of the liver using the apotryptophanase method [9]. After more than 2 months on the synthetic diet without pyridoxine hydrochloride, the pyridoxal phosphate level in liver fell to about 30 % of the control value obtained from rats fed with pyridoxine.

Measurements of glycolytic enzymes: Femoral muscles were dissected out immediately after decapitation from the rats which had been fasted for 16 - 20 hr. Muscle specimens were homogenized in an all glass homogenizer with cold Tris-EDTA buffer (0.05M Tris, 5mM EDTA, pH 7.4). The percentage of the homogenate was 20% for phosphorylase assay and 5 % for phosphofructokinase and pyruvate kinase assay.

TABLE 2 — **SYNTHETIC DIET**

With or Without

Pyridoxine hidrochloride	1.0 mg
Casein Vitamin free	18.0 g
Sucrose	73.3 g
Corn oil	4.0 g
McCollum's salt	4.0 g
Choline chloride	0.2 g
Vitamin A	1200 I.U.
Vitamin D	120 I.U.
Thiamine hydrochloride	1.0 mg
Riboflavin	1.0 mg
Nicotinic acid	4.0 mg
Calcium pantothenate	6.0 mg
Inositol	13.0 mg
p-Aminobenzoic acid	20.0 mg
Biotin	20.0 μg
Folic acid	20.0 μg

Values indicate each weight in 100 g of diet.

Then, centrifugation was performed for 30 minutes at 11,000 x g. For phosphorylase assay the homogenate was immediately diluted with the equal volum of 0.2M NaF before centrifugation. Supernatants were assayed at 24° C in a 1 ml cuvette in coupled

systems in which the enzymes to be measured were rate-limiting; phosphorylase activity was measured in a system coupled with glucose-6-phosphate dehydrogenase; phosphofructokinase was coupled with aldolase, triose-phosphate isomerase and α - glycerophosphate dehydrogenase; pyruvate kinase was coupled with lactate dehydrogenase. The protein in the sample fluids was measured by the biuret method [10].

Measurements of glycogen and glycolytic intermediates: The lower limbs of rats were plunged into liquid nitrogen simultaneously with amputation. Femoral muscles were separated from the frozen materials. The glycogen was isolated from the frozen muscles with the method of Somogyi [11] and determined using anthrone reagent as described by Hassid and Abraham [12].

Perchloric acid extracts of the muscles were assayed enzymatically for hexose-monophosphates using coupled systems linked to reduction of NADP.

Results and Discussion

The glycolytic enzyme activities in the skeletal muscle of rats maintained on a pyridoxine - deficient synthetic diets for 3 months are shown in Table 3. The activities of phosphofructokinase and pyruvate kinase, two of the key glycolytic enzymes were not changed in pyridoxine deficiency from the normal levels. On the contrary, a significant decrease was observed in the phosphorylase activity. As evidenced by Cori and Illingworth [13], phosphorylase a contains 4 moles of pyridoxal-5-phosphate per mole of enzyme. The activities of hepatic enzymes possessing pyridoxal-5-phosphate as the prosthetic group such as serine dehydratase, homoserine dehydratase, kynureninase, tyrosine transaminase, and soluble

TABLE 3 — **Glycolytic enzyme activities in the skeletal muscle of pyridoxine-deficient rats.**

	Control	VB_6-deficient	
Phosphorylase (7)	0.153 ± 0.037 *	0.074 ± 0.036	$p < 0.005$
Phosphofructokinase (5)	0.432 ± 0.118	0.454 ± 0.114	
Pyruvatekinase (3)	3.73 ± 0.11	3.42 ± 0.07	

unit: μmoles substrate converted/min./mg protein * Mean ± s.d.

The assay mixture had the following contents and was adjusted to 1 ml with distilled water.

Phosphorylase: 0.5M Tris (pH 7.4) 0.1ml; 0.1M sodium phosphate (pH 7.4) 0.1ml; 0.1M $Mgcl_2$ 0.1ml; 0.1M 1-cysteine hydrochloride 0.1ml; phosphoglucomutase 0.27 μ; glucose-6 phosphate dehydrogenase 0.7μ; 10mg/ml NADP 0.05ml; 2% glycogen 0.1ml; 0.1M 5'-AMP 0.01ml; supernatant 0.2ml. Reaction was started by the addition of glycogen.

Phosphofructokinase: 1M Tris (pH 8.0) 0.05ml; 0.2M thioethanol 0.05ml; 0.1M $Mgcl_2$ 0.01ml; 0.1M ATP 0.01ml; 0.1M fructose-6-phosphate 0.01ml; 2mg/ml NADH 0.06ml; aldolase 0.5μ; triose phosphate isomerase 1.0μ; α-glycerophosphate dehydrogenase 0.33μ; supernatant 0.01ml. Reaction was started by the addition of fructose-6-phosphate.

Pyruvate kinase: 0.1M Tris (pH 7.4) 0.49ml; 1M Kcl 0.1ml; 0.1M $Mgcl_2$ 0.05ml; 0.02M ADP 0.1ml; 0.02M phosphoenol pyruvate 0.1ml; 2mg/ml NADH 0.12ml; lactate dehydrogenase 0.9μ; supernatant 0.01ml. Reaction was started by the addition of ADP.

and mitochondrial glutamate alanine transaminase were decreased below one-third of the control levels when assayed without added pyridoxal-5-phosphate (not shown). Rates of glucose utilization by muscle homogenate and apparent activity of uridine diphosphoglucose-glycogen transferase were already shown not to be changed in pyridoxine deficiency (Beaton [14]; Illingworth et al. [8]) Phosphorylase is the only enzyme which requires pyridoxal-5-phosphate among the enzymes in the pathway of glucose and glycogen metabolism. Therefore, the step between glycogen and glucose-1-phosphate in the glycogenolysis was specifically blocked in the pyridoxine-deficient rats. In this sense it is possible to say that these rats had "experimental McArdle's disease".

Table 4 shows the changes in the concentration of glycogen and glycolytic intermediates in muscle specimens of the pyridoxine-deficient rats. The glycogen content of the muscles was found to be inclined to increase but the difference between the control and the pyridoxine-deficiency was not statistically significant. According to

TABLE 4 — **Glycogen and glycolytic intermediates in the skeletal muscle of pyridoxine-deficient rats.**

	Control	VB_6-deficient	
Glycogen [1] (5)	0.114 ± 0.125 [3]	0.135 ± 0.037	$p > 0.1$
Glucose-6-PO [2]$_4$ (8)	0.998 ± 0.175	0.600 ± 0.231	$p < 0.005$
Fructose-6-PO [2]$_4$ (8)	0.230 ± 0.069	0.126 ± 0.059	$p < 0.005$

1) mg/100mg wet weight.
2) μmoles/g wet weight.
3) Mean ± s.d.

Glycogen was determined as described in "Methods".

The frozen muscles were homogenized in the equal volume of cold 10 % perchloric acid; then, a neutralizing mixture containing 1M Tris (pH 8.0) and 4M KOH was added; after removal of $KC10_4$, glucose-6-phosphate and fructose-6- phosphate were assayed in two steps in the same medium enzymatically. Glucose-6-phosphate was determined first in a mixture containing 0.05M Tris (pH 7.5) 0.53 ml; 0.1M $Mgcl_2$ 0.05ml; 10mg/ml NADP 0.1ml; glucose-6-phosphate dehydrogenase 0.28μ; sample fluid 0.8ml. After this reaction was complete, phosphoglucoisomerase (0.39μ) was added to measure fructose -6-phosphate.

the available papers [8, 14, 15] muscle and liver glycogen levels were reported to be normal, moderately increased, or only slightly depressed in pyridoxine deficiency. However, the tissue concentrations of glucose-6-phosphate and fructose-6-phosphate were markedly decreased in the pyridoxine-deficient rats ($p<0.005$; $p<0.005$). Since phosphorylase is considered to be the rate-limiting enzyme controlling glycogenolysis, it is quite plausible that a depressed phosphorylase activity could lead to the decrease in the tissue concentration of hexose-monophosphates. It is in a marked contrast to muscles missing phosphofructokinase activity in which an excessive accumulation of glucose-6-phosphate and fructose-6-phosphate and an extreme fall in fructose-1,6-diphosphate concentration were demonstrated [4] (Table 5). An increased rate of glucose

TABLE 5 — **Glycogen and glycolytic intermediates in muscle specimens of patients with muscle phosphofructokinase deficiency.**

	Control (Mean ± s.d., 6 cases)	PFK - deficient (Mean of 3 cases)
Glycogen [1]	0.96 ± 0.18	2.95
Glucose-6-PO[2]$_4$	0.50 ± 0.30	5.4
Fructose-6-PO[2]$_4$	0.10 ± 0.05	0.95
Fructose-1,6-di PO[2]$_4$	0.61 ± 0.23	0.03

1) mg/100mg wet weight.
2) μmoles/g wet weight.

uptake in an isolated diaphragm of a pyridoxine-deficient rat, demonstrated by Guggenheim and Diamont [15], might be due to an increased activity of hexokinase caused by the lowered concentration of glucose-6-phosphate.

In Type V and Type VII glycogenoses the tissue concentrations of hexose-monophosphates change in the opposite direction. It certainly constitutes one of the crucial points for differential diagnosis. Besides this, the other several conditions presented in Table 6 are thought to be important for the differentiation of Type V and Type VII. In particular, the determination of erythrocyte phosphofructokinase activity seems to have an essential value in the clinical investigation in the viewpoint of the ease with which blood cells can be obtained from patients. The decreased activity of erythrocyte phosphofructokinase in Type VII glycogenosis was first demonstrated in this laboratory [4] and further characterization of muscle and erythrocyte phosphofructokinases has been performed [16, 17]. Muscle and erythrocyte phosphofructokinases are apparently separate isozymes [17]. However, the biosynthesis of enzyme protein of human erythrocyte phosphofructokinase appears to be in part under the same genetic control as muscle phosphofructokinase [16].

As causes for muscle glycogenoses, enzyme deficiencies of phosphorylase, phosphofructokinase and debrancher have been already described. What of other enzyme deficiencies as causes? The presence of a disease with phosphoglucomutase or phosphoglucoisomerase would be possible. since Thomson et al [18] and Satoyoshi and Kowa [19] presented case-reports in which data from *in vitro* studies of anaerobic glycolysis were compatible with such deficiency (No direct assay of the enzyme was made). The measurement of glycolytic intermediates would also provide a definitive diagnosis of phosphoglucomutase or phosphoglucoisomerage or phosphoglucoisomerase deficiency.

Is it possible that muscle glycogenosis is caused by the defect of a single enzyme in the glycolytic chain after the stage of fructose-1,6-diphosphate? The abnormal accumulation of glycogen in muscle could be brought about through the following two mechanisms.

(A) The defect of the enzymes which catalyze the reaction in which glycogen is substrate.

(B) The allosteric effect of glucose-6-phosphate in the high tissue concentration.

(1) activation of uridinediphosphoglucose-glycogen transferase

(2) competitive inhibition of 5'-AMP effect on phosphorylase [20, 21] (5'-AMP

TABLE 6 — **Differential diagnosis of Type V and Type VII glycogenoses on biochemical investigations.**

	Type V	Type VII
muscle hexose-monophosphates	decreased	markedly increased
breakdown of hexose-monophosphates by muscle homogenate	Normal	markedly decreased
muscle phosphorylase	deficient	normal
muscle phosphofructokinase	Normal	deficient
muscle pyruvate kinase	normal ?	increased
erythrocyte phosphofructokinase	Normal	decreased

is essential for the activity of phosphorylase b and 5'-AMP can also affect the activity of phosphorylase a in the low levels of substrates [22]).

Glycogen storage is caused through (A) in glycogenosis Type V and through (B) in Type VII. However if glycolysis is blocked after the stage of fructose-1·6-diphosphate in muscles neither (A) nor (B) might be as such expected. Presumably there would not be so many kinds of muscle *glycogen-storage* disease.. Since abnormalities of triose-phosphate isomerase, phosphoglycerate kinase and pyruvate kinase in erythrocytes have been demonstrated in non-spherocytic hemolytic diseases [23] and neuromuscular disorders were found to be associated with some of these abnormalities [24], it is quite possible that there could exist several disease entities due to the defect of the enzymes in Embden-Meyerhof's pathway after the stage of 1,6-diphosphate in muscles. If we would be confronted with a patient with long-standing muscle weakness associated with lack of rise in venous lactate at ischemic exercise and negative or minor glycogen storage in muscles, these possibilities must be taken into considerations.

SUMMARY

A significant decrease in the concentration of glucose-6-phosphate and fructose-6-phosphate was demonstrated in skeletal muscles of rats maintained on a pyridoxine-deficient synthetic diet for 3 months. Muscle specimens of the rats had specifically decreased activity of phosphorylase and normal activity of phosphofructokinase and pyruvate kinase. The direction of the change in hexose - monophosphates concentration in phosphorylase-deficient muscles is in a marked contrast to that in phosphofructokinase-deficient muscles, in which an extreme accumulation of hexose-monophates is observed. This point would provide a specific diagnosis of muscle glycogenoses. In addition, the other several important conditions were discussed for the differential diagnosis of glycolytic disorders in muscles.

The authors wish to thank to Prof. H. Wada and Dr. M. Takami for their helps in the preparation of the synthetic diet and to Prof. M. Nishikawa for guidance in this study.

RESUMEN

Una disminución significativa en la concentración de glucosa-6-fosfato y fructuosa-6-fosfato fue demostrada en músculos esqueléticos de ratas mantenidas con una dieta sintética deficiente en piridoxina durante 3 meses. Muestras de músculos de las ratas mostraban actividad específicamente disminuida de fosforilasa y actiidad normal de fosfo-fructoquinasa y piruvatoquinasa. La dirección del cambio en la concentración de hexosa-monofosfatos en músculos deficientes en fosforilasa está en marcado contraste con aquel de los músculos deficientes en fosfofructoquinasa en los cuales se observa una notable acumulación de hexosa-monofosfatos. Este punto brindaría un medio de diagnóstico específico de la glucogenosis del músculo.

Además variadas e importantes comprobaciones se discutieron para el diagnóstico diferencial de desórdenes glucolíticos en los músculos.

RÉSUMÉ

Une diminution significative de la concentration de glucose-6-phosphate et de fructose-6-phosphate a été mise en évidence dans des muscles squelettiques de rats maintenus avec une diéte synthétique pauvre un pyridoxine pendant 3 mois. Des échantillons de muscles de ces rats présentaient une activité spécifiquement diminuée de phosphorilase et une activité normale de phosphofructokinase et de pyruvatokinase. L'orientation du changement dans la concentration d'exose-monophosphates dans les muscles pauvres en phosphorilase est en réel contraste avec celui des muscles pauvres en phospho-fructokinase où on observe une remarquable accumulation d'hexo-monophosphates.

Ceci procurerait un moyen de diagnostic spécifique de la glucogénèse du muscle.

En outre, des resultats importantes ont té discutées pour le diagnostic differéntiel de désordres glucolitiques dans les muscles.

ZUSAMMENFASSUNG

Eine massgebende Minderung im der Konzentration von Glucose-6-phosphat und ruktose-6-phosphat wurde in Sklettmuskeln von Ratten gezeigt, die unter einer synthetischen Diät mit Mangel an Pyridoxin während 3 Monate gehalten worden waren. Muster von Muskeln der Ratten zeigten eine spezifisch verminderte Axtivität der Phosphorilase und normale Aktivität der Phospho-fruktokinase und Pyruvatokinase. Die Direktion des Konzentrationswechsels der Rexo-monophosphate in Muskeln mit Mangel an Phosphorilase steht in markiertem Kontrast mit denen der Muskeln mit Phosphofruktokinase, bei denen eine wesentliche Akkumulation von Rexosamonophosphaten nachweisbar ist. Dieser Punkt stellt ein Mittel dar zu spezifischer Diagnose der Glucogenese des Muskels.

Ausserdem wurde diskutiert über verschiedene und wichtige Nachweise bei der Differentialdiagnose der glucolytischen Störungen in den Muskeln.

The authors wish to thank to Prof. H. Wada and Dr. M. Takami for their helps in the preparation of the synthetic diet and to Prof. M. Nishikawa for guidance in this study.

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Terapeutic Approaches to Selected Disorders of Inborn Errors of Metabolism with Neurological Involvement

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INTRODUCTION

Growth and sustenance of life depend on a great number of general and specific metabolic processes. For the optimal benefit to the organism, these processes are under constant control of complex genetic systems transmittable with great regularity from generation to generation, to assure preservation of species. Nevertheless, the evolutionary process and variability within the species depend largely on chance occurrence of a sudden change (mutation) in the make-up of one of many genes. The change may be a blessing, conveying a specific new advantage to the individual and through him to a future segment of the species, or it may be disastrous, leading to a lethal, sublethal or a distinctly morbid trait.

There are many known disorders of inborn errors of metabolism; certain of them, as for example, hemoglobinopathies, are common to specific ethnic groups, other genetic diseases affect a very small fragment of the population but their importance rests on the great numbers of these disorders. In the catalog of mendelian characteristics of man, McKusick [47] recorded 1,545 genetically determined notable variations, most of which are diseases. It may be recalled that a given genetic mutation may manifest itself by the dysfunction of many organ systems, or the abnormality may be confined to one or a few traits of the phenotype. The presence of an abnormal gene is usually suggested by the Mendelian distribution of the phenotype but the specific product of the mutant gene or deficiency of normal product is still to be determined in the majority of the hereditary disorders.

Transmission of demonstrable chromosomal aberrations is a special subgroup in the inheritance of diseases [22, 48, 65]. More commonly, the mutation involves one locus and it may lead to alteration in the quality or availability of a specific enzyme protein [3, 5, 14, 15]. If the detailed structural alteration of such protein is demonstrated, the corresponding specific change in DNA structure can be assumed. The field of genetics has been further expanded by the discovery of the existence of structural as well as control genes [37, 50, 58]. The latter can alter the function of more than one structural gene and thus produce change in the amount of more than one protein without affecting the protein structure.

In this communication we will be concerned with a selected number of genetic disorders in which the nervous system is affected and we have participated in therapeutic trials compensating for the deranged metabolism. The following groups of diseases will be considered in some detail:

A. Abnormalities of copper metabolism: kinky hair disease and hepatolenticular degeneration
B. Mucopolysaccharidosis: Types I - VII
C. Certain sphingolipidoses: Fabry's disease; Gaucher's disease

INBORN ERRORS OF COPPER METABOLISM

Menkes kinky hair disease (KHD) and Wilson's hepatolenticular degeneration (HLD) are two genetically determined disorders of copper metabolism. KHD is inherited as an X-linked recessive and HLD as an autosomal recessive trait.

Kinky Hair Disease

KHD is a sublethal condition with the onset of symptoms between 2-4 months of age in term infants and earlier in premature babies. The main clinical features consist of frequent and uncontrollable seizures, signs of progressive cerebral degeneration with motor weakness spasticity, lack of response to social stimulation deficient respiratory efforts and characteristic pili torti (Fig. 1). The course of the disease is punctuated by respiratory infections episodes of hypothermia and preterminal decerebrate state. Pathological abnormalities include

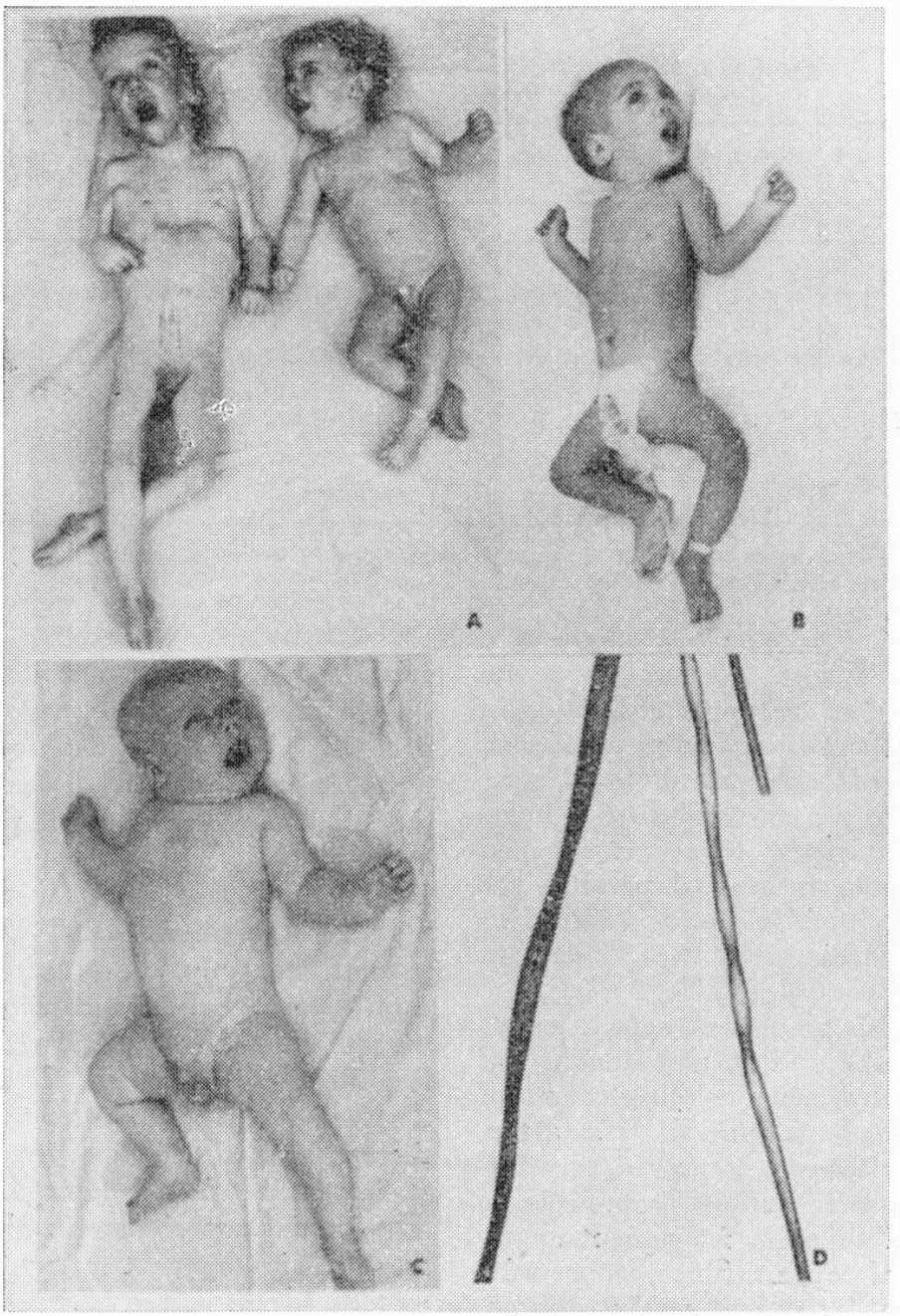

Fig. 1. — *Four male patients with KHD. The infant in the lower panel (C) was started on copper sulfate infusion at the age of 15 weeks, the progression of neurological deterioration was arrested and he even showed a slight improvement. D- shows variable diameter and characteristic twisting of hair.*

widespread cerebral atrophy, spongy lesions in the white matter, tortuosity, dilatation and degeneration of arterial blood vessels leading to aneurysmal dilation, stasis and tissue ischemia. It is probable that brain abnormality in the late phase depends on both impairment of oxidative processes from deficient activity of cytochrome C oxidase (requires copper) and widespread ischemia. The main laboratory findings consist of abnormally low copper levels in the plasma and tissues and low values of ceruloplasmin (Table 1). Danks *et al* [18, 20] and, more conclusively Dekaban *et al* [29], demonstrated that the principal abnormality in KHD is impairment of copper absorption from the

TABLE 1

SERUM COPPER (μg/100 ml) AND CERULOPLASMIN (mg/100 ml) (MEAN ± S.D.) IN THREE PATIENTS WITH KHD. PRETREATMENT AND DURING PARENTERAL ADMINISTRATION OF COPPER SULFATE INTRAVENOUSLY AND SUBCUTANEOUSLY

(expanded data from Dekaban & Steusing [29])

CASE NO.	PRETREATMENT		TREATMENT BY I.V. COPPER SULFATE 0.2-0.3 mg/kg body wt. every 4-7 d*		SUBSEQUENT TREATMENT WITH COPPER SULFATE SUBCUTANEOUSLY EVERY 3-4 d**	
	Copper	Ceruloplasmin	Copper	Ceruloplasmin	Copper	Ceruloplasmin
1	N4) 19.2 ± 2.1	5.8 ± 1.4	(N8) 53.1 ± 7.3	(N6) 13.8 ± 2.5	(N19) 80.0 ± 7.5	(N12) 19.2 ± 3.1
2	(N4) 23.2 ± 1.8	6.1 ± 0.8	(N11) 59.2 ± 5.9	(N9 19.0 ∓ 1.9	(N5) 82.0 ∓ 6.1	(N4) 18.8 ± 3.4
3	(N3) 17.5 ± 1.6	4.6 ± 0.7	(N15) 61.0 ± 9.8	(N6) 22.0 ± 3.1	—	—
Normal Controls	90 — 130	20 — 35	—	—	—	—

* Treatment for 9 weeks in Case 1, 18 weeks in Case 2 and 29 weeks in Case 3

** Treatment for 48 weeks in Case 1 and 9 weeks in Case 2

digestive tract. Figure 2 shows the actual extent of the defect in copper absorption in patients with HD as compared to unaffected controls and HLD. Because of low copper concentration in plasma and tissue, intraveneously administered copper is retained much longer than in normal persons, the biological half-life of ^{67}Cu being 72.8 - 89.5 days in KHD as compared to 27.1 days in the unaffected control (Table 2).

Because of this absorptive defect, it was natural for Danks *et al* [19] and Bucknall *et al* [13] to try intravenous administration of copper to the patients with KHD as a therapeutic trial. However it proved very difficult and in some infants impossible to find superficial veins for frequent maintenance infusion of copper. Since copper salts are toxic to tissues unless greatly diluted, we have determined experimentally the suitable concentration for subcutaneous infusion of copper by slow drip method. The subcutaneous infusion of 25-50 ml of copper sulfate in saline (0.04 mg/ml) are given over 1-2 hours every 3-4 days in four alternate sites: two beneath each scapula and two at the level of the 8th rib in mid-scapular line [28]. The infusion can be given in the outpatient clinic or at home by a public health nurse. Table 1 shows the effects of such infusion on serum copper and ceruloplasmin. Although biochemical correction has been accomplished, the clinical benefit is still uncertain. This is related to the fact that all patients who received the therapy have already shown varying degrees of irreversible brain damage. The proper test for efficacy of parenteral copper treatment will be to start treatment of the affected term infant within his first weeks of life. This could be done by screening all newborn brothers of the patients with KHD and identifying who are affected.

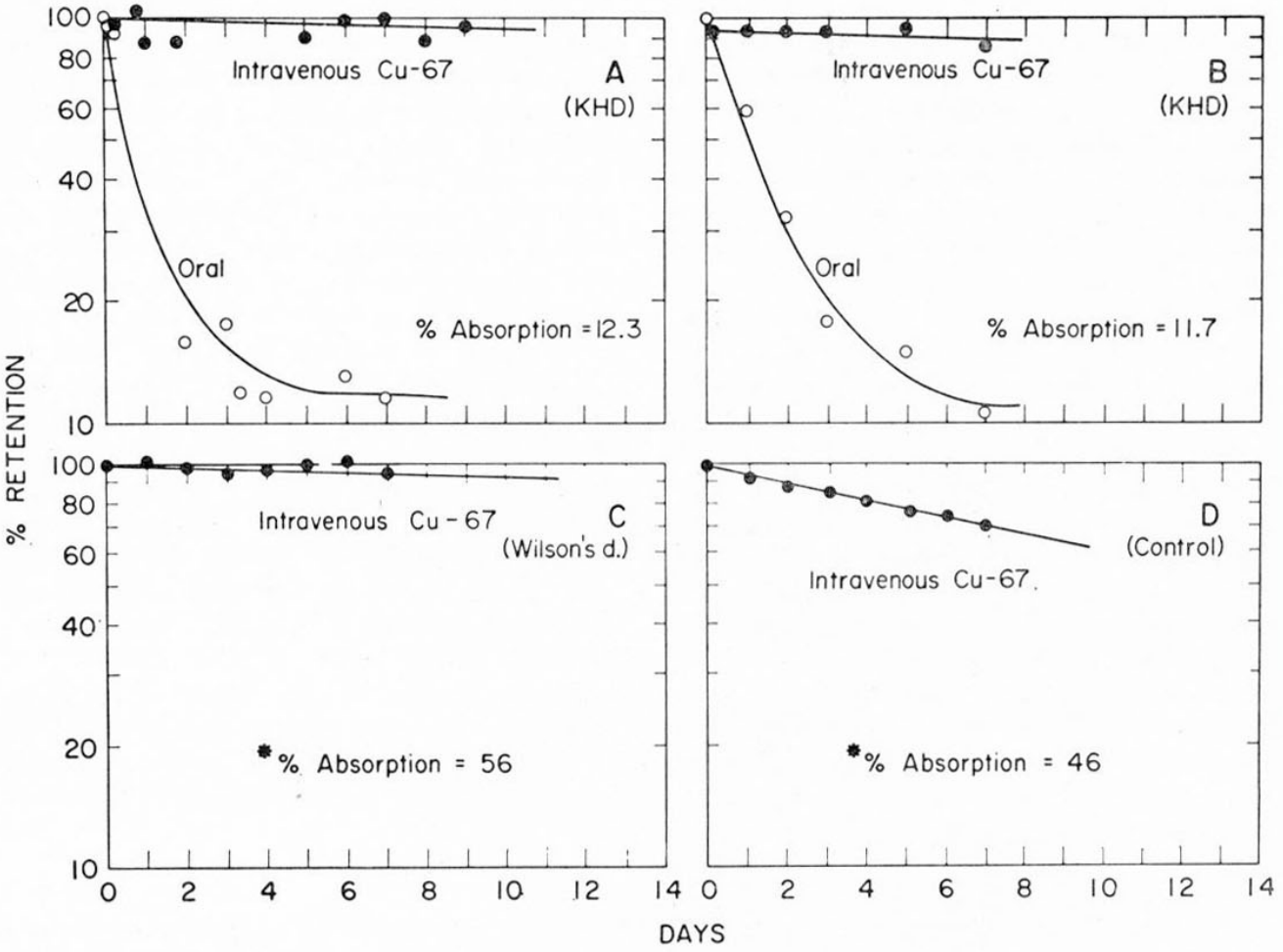

Fig. 2. — *Levels of ^{67}Cu retention after I. V. injection and after oral ingestion permitting calculation of percent absorption; A and B— in patient with KHD; C— in HLD and D— unaffected control. The values indicated by an asterisk in C and D represent a mean of oral absorption in patients with HLD and unaffected controls as determined by Strickland et al* [59]. *[This figure is slightly modified from the Figure 2 of Dekaban et al* [29].]

TABLE 2

EXCRETION OF ^{67}Cu OVER THE FIRST 8 DAYS AFTER I. V. INJECTION OF 50 μCi of ^{67}Cu

(expanded data from Dekaban **et al** [28])

PATIENT'S CASE NO.	SEX/AGE	DIAGNOSIS	^{67}Cu% OF DOSE STOOLS/URINE	^{67}CuHALF-LIFE BIOLOG. DAYS
1	M/1.2 y	Kinky hair disease	3.1/1.4	87.3
2	M/2.8 y	Kinky hair disease	4.2 (S+U)	89.5
3	M/1.6 y	Kinky hair disease	3.3 (S+U)	72.8
4	M/14 y	Wilson's disease	3.8/1.9	79.5
5	M/52 y	Control	24.7/0.5	27.1

Hepatolenticular degeneration (Wilson's disease)

HLD is characterized by excessive accumulation of copper in tissues which causes degenerative changes, especially in the brain, liver and cornea. Abnormal laboratory findings include greatly increased urinary excretion of copper, low total serum

copper (although albumin-bound serum copper may be increased) and low level of the copper containing serum protein-ceruloplasmin. The principal clinical manifestations in a fully developed case consist of liver cirrhosis, involuntary movements with tremor and rigidity, mental deterioration and corneal hyperpigmentation (Kayser-Fleischer ring). The onset of symptoms is commonly between 12 and 25 years of age, but it may occur in early childhood and during the third or fourth decade. If not appropriately treated, the disease progresses to death within 8-20 years.

Current therapy with orally administered chelating agent —penicillamine (β, β-dimethylcysteine)— introduced by Walshe [66] proved to be quite successful in improving most symptoms in patients with advanced disease and preventing occurrence of abnormal manifestations in patients in an early phase of HLD [6, 36]. The usual maintenance dose for penicillamine is 1-2 g/day, depening on the body weight and the patient's response to the therapy. Prolonged administration of this preparation may lead to various side effects and complications in a proportion of patients. The side effects may include skin exanthemas, fever, lymphadenopathy, mild thrombocytopenia and leukopenia and others [39]. Temporarily stopping the medication usually alleviates these symptoms. Occurrence of optic neuritis can be reversed by pyridoxine [63]. More severe complications such as lupus-like syndrome and predisposition to neoplastic conditions are rare but the attending physician must keep these in mind during long range maintenance therapy with penicillamine.

For the purpose of successful therapy, it is important to identify early all homozygous subjects for HLD, even prior to overt clinical manifestations. This can be done by studying clinically, and with laboratory tests (including, if necessary, determination of copper in liver needle biopsy) all siblings of the affected patients [57]. The pedigree presented in Fig. 3 exemplifies this situation. The parents are presumed heterozygotes for Wilson's disease: 1/1 (propositus) has symptomatic disease, currently under satisfactory control. His four younger siblings are homozygous for Wilson's disease by laboratory tests (low ceruloplasmin, high excretion of urinary copper, high hepatic copper as judged by liver needle biopsy), but had not developed clinical signs at the time their oldest brother was diagnosed. These four siblings were also started on treatment consisting of a moderate restriction of foods rich in copper and administration of penicillamine. Follow-up examination several years later showed that the propositus was in a good health and none of his homozygous siblings for HLD had developed clinical signs of the disease. It must be recognized, however, that the gene frequency in the general population will be on the increase since many of these homozygotes for HLD will reproduce. The current gene frequency for heterozygotes of HLD was calculated as 0.002 [1, 4]. The underlying pathogenesis in HLD has not yet been determined, but the failure of the liver to incorporate copper into specific complex of apoprotein - glycolipid to form ceruloplasmin seems to be at the root of the problem.

Comment

These two inherited disorders of copper metabolism clearly show a number of similarities and differences. In both there is a reduction in plasma copper and impaired biosynthesis of ceruloplasmin. In KHD ceruloplasmin deficiency is related to the reduced availability of copper in the bloodstream because of the reduced intestinal absorption, while in HLD the absorption of copper is normal but this element is either not available at the proper sites in the liver or it cannot be utilized to form ceruloplasmin. Copper not utilized in HLD is stored excessively in tissues (causing damage) and is excreted in large quantities in the urine as "free" plasma copper (non-ceruloplasmin). As demonstrated in Fig. 2 and Table 2, retention of the radioactive copper in these two conditions is greatly increased and consequently the biological halflifetime of the copper is 3-4 times greater in these patients than in normal subjects.

It is of interest that the therapeutic approach to these two disorders of copper metabolism is quite different. In KHD we supply the necessary quantity of copper by

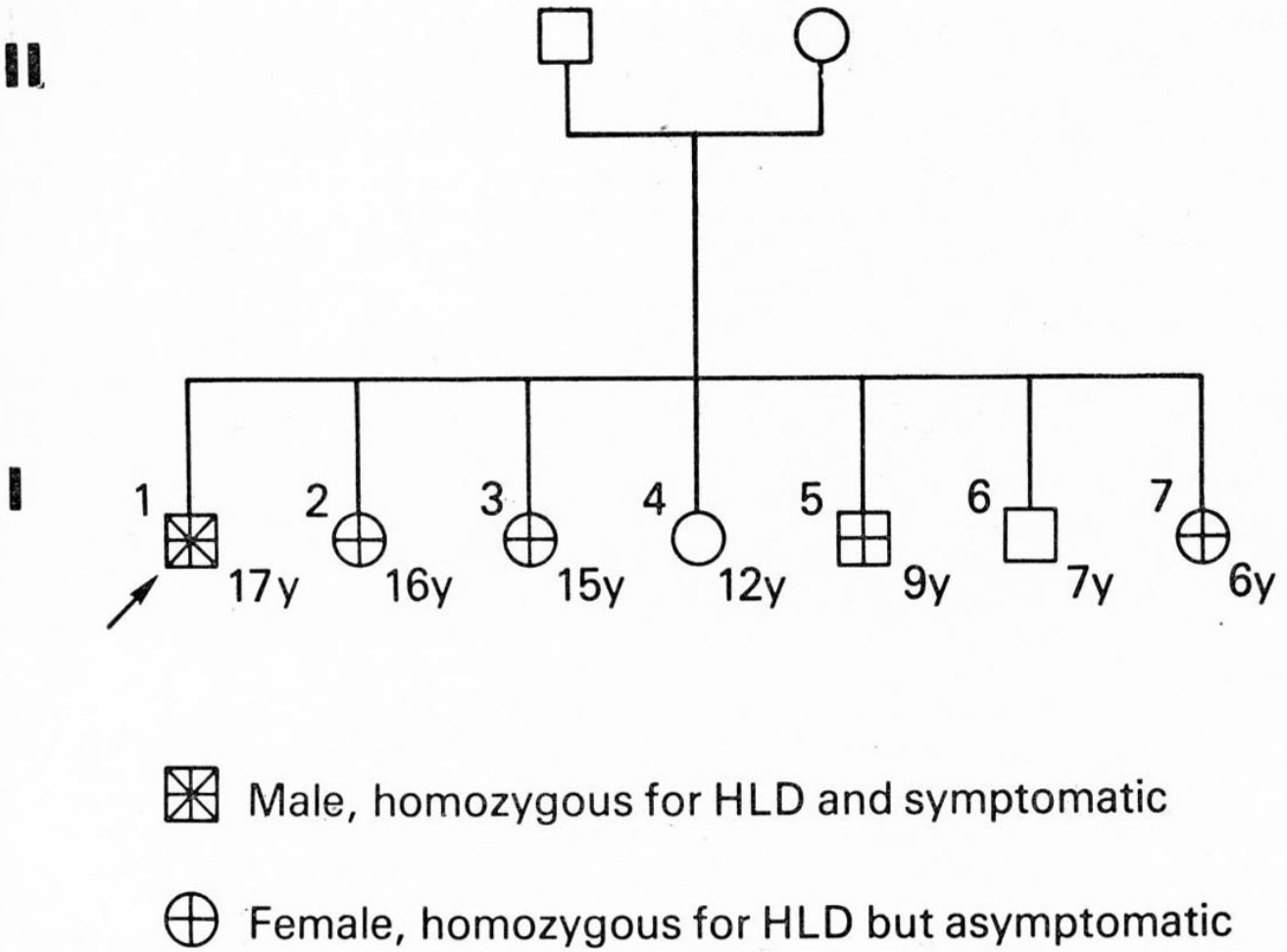

Fig. 3. — *Pedigree of a family with hepatolenticular degeneration. The propositus* (I/1) *showed signs of the disease when started on treatment; his four homozygous, but asymptomatic, siblings are receiving prophylactic therapy.*

parenteral route to overcome absorptive defect, whereas in HLD we remove the excess of copper stored in tissues to prevent cellular damage. In neither of these disorders has the presumably deficient enzyme been identified, yet the therapeutic management of patients in HLD is satisfactory and in KHD promising. Application of the knowledge derived from the study of the pathophysiology of these disorders was instrumental in devising the respective therapies.

MUCOPOLYSACCHARIDOSES

Mucopolysaccharidoses (MPS) are inborn errors of mucopolysaccharide metabolism. The genetic defect is manifested by excessive deposition in organs and tissues of mucopolysaccharides, more properly called glycosaminoglycans (GAG) and the excretion of large quantities of these compounds in the urine; the principal GAG involved are the partially degraded dermatan sulfate (DS) and heparitin sulfate (HS). Seven different variants of the syndrome have been identified [17, 44, 46, 62]. Clinical manifestations are generally severe, and the condition is sublethal in the majority of the affected patients. Skeletal deformities, hepatosplenomegaly, corneal opacities, cardiac valvular disease and neurological and mental deterioration are among the principal features (Fig. 4) of various forms of MPS [24, 32]. Although much has been accomplished during the past decade in the elucidation of specific enzyme deficiencies and in unravelling the pathogenesis of MPS [17, 27, 45, 51], only isolated approaches at therapeutic trials were made.

The experimental basis for use of corticosteroids in the treatment of patients with MPS was provided by Layton [42], Dorfman

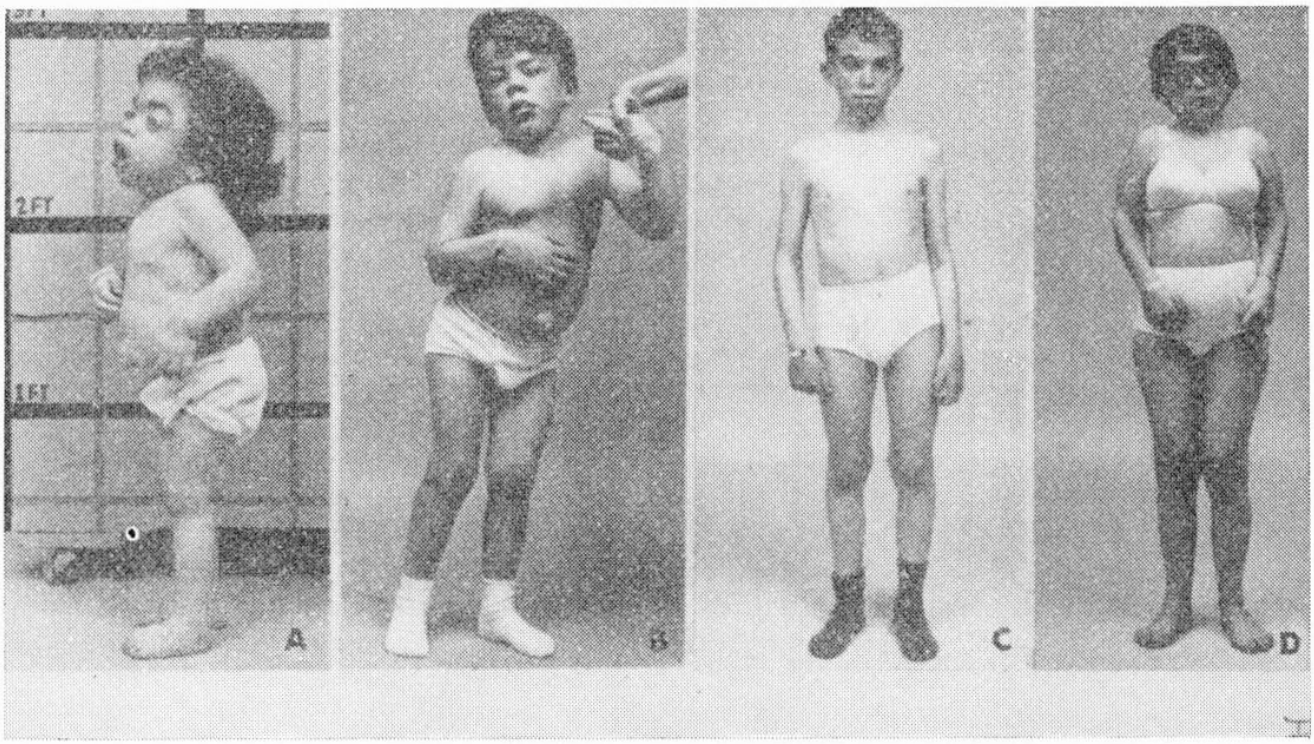

Fig. 4. — *Physical abnormalities in various types of MPS patients. A- patient with MPS Type I (Hurler); B- patient with MPS Type II (Hunter); C- patient wiht MPS Type III (Sanfilippo); and D- Patient with MPS-V (Scheie). Note that the patient with MPS Type III has essentially normal physical appearance although his mental status declined from normal to I. Q. of* 41.

and Schiller [33], and Davidson *et al* [21]. These investigators showed that corticosteroids suppress incorporation of labelled ^{35}S into GAG of skin of animals and also slow the turnover of hyaluronic acid. Usui *et al* [64], administered corticosteroids to patients with MPS but could not show any discernable changes in urinary GAG, but Wolfson *et al* [67] found a substantial decrease in the urinary output of GAG in Hurler's patients and some clinical amelioration, although worsening of cardiac function occurred. Our earlier studies [55] revealed decrease of the urinary GAG in the patients receiving Prednisone while the administration of salicylates and human growth hormone had no effect. In subsequent long-term studies [23, 25] in the patients with Sanfilippo variant of MPS (minor or no cardiac involvement) we have administered on alternate days Prednisone for 6-12 months. This was associated with demonstrable improvement in their mental performance and in behavior. After several days on Prednisone their urines showed decline of GAG which plateaued at the level 20 % lower than basic values. Further evaluation of the role of corticosteroid therapy in MPS is in progress.

More recently, DiFerrante *et al* [31] and Knudson *et al* [40] reported an induced degradation of urinary GAG in patients with Hurler (MPS Type I) and Hunter (MPS Type II) by repeatedly infusing frozen plasma or white blood cells over 32 to 68 hours. They also noted an increased ratio in CPC-non-precipitable to the precipitable fragments. Subsequently, Dekaban *et al* [26] infused repeatedly fresh plasma or whole blood to Hurler's, Hunter's or Sanfilippo's patients. The two patients who received plasma showed no change in the amount or composition of the urinary GAG; of the three who were given whole blood, two excreted only slightly smaller amount of GAG after infusions. The distribution of molecular weights in the isolated GAG prior to and after infusion did not change. Erickson *et al* [34] were also unable to demonstrate any significant change in MPS patients receiving fresh-frozen plasma while Moser *et al* [49] showed only slight and transient change in the ratio of small to large molecules

of urinary GAG after leukocyte infusions without a clear change in the clinical status of the patient. It is probable that the amount of active enzyme present in the donor's blood is not sufficient to effect reversal of biochemical abnormality and to ameliorate the clinical condition. However, further efforts in this direction are warranted.

FABRY'S DISEASE

The underlying abnormality in Fabry's disease is deficiency of an enzyme, α-galactosyl hydrolase, which is necessary for catabolism of a glycosphingolipid: galactosyl-galactosyl-glucosylceramide [9]. The disorder is transmitted as an X-linked trait and is characterized clinically by telangietatie skin lesions (angiokeratoma corporis diffusum), corneal opacities, intractable burning pain in the extremities, progressive renal dysfunction and general malaise. The life span is shortened, death occurring in early and mid-adult years usually from renal or cardiac failure or cerebrovascular disease. A small proportion of heterozygous females may show milder manifestations of the disease. The documented pathological abnormality consists of excessive accumulation of the lipid ceramidetrihexoside in the cellular elements of blood vessel walls, autonomic ganglia, epithelial cells of the kidney and cornea and also in cardiac muscle, reticuloendothelial and connective tissue cells as well as characteristic lesions in blood vessels [38, 61, 56].

Recently, several far-sighted attempts were made to control the disease by supplying the deficient enzyme, ceramidetrithxosidase. In 1970 Mapes *et al* [43] carried infusion of normal plasma into patients with Fabry's disease following which increased levels of enzymatic activity in the plasma of these patients was demonstrated; this was paralleled by a decrease in the concentration of ceramidetrihexoside in the blood. An attempt to provide the missing enzyme on a continuous basis was made by Desnick *et al* [30], Philippart *et al* [53], and Clarke *et al* [16]. These investigators from different medical centers transplanted kidneys to the patients with Fabry's disease; this led to beneficial effects in some, but not all, recipients.

In 1973, we have infused highly purified enzyme, ceramidetrihexosidase, obtained from human placental tissue to two patients with Fabry's disease [10]. The enzyme was rapidly cleared from the blood (Fig. 5) and

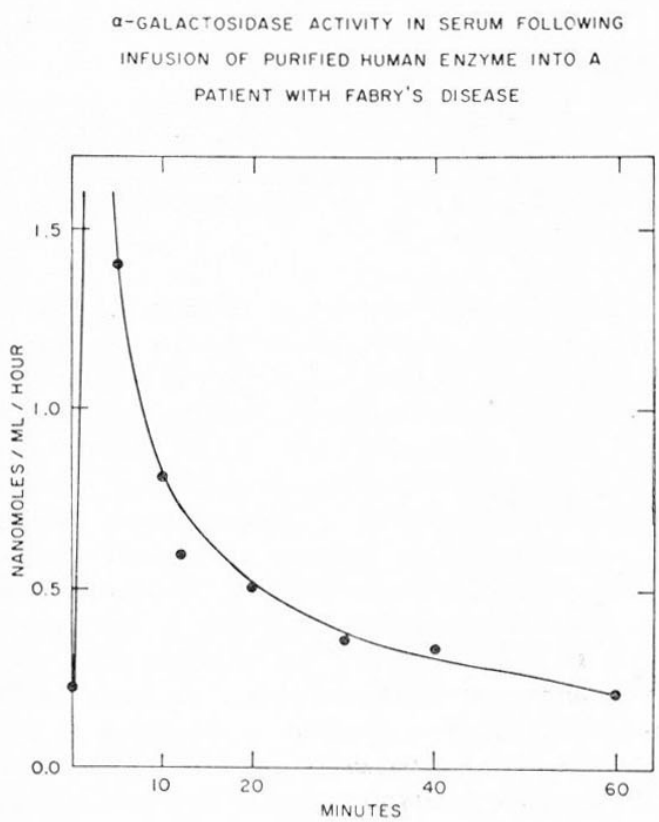

Fig. 5. — *α-galactosidase activity in serum following infusion of purified human enzyme into a patient with Fabry's disease*

was taken up to a major extent by the liver. In one patient, the level of the circulating glycolipid - ceramidetrihexoside - decreased by more than 50 % and in the other patient by about 30 % in 40 minutes after infusion (Fig. 6). Infusion of leukocytes and platelets in plasma gave rather comparable results (Table 3). However, infusion of leukocytes for extended therapy would be impractical. We surveyed 65.000 potential

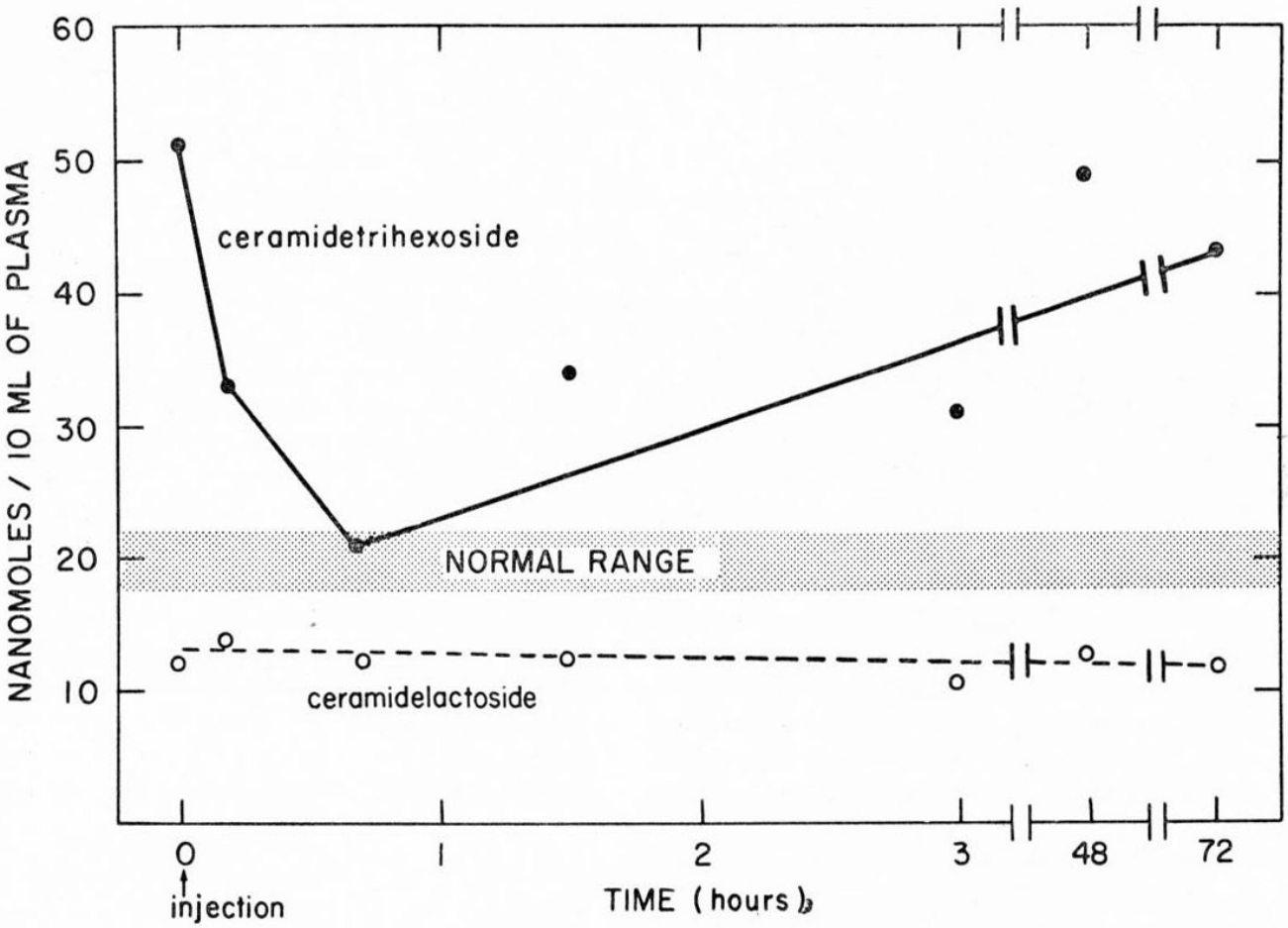

Fig. 6. — *Effect of infusing purified ceramidetrihexosidase on the level of circulating ceramidetrihexoside in a patient with Fabry's disease. Note that the level of ceramidelactoside, the immediate product of the enzymatic reaction, was unchanged. This finding and kinetic observations suggest that the catalytic effect of the administered enzyme occurred extracirculatorily.*

donors by computer matching and found only two subjects whose white cell antigens were completely compatible with the patient under investigation. For this and other reasons, trials at replacement therapy with placental ceramidetrihexosidase are preferable and we feel that this approach will become increasingly useful in Fabry's disease. Of course one has to be aware of the fact that infusion of exogenous protein may lead to formation of antibodies and sensitization of the recipient. So far skin testing of the patient who received placental ceramidetrihexosidase was negative.

The machanism of action of the infused exogenous enzyme is unknown. Among others, it is possible that some enzyme deficiency diseases are due to the synthesis of a faulty, catalytically inactive protein because of an alteration in the genetic code. Although there is no published evidence for this in Fabry's disease, a clear demonstration for the presence of a protein which cross-reacted with antibody produced against normal human Arylsulfatase A was reported by Stumpf *et al* [60] in patients with metachromatic leukodystrophy. In spite of the lack of demonstrable Arylsulfatase A activity, these tissues appeared to synthesize the inactive enzyme protein. It is of interest

TABLE 3

EFFECT OF INFUSION OF PLASMA, LEUKOCYTES AND PLATELETS SUSPENDED IN PLASMA AND PURIFIED CERAMIDETRIHEXOSIDASE ON CIRCULATTNG CERAMIDETRIHEXOSIDE IN PATIENTS WITH FABRY'S DISEASE

(Summary of data from Brady **et al** [10])

CASE NO.	MATERIAL INFUSED	CERAMIDETRIHEXOSIDE % OF PREINFUSION LEVEL	TIME OF MAXIMAL EFFECT (minutes)
1	Plasma	100	—
1	Plasma enriched with leukocytes & platelets	54	40
2	Ceramidetrihexosidase (3 mg of protein)	67	60

that we have obtained a 3.6-fold increase in liver trihexosidase activity one hour after the second intravenous infusion of the exogenous enzyme. A similar augmentation of hexosaminidase activity in the liver was also observed in a patient with Tay-Sachs disease who was infused with human hexosaminidase A [12].

GAUCHER'S DISEASE

The basic abnormality in Gaucher's disease is a deficient enzyme activity of β-glucosidase (glucocerebrosidase) which catalyzes removal of glucose from glucosyl ceramide [7, 8]. This results in excessive accumulation of this compound in the cells of reticular system [41], leading to their characteristic enlargement (Gaucher's cells) and their extensive proliferation in various organs and tissues. The principal clinical manifestations consist of hepatosplenomegaly, skeletal abnormality in a form of focal replacement of focal replacement of bony structure by nests of Gaucher's cells [54], and in certain variants of the disease, severe cerebral involvement [2]. There probably exist at least three genetic types of Gaucher's disease [35], all transmittable as autosomal recessive traits. Type I or chronic non-neuronopathic form of adults may manifest itself at any age with a quite frequent survival to adult years; anemia and bleeding tendency due to hypersplenism and bony abnormality are the main manifestations. Type II or acute neuronopathic or malignant form has onset during early months of life and is associated with hepatosplenomegaly and severe brain involvement which leads rapidly to decerebrate state and death under two years of age. Type III, juvenile neuronopathic form is the least common of the three variants. It is characterized by the age of onset during childhood, hepatosplenomegaly, bony lesions, epilepsy and involuntary movements. In Types II and III the principal pathological lesions in the brain consist of marked proliferation and hypertrophy of periadventitial cells leading to impairment of circulation; there is also a rather mild storage of glucosyl ceramide in the nerve cells.

The treatment of Gaucher's disease has been entirely supportive. Splenectomy generally alleviates anemia and thrombocytopenia. Frequent bony fractures, especially of the head of the femur (asceptic necrosis) and of the vertebral bodies require appropriate orthopedic treatment. A major advance in enzyme replacement therapy has recently been attained in a clinical trial with purified glucocerebrosidase infused to patients with Gaucher's disease [11]. The enzyme was isolated in homogenous form from human placental tissue [52], and found to be nonpyrogenic on appropriate testing. Infusion of this enzyme into two patients with Gaucher's disease resulted in 26 % reduction in the amount of accumulated glucocerebroside in the liver of each recipient (Table 4). The excessively accumulating glucocerebroside in patients' erythrocytes returned to normal by 72 hours after the injection (Fig. 7). Subsequent testing showed long lasting effects of the enzyme infusion on the level of glucocerebrosidase in erythrocytes.

TABLE 4

EFFECT OF INTRAVENOUS INJECTION OF PURIFIED GLUCUCEREBROSIDASE ON GLUCOCEREBROSIDE LEVELS IN THE LIVER OF TWO PATIENTS WITH GAUCHER'S DISEASE. (Summary of data from Brady et al [11])

CASE Nº	AMOUNT OF ENZYME INFUSED IN UNITS OF ENZYME ACTIVITY *	GLUCOCEREBROSIDE IN LIVER MICROGRAMS PER GRAM OF LIVER **		
		Before Infusion	After Infusion	Change
1	1.65×10^6	702	519	—183
2	3.3×10^6	1634	1214	—420

* One unit of enzymatic activity represents the hydrolysis of 1 nanomole of glucocerebroside per hour under standard conditions [52].

** The normal value is 31 μg/g wet weight in males and 46 μg/g wet weight in females.

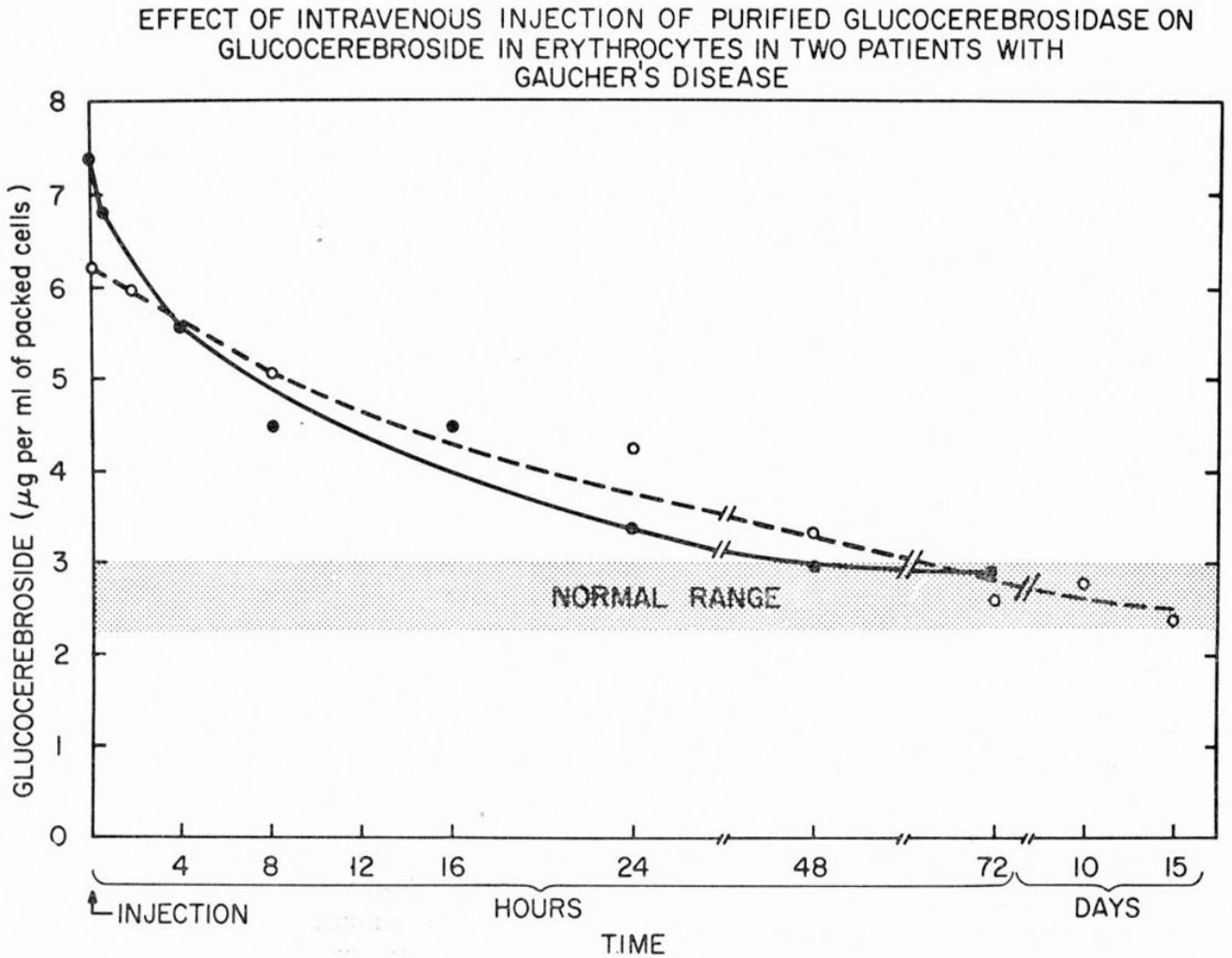

Fig. 7. — *Effect of intravenous injection of purified glucocerebrosidase on glucocerebroside in erythrocytes in two patients with Gaucher's disease.*

SYPNOPSIS ON THE TREATMENT OF INBORN ERRORS OF METABOLISM

The therapeutic approaches to heritable metabolic diseases have been manifold and ingenious; usually it was an insight into the pathophysiology of a given disorder that supplied the necessary clue. The results to date proved successful in some of these con-

ditions, but in the majority of the remaining genetic diseases, no specific treatment can be offered at the present time. The type of therapy or therapeutic trials for correction of metabolic defects can be put into 7 tentative categories as outlined in Table 5.

We have utilized several therapeutic approaches listed in Table 5. Thus, parenteral administration of copper sulfate normalized biochemical defect in KHD and prevented further deterioration of the disease. It is imperative to begin future therapy before irreversible damage to the nervous system is done. In HLD, removal of the excess of tissue copper, which generally leads to cerebral and hepatic damage, ameliorates the condition of the affected patients and prevents the occurrence of the disease in young, asymptomatic siblings who are homozygous for the trait. Therapeutic administration of Prednisone every other day to patients with MPS provided a limited improvement in Type III (Sanfilippo) variant; there was also a mild biochemical improvement following repeated infusions of blood products. Further therapeutic trials are indicated.

In Fabry's disease, beneficial effects were produced by kidney transplant in some of these patients. Intravenous infusion of the isolated and purified human enzyme, ceramidetrihexosidase, to two patients with the disease opened a hope that this could become a successful approach to future treatment of many inborn errors of metabolism. Results obtained from the infusion of glucocerebrosidase to the patients with Gaucher's disease further supports this contention. Clearly, enormous investigative and preparatory work remains to be done before enzymatic maintenance therapy in inborn errors of metabolism becomes a reality.

TABLE 5

GENERAL APPROACHES TO THE TREATMENT OF INBORN ERRORS OF METABOLISM

CATEGORIZED THERAPY OR THERAPEUTIC TRIALS	EXAMPLES OF DISEASES
A. Supply of missing metabolite	Kinky hair disease - parenteral supply of copper; Macrocytic anemia of orotic aciduria - supply of oral uridine Congenital goitrous hypothyroidism - supply of thyroxin
B. Supply of vitamin cofactor or hormone	Homocystinuria - therapeutic correction by pyridoxine; Sanfilippo (MPS-III) - partial improvement by glucosteroids
C. Limitation of precursor intake	Hepatolenticular degeneration - limitation of foods high in copper; PKU - limitation of dietary phenylalanine; Galactosemia - limitation of dietary galactose
D. Depletion of excessively stored metabolite	Hepatolenticular degeneration - chelation of copper with Penicillamine; Hyperlipoproteinemia, familial - administration of cholesteramine; Cystinuria - administration of Penicillamine
E. Supply of deficient enzyme by infusion of blood product or organ transplants	Mouth lesions in acatalasia - transfusion of catalase-containing RBC; Fabry's disease - infusion of blood products, transplant of kidney
F. Infusion of purified missing enzyme	Fabry's disease - infusion of purified ceramidetrihexosidase Gaucher's disease - infusion of purified glucocerebrosidase
G. Prospects for future therapy	Introduction to the patients of deficient enzyme synthesized **in vitro;** Transduction of human cells; Use of certain RNA viruses to code for a specific DNA system; Suppression of inactivation of the deficient enzyme.

SUMMARY

The medical importance of inborn errors of metabolism rests on their great numbers, although only a small fraction of the population is affected by the individual genetic disorder. The clinical manifestations are generally severe. With several notable exceptions, the treatment and long-term management of these diseases is very unsatisfactory. In this communication, we have outlined and elaborated on the therapeutic approaches to three categories of inborn errors of metabolism with which we had personal experience or introduced a new form of therapy. These three categories include A. abnormalities of copper metabolism; B. mucopolysaccharidoses; and C. certain sphingolipidoses - Fabry's disease and Gaucher's disease. In particular, treatment of kinky hair disease with copper sulfate by slow drip subcutaneous administration, and intravenous infusion of isolated enzymes —trihexosidase and glucocerebrosidase which are deficient in Fabry's disease and Gaucher's disease respectively— received special consideration.

RESUMEN

La importancia médica de errores innats de metablismo reside en su gran número, aunque sólo una pequeña fracción de la población es afectada por el desorden genético individual. Las manifestaciones clínicas son generalmente severas. Con varias excepciones notables, el tratamiento y el procedimiento a largo plazo de estas enfermedades es muy insatisfactorio. En esta comunicación, hemos trazado y elaborado el acceso terapéutico a tres categorías de errores innatos de metabolismo con los que hemos tenido experiencia personal, o en los que hemos introducido una nueva forma de terapia. Estas tres categorías incluyen: A) Anormalidades de metabolismo del cobre) Mucopolisacaridosis; y C) Ciertas esfingolipidosis: enfermedad de Fabry y enfermedad de Gaucher. En particular, el tratamiento del mal del cabello ensortijado con sulfato de cobre mediante administración lenta de gotas subcutáneas e infusión intravenosa de enzimas aisladas —triexcsidasa y glucocerebrosidasa las cuales son deficientes en las enfermedades de Fabry y de Gaucher respectivamente— han recibido especial consideración.

RÉSUMÉ

Le grand nombre de perturbations innées du métabolisme est important du point de vue médical, alors que seulement une faible partie de la population est affectée par quelque désordre génétique individuel. Les manifestations cliniques sont généralement sévères. A l'exception de cas remarquables, le traiterment et les soins à long terme de ces maladies sont très peu satisfaisants.

Dans ce travail nons avons tracé et réalisé la voie thérapeutique à trois sortes de perturbations innées du métabolisme desquelles nous avons eu une expérience personnelle, on dans lesquelles nous avons introduit une nouvelle forme de thérapie. Ces 3 catégories comprennent: A) Anomalies du métabolisme du cuivre; B) Mucopolysacharidoses et C) Certaines Sphingolipidoses: maladies de Fabry et Gaucher. En particulier, certains traitements ont été particulièrement étudiés, tels celui de la maladie des cheveux crépus, traitée par le sulfate de cuivre administré sous forme lente par gouttes sous cutanées et injections intra — voineuses d'enzymes isolées: Trihexosidase et glucocerebrosidase, qui sont peu abondantes dans les maladies de Fabry et Gaucher respectivement.

ZUSAMMENFASSUNG

Die medizinische Wichtigkeit von angeborenen Fehlern des Metabolismus beruht in seiner grossen Mehrheit, wenn auch nur eine kleine Fraktion der Bevölkerung da-

von betroffen wird, in der genetisch individuellen Unordnung. Die kleinen Manifestationen sind allgemein ernst. Mit einigen notablen Ausnahmen ist die Behandlung und die Aussicht auf lange Zeit bei diesen Krankheiten sehr unbefriedigend.

In diesem Bericht haben wir aufgezeichnet und den therapeutischn Weg ausgearbeitet für drei Kathegorien von angeborenen Fehlern des Metabolismus, bei denen wir selbst persönliche Erfahrungen hatten oder bei denen wir eine neue Behandlungsform eingeführt haben. Diese drei Kathegorien schliessen ein: A) Anormalitäten des Metabolismus des Kupfers; B) Mucopolysaccharidosis; und C) gewisse Sphingolipidosis: Krankheit von FABRY und Krankheit von GAUCHER. Besonders die Behandlung der Kräuselkrankheit des Haares mit Kupfersulfat mittels langsamer subkutaner Tropfinfussion und die intravenöse Infussion von isolirten Enzymen-Trihexosidase und Glucocerebrosidase, welche bei der dr Fabrykrankheit und der Gaucherkrankheit respektiv fehlen, haben eine besondere Berücksichtigung erhalten.

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Minamata disease with a long-term follow-up

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Minamata disease which occurred in Minamata, Japan, is an organic mercury poisoning caused by ingestion of fish or shellfish contaminated with the effluent from a factory. The clinical pictures of the disease were described in the series of the previous documentations [1-8]. Though the organic mercury poisoning was first reported in 1865 [9] and its clinico-pathological documents were confirmed by Hunter et al. in 1940 and 1954 [10, 11], the term of Minamata disease was given attention over the world because it occurred as a public nuisance with a peculiarity of the mode of invasion.

Minamata disease occurred between 1953 and 1960, so most living patients had about 10 years' history. In 1969 all the patients were examined by the staffs of the Kumamoto University. This report deals with a long-term (8-14 years) follow-up study of the disease.

1. Follow-up of capabilities in daily activities

Results of 8 to 14 years' follow-up of 50 cases which were examined at the initial stage are summarized in Table 1. These cases were divided into three groups: severely, moderately and mildly affected groups, according to the grade of neurological findings; and into two subgroups: adult and non-adult with the age of onset below 15.

Among 22 cases of the severely affected group, 14 died; 7 in the acute, 6 in the chronic stage and one with carcinoma of the liver. Most of the survivors (6 out of 8 cases) of this group still required help in their daily lives, and the number of cases improved was only 2 (9.5 %): they were one out of 15 in adult and one out of 6 in non-adult group. Among the moderately affected group 5 out of 13 cases also had considerable difficulties in their daily activities, and the number of cases improved was 8 (61.5 %): 7 out of 9 in adult and 1 out of 4 in non-adult group. Among 15 cases of the mildly affected group one died with carcinoma of the stomach and the number of cases improved was 4 (28.6 %): none in adult and 4 out of 7 in non-adult group. These 4 cases had little difficulties in their daily activities.

In the congenital cases (see APPENDIX), the neurological deficit was generally severe and 12 of 21 cases required help in their daily activities.

2. Follow-up of clinical manifestations

In 22 adult cases clinical manifestations were compared with those observed at the previous investigation 10 years before (Fig. 1).

1) Mental impairment. — The number of cases with mental impairment was reduced from 13 (59.1 %) to 7 cases (31.8 %). Regarding the grade of each symptom, improvement was recognized in 8 (61.5 %), no change in 5 cases.

Intellectual disturbance was most prominent. Although I.Q test was not made in the previous investigation, 5 out of 16

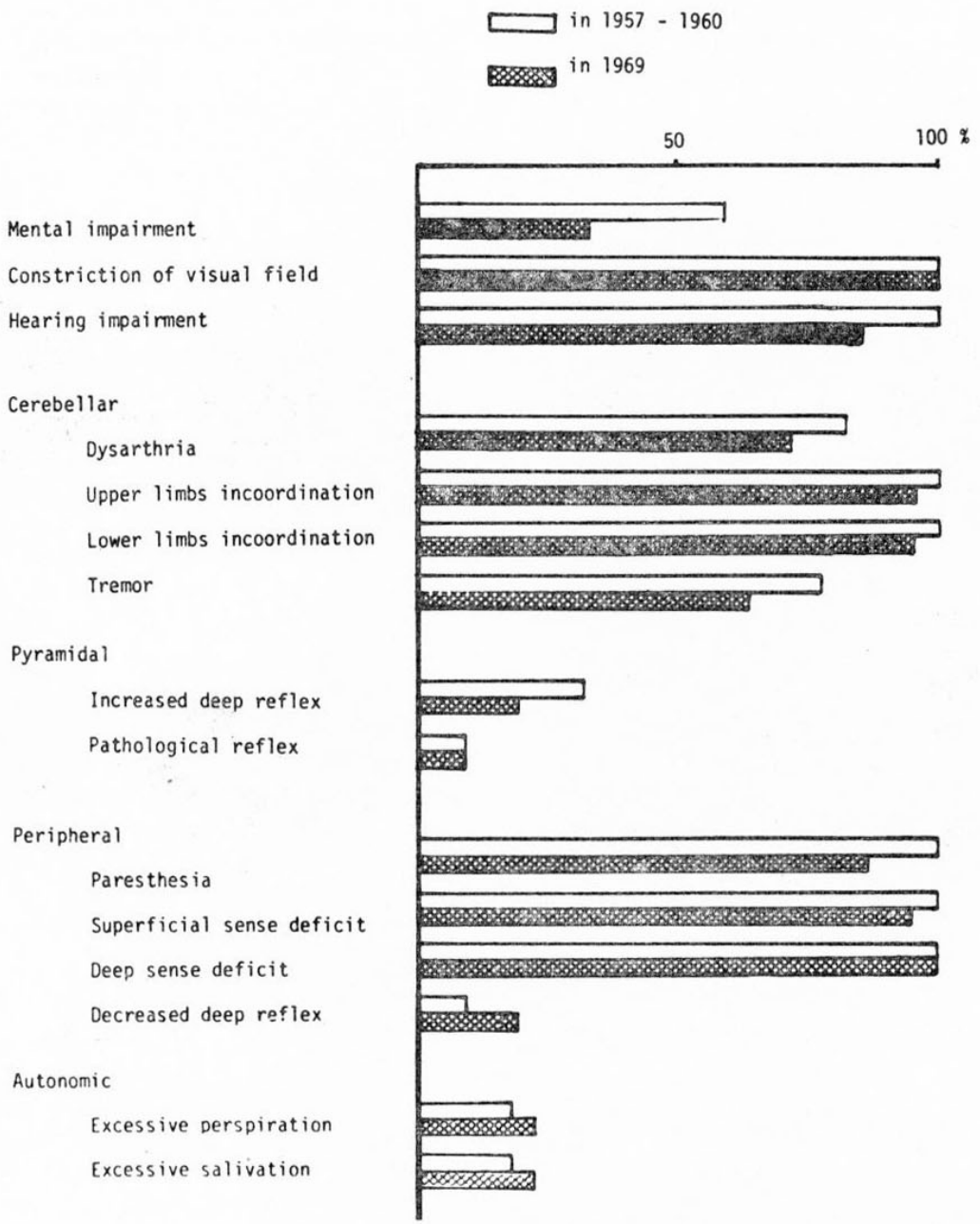

Fig. 1. — *Frequencies of symptoms and signs in* 22 *cases.*

cases tested at the present investigation showed I.Q below 70 (Tanaka-Bient method). Other 6 cases were unable to be tested because of difficulty in communication.

Emotional disturbances such as euphoria, anxiety or extreme shyness were inconstantly observed. In one fits of generalized clonic convulsion elicited by emotional excitation still sustained. The fits were not accompanied by loss of consciousness but frequently by oculogyric spasms. It was thought to be of a psychogenic disturbance.

2) Constriction of visual fields. — Concentric constriction of the visual fields which is one of the most characteristic features of this disease was determined in all of 21 cases tested in the previous investigation. Repeated perimetric examination of 19 cases revealed improvement (Fig. 2, A), but not complete recovery, in 9 (47,9 %), no change in 6, deterioration (Fig. 2, B) in 2 and indefinite fluctuation in 2 cases, during the 10 years' period. The other 3 cases were not tested because of difficulty in communication or of visual disturbance due

to cataract. One among slightly affected 5 cases which were investigated for the first time in this study was found to have normal visual fields.

3) Hearing impairment. — In the previous report hearing impairment was stated to be in 85 %, but thereafter audiometric examination disclosed the hearing deficit

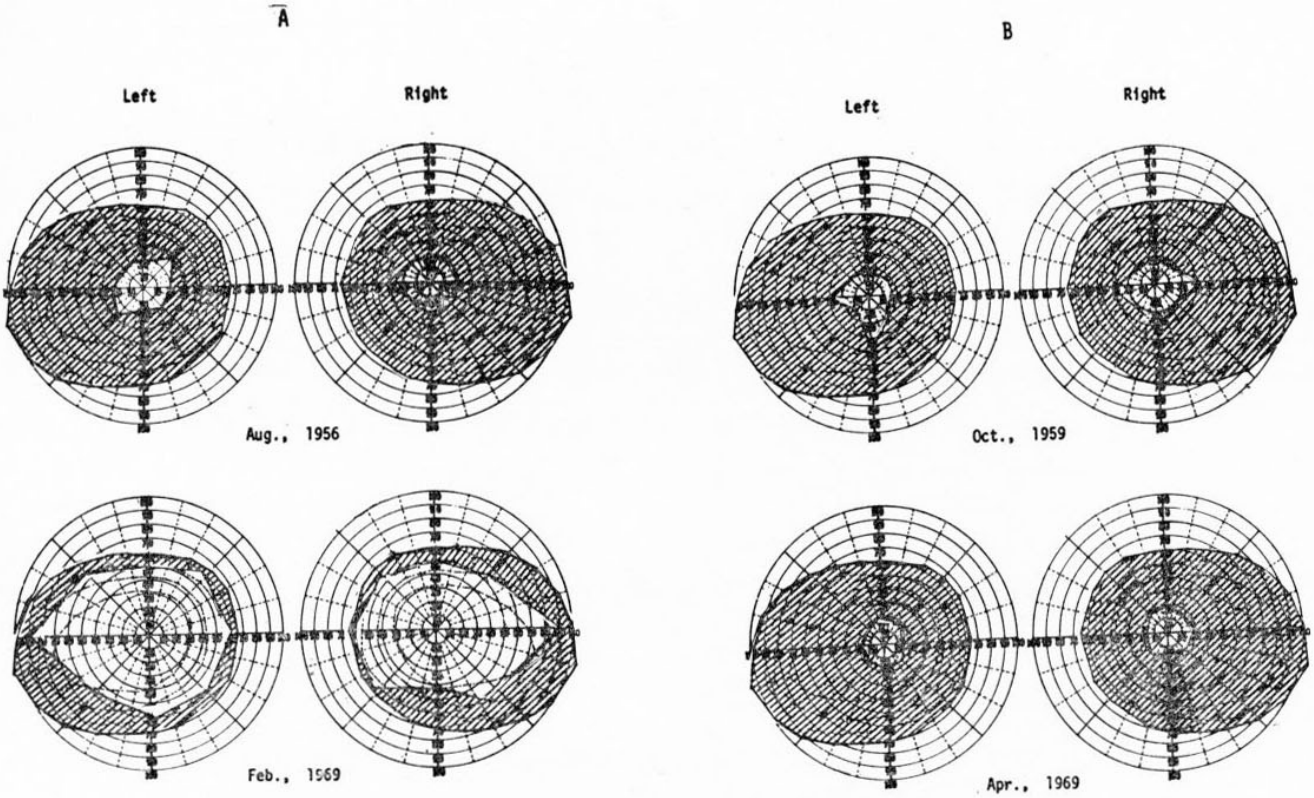

Fig. 2. — *Course of visual field constriction.*
A: *Moderate improvement.*
B: *Slight deterioration.*

even in cases with practically normal hearing acuity. In the present investigation audiometry demonstrated the hearing deficit in 17 of 20 cases (85 %), too. Repeated audiograms showed evidence of improvement (Fig. 3, A) in 11 (55 %), no change in 7 and deterioration (Fig. 3, B) in 2 cases. Two cases deteriorated belonged to the third decade of life. The other 2 cases could not be tested because of difficulty in communication.

4) Cerebellar dysfunction. — Dysarthria was observed in 15 (71.4 %), initially in 18 cases (81.8 %), and showed progressive improvement in 9 cases (50 %). Two cases showed considerable difficulty of vocalization due to unknown cause. Tongue movement was generally slow even in cases with practically normal speech.

There were little changes in the frequency of limbs incoordination which reduced from 100 to 95 % both in the upper and lower limbs, but their severity was more or less improved in 12 (54.5 %) and 11 cases (50 %), unchanged in 9 and 8 cases, and deteriorated in one and 2 cases, respectively.

Tremor of either rest or intention type reduced in number from 17 (77 %) to 14 cases (64 %), and 2 cases for each type and 10 cases for combined type were observed.

5) Pyramidal dysfunction. — The number of cases with exaggerated tendon reflexes reduced from 7 (32 %) to 4 cases (19 %). Among these, a single case showed weakness with pathological reflexes. The details of this case will be stated later.

6) Peripharal nerve dysfunction. — At the initial stage all cases complained of paresthesia of the extremities, and in addition, 12 cases that of the perioral region and 5

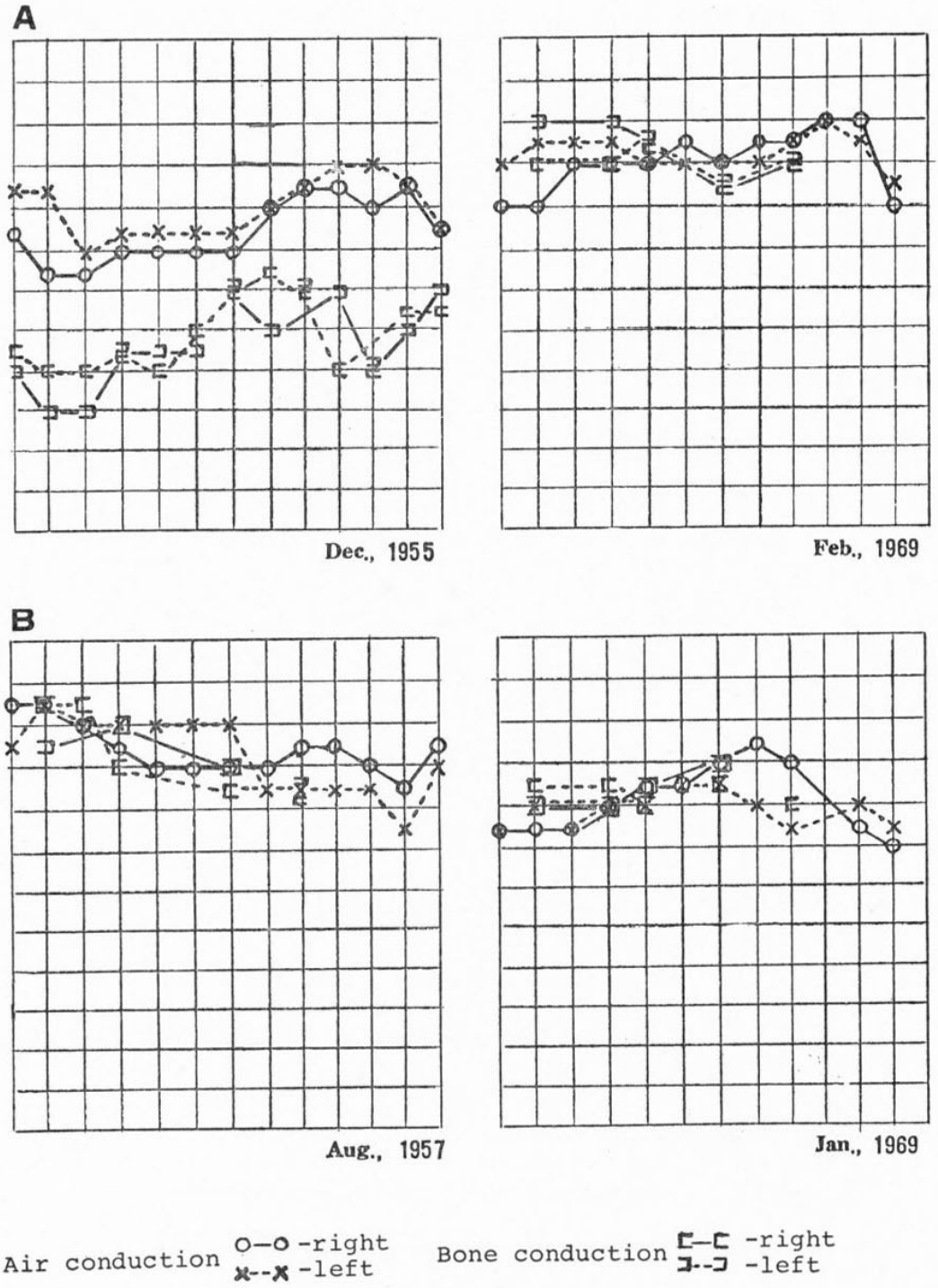

Fig. 3. — *Course of audiometric hange.*
A: *Improvement.*
B: *Deterioration.*

cases that of the tongue. Sensory disturbance of all modalities was also demonstrated in all cases, especially in the extremities, and in some cases over the whole body. At the present study all but 3 cases (86.4 %) still had paresthesia of the extremities, 4 cases of the perioral region and 2 cases of the tongue. Objective sensory disturbance was demonstrated in all but 1 case, and the extent and/or severity were improved in 10 (45.5%), unchanged in 8 and progressed in 3 cases. In another one case satisfactory data were not obtained because of difficulty in communication. Disturbance of vibration sense was most hard to improve. The number of cases with diminished deep

tendon reflex changed from 2 (9 %) to 4 cases (19 %).

7) Autonomic nerve dysfunction. — Excessive salivation and perspiration were still observed in 5 cases (22.7 %), initially in 4 cases (18.1 %). A few cases were unable to open their mouth because of excessive salivation.

3. Laboratory studies

There were no definite changes in the blood, urine and serum as had been reported [1-8] in the previous investigation.

1) Electroencephalography. — Among 34 cases tested, abnormal or slightly abnormal records were obtained in about one half of the cases. Low voltage, slow alpha, diffuse alpha or slow wave burst or train of theta or delta range without lateralization were predominant findings of abnormal EEG (Fig. 4). There were no significant changes in EEG findings in 9 cases between the previous and present records (Table 2). Initial EEG recording was not available in other patients.

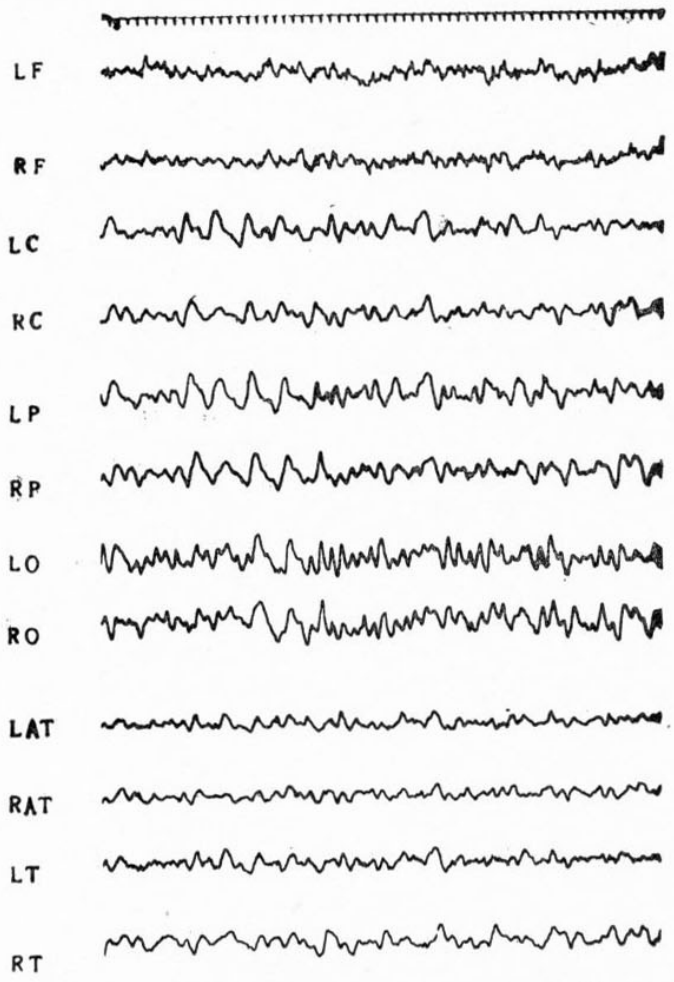

Fig. 4. — *EEG of the 5th case shown in table 2.*

2) Electromyography. — Among 29 cases tested, fibrillation voltage in 2, diminution of discharge in 11, grouping voltage in 15 and high amplitude NMU voltage in 7 cases were observed as the abnormal recording (Fig. 5).

In the previous investigation, EMG, examined only in 5 cases, showed no abnormal discharge except a grouping voltage in one case. This case showed similar finding at the present recording and one of the other cases showed a high amplitude NMU voltage as a newly obtained finding.

3) Motor nerve conduction velocity. — Motor nerve conduction velocity was exa-

TABLE 1

RESULTS OF LONG-TERM FOLLOW-UP

Initial grade of illness	Age group	Death	Difficulty in daily activities, at present (8-14 years after onset)				Cases improved / Initial	Almost none / Initial
			Severe	Moderate	Mild	Almost none		
Severely affected 22	Adult 16	11(1)*	4	1	0	0	1	0
		14(1)*	6	2			2/21# (9.5%)	
	Non-adult 6	3	2	1	0	0	1	
Moderately affected 13	Adult 9	0	0	2	7	0	7	0
				5	8		8/13 (61.5%)	
	Non-adult 4	0	0	3	1	0	1	
Mildly Affected 15	Adult 8	1(1)*	0	0	7	0	0	4/14# (28.6%)
		1(1)*			10	4	4/14# (28.6%)	
	Non-adult 7	0	0	0	3	4	4	
Total 50	Adult 33	12(2)*					14/48# (29.2%)	4/48# (8.3%)
	Non-adult 17	15(2)* 3	6	7	17	4		

* Death unrelated to Minamata disease

\# Cases died with unrelated disease were excluded

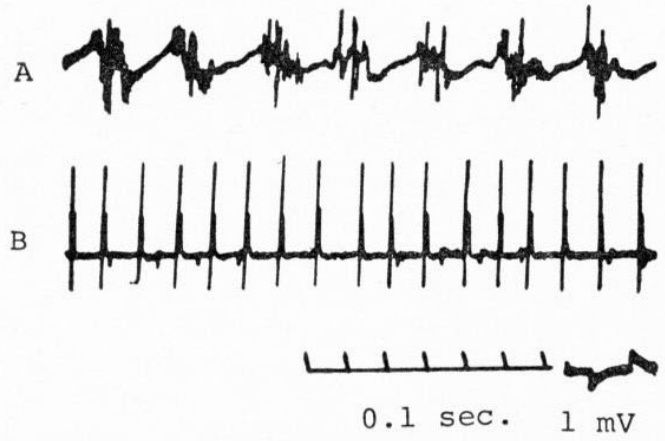

Fig. 5. — *EMG of selected cases.*
A: *Grouping voltage.*
B: *High amplitude NMU voltage.*

mined at the ulnar nerve for the first time. Slowing of the conduction velocity (below normal: 50 m/sec.) was obtained in 2 of 30 cases tested.

4) Mercury content in the hair. — The mercury content in the hair of patients, examined within 10 months after the onset, was of a high value ranging from 96 to 705 ppm, but at the present investigation, it was within normal limits in all cases (Fig. 6).

4. An unusual case

A man engaged in fishing with his family was healthy until the end of July, 1955, at age 20 when he noticed numbness and tremor in his arms. One week later he also felt numbness in his legs. However,

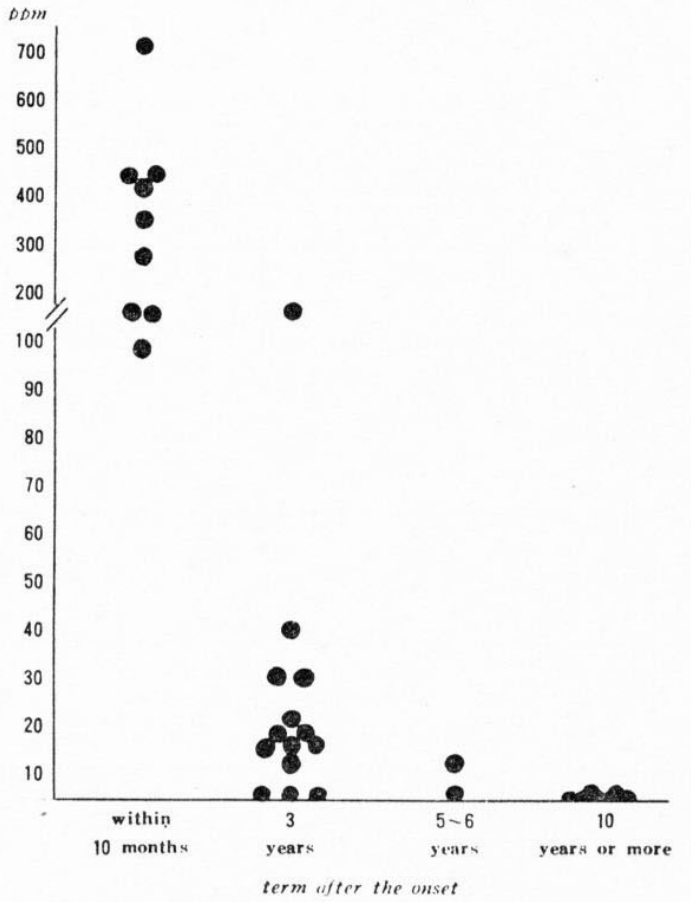

Fig. 6.— *Mercury content in the hair of patients.*

these symptoms were somewhat improved, so he went on fishing. In August, 1956, he was admitted to our clinic because of increased tremor. Examinations revealed concentric constriction of the visual fields, slight dysarthria, loss of hearing, coarse tremor of hands, ataxia and sensory disturbance in arms and legs. Deep reflexes in the upper limbs were slightly increased and those in the lower ones moderately exaggerated. Babinski's and Rossolimo's signs were elicited. In January, 1957, when the numbness was disappeared and his gait became better, he was discharged. During this period his father and 2 years later his mother died. Both of them had been suffering from the same illness since summer, 1966. He was engaged in packing of products, about 25 kg in weight, at a factory for several years, but was readmitted to the Minamata City Hospital at the end of 1968, with complaints of progressive gait disturbance and tremor. Examination disclosed concentric constriction of the visual fields and impairment of hearing as previously investigated. No dysarthria was noted. There was no muscular atrophy but slight weakness in the legs. Though dysdiadochokinesis and clumsiness in finger to nose test were slight, intention tremor was prominent.

Disturbances both in superficial and deep sensations were detected . Jaw reflex was within normal limits but all deep reflexes in the extremities were markedly increased with ankle clonus. Pathological reflexes were also elicited.

The outstanding feature of this case was the pyramidal tract involvement.

5. Comment

The characteristics of the clinical manifestation in Minamata disease are the constriction of the visual fields, hearing impairment ,cerebellar dysfunction, such as dysarthria, incoordination and tremor, and peripheral nerve dysfunctions. Mental impairments are also common and autonomic

TABLE 2

EEG FINDINGS IN 9 CASES EXAMINED AT

THE PREVIOUS AND PRESENT INVESTIGATIONS

Name	Sex	Year of birth	EEG at previous investigation	EEG at present investigation in 1969
Y.T.	M	1930	6.5 - 8 c/s theta activity, frontally predominant*	6.5 - 8 c/s theta activity, frontally predominant
T.M.	M	1910	frontal - 8 c/s alpha rhythm with 24 c/s beta components*	diffuse 7 - 8 c/s alpha rhythm with 15 - 25 c/s beta components
T.H.	M	1936	10 - 11 c/s alpha rhythm*	10 - 11 c/s alpha rhythm
T.K.	F	1914	8 - 10 c/s alpha rhythm*	diffuse 6 c/s theta activity
S.Y.	F	1942	7 - 8 c/s alpha rhythm with paroxysmal 3.5 c/s theta components**	7 - 8 c/s alpha rhythm with paroxysmal 3 - 4 c/s delta components#
T.S.	F	1939	9 c/s alpha rhythm in the occipital region and diffuse 6 c/s theta activity in the other areas**	8 - 9 c/s alpha rhythm with paroxysmal 3 - 4 c/s delta components
T.S.	F	1941	9 c/s alpha rhythm with sporadic 6 - 7 c/s theta activity**	9 c/s alpha rhythm with sporadic low voltage 5 - 6 c/s theta components
T.N.	F	1937	8 - 9 c/s alpha rhythm**	8 - 10 c/s alpha rhythm with sporadic 6 c/s theta components
S.M.	M	1912	frontal 7 - 8 c/s alpha rhythm with 15 - 25 c/s beta components***	generalized low voltage 15 - 25 c/s beta activity

* examined in 1956 ** examined in 1958 *** examined in 1959

\# This EEG record was shown in Fig. 4.

TABLE 3

FREQUENCY OF CEREBRAL PALSY
IN SEVERAL VILLAGES WHERE A NUMBER OF
CASES OF MINAMATA DISEASE WERE FOUND

Year	Number of Cerebral Palsy*	Number of Birth*	Number of Cerebral Palsy per 1,000 Births
1956	4	95	42.1
1957	7	90	77.8
1958	6	83	72.3
1959	2	89	22.5

* Data from the Dept. of Pediatrics (19)

nerve dysfunction are sometimes observed. Pathologically this disease is a diffuse encephalo-neuropathy with involvements of the cerebrum, especially visual cortex, cerebellum, especially granular layer, and peripheral nerves, enough to elucidate the clinical findings [12].

There have been several reports on the organic mercury poisoning, but follow-up study was not reported except an autopy case of Hunter and Russell [11]. The authors were able to follow up 50 cases of Minamata disease periodically for about 10 years.

Most of the survivors of the severely affected group still required help in their daily lives and 5 of 13 cases of moderately affected group also had considerable disabilities in their daily activities, and the number of cases improved in these groups were 2 out of 21 (9.5 %) and 8 out of 13 (61.5 %), respectively. Among 14 cases of mildly affected group, the number of cases improved was 4 (28.6 %), and these 4 cases had little difficulties in their daily activities. Summarizing the above results, exclusive of 2 cases which died of unrelated disease, 14 cases (29.2 %) showed an improvement and only 4 (8.3 %) attained satisfactory capabilities during the 10 years' period. There were no obvious difference in the clinical course, between the adult and non-adult groups.

In the congenital cases the neurological deficit was generally severe and 12 of 21 cases required help in their daily activities.

Examination was carried out with cooperation of the Pediatric staffs, so the authors investigated the clinical manifestations mainly in adult cases.

Summarizing the results in regard to each clinical manifestation, there were some improvement in the mental impairment, cerebellar and peripheral nerve dysfunctions, and in the audiometric findings. Concentric constriction of visual fields which is one of the most characteristic features of this disease was also improved to various extents in 47.4 %, but no case showed complete recovery. On the other hand, there were a few cases which showed a deterioration in some clinical manifestations. In some cases it was probably due to aging, but in the others the cause couldn't be determined. As a conclusion the neurological disabilities were considered, as a whole, slightly improved, but very slowly, during the past 10 years' period. In EEG there were also no significant changes between the previous and present investigations. Among the new findngs obtained from the present survey, there was one case with a feature of pyramidal tract involvement and, in some cases, EMG showed diminution of discharge or high amplitude NMU voltage. These findings suggest the involvement of the motor neurons which was never seen in the previous investigation [13]. Though such findings have not been confirmed by autopsy yet, there have been some reports of organic mercury poisoning indicate the presence of symptoms and signs resembling those of amyotrophic lateral sclerosis [14, 15]. Follow-up study of the unusual case referred will give a valuable answer.

APPENDIX

Congenital Minamata disease

Congenital Minamata disease is caused by organic mercury which had been transported through the placenta. Since 1955 many cases of infantile cerebral palsy of undetermined type were encountered in some villages where a number of cases of typical Minamata disease were detected. The frequency of the infantile cerebral palsy was said to be 0.6 to 6 per 1,000 births in the U. S. A. and in Europe and the prevalence rate for 5 to 9 years of age was reported as 203 to 634 per 100,000 population [16]. In Japan the frequency is usually not so high as compared with these data, showing 240 per 100,000 population of the same age group [17] or 190 per 100,000 population less than 5 years of age [18]. In the Minamata district the frequency was extremely high during a period from 1956 through 1959, when it was estimated to be 22.5 to 77.8 per 1,000 births (Table 3). The symptoms and signs frequently observed were mental retardation, visual disturbance, loss of hearing, anarthria, disturbances in daily performances and pyramidal signs. Palsy of ocular muscles, nystagmus, dysphagia or involuntary movements were occasionally observed. Such widespread le-

sions in the central nervous system are rarely seen in the usual cerebral palsy, and it was suggested that the relationship to the Minamata disease could exist. Later, two of these cases died and the autopsy disclosed almost similar findings to those seen in the acquired Minamata disease. Thus extensive survey was carried out on those paralized infants and 23 cases were diagnosed as congental Minamata disease.

SUMMARY

A long-term (8-14 years) follow-up study was made in the cases of Minamata disease. Most survivors still had difficulties of various grade in their daily activities and only 4 cases (8.3 %) attained satisfactory physical capabilities. There was some improvement in the mental impairment, cerebellar and peripheral nerve dysfunctions, and in the audiometric findings. Concentric constriction of visual fields was also improved in about one half cases but no case showed complete recovery. Thus, it was considered that the activities in daily life as well as each clinical manifestation were, as a whole, somewhat improved, but very slowly and only slightly during the past 10 years' period. EEG also showed no improvement. An unusual case and the findings of EMG showed an evidence of the involvements of motor neurons.

RESUMEN

El estudio de una prolongada evolución (8-14 años), fue realizado de casos de enfermedad de Minamata.

La mayoría de los sobrevivientes tuvieron aún dificultades de grado variable en sus actividades diarias y sólo 4 casos (8,38 %) alcanzaron aptitudes físicas satisfactorias. Hubo alguna mejoría en el deterioro mental, las disfunciones cerebelosas y de los nervios periféricos y en los hallazgos andiométricos.

La disminución concéntrica de los campos visuales fue también mejorada en alrededor de la mitad de los casos, pero ninguno de ellos mostró recuperación completa.

De este modo fue considerado que las actividades en la vida diaria, igual que cada manifestación clínica eran en general algo mejoradas, pero muy lentamente y sólo levemente durante el período evolutivo de 10 años.

El electroencefalograma no mostró mejoría. Un caso desusado y los hallazgos del EMG mostraron la evidencia de afectarse las neuronas motoras.

RÉSUMÉ

On a étudié l'évolution prolongée (8 a 14 ans) de cas de la maladie de Minamata.

La plupart des survivants ont gardé des difficultés de degré variable dans leurs activités journalières et 4 cas seulement (8,38 %) ont atteint des aptitudes physiques satisfaisantes, on constate une amélioration dans l'affaiblissement mental, les disfonctions cérábelleuses et les nerfs périphériques et les observations audiométriques. La diminution concentrique des champs visuels a été également améliorée dans environ la moitié des cas, mais aucun n'a montré de récupération complète.

On peut considérer que les activités de la vie journalière de même que chaque manifestation clinique étaien généralement un peu améliorées, mais trés lentement, et à peine, perdant une période évolutive de 10 ans.

L'électroencéphalogramme n'a pas été amélioré.

Dans un cas spécial on a pu trouver un EMG anormal montrant que les neurones moteurs étaient affectés.

ZUSAMMENFASSUNG

Das Studium einer varlängerten Evolution (8-14 Jahre) wurde in Fällen von der Minamatakrankheit durchgeführt.

Die Mehrheit der Überlebenden hatten

noch Schwierigkeiten in verschiedenem Grad bei ihren täglichen Aktivitäten, und nur 4 Fälle (8,38 %) erreichten physische ausreichende Fähigkeiten. Einige Besserung fand sich bei der mentalen Minderung, den cerebelosen Disfunktionen und den peripheren Nerven und den andiometrischen Befunden.

Die konzentrische Minderung der Visualfelder wurde auch gebessert in etwa der Rälfte der Fälle, aber keiner von ihnen zeigte komplette Wiederherstellung.

Auf diese Art wurde angenommen, dass die Aktivitäten im täglichen Leben, ebenso wie jede klinische Manifestation, im Allgemeinen etwas gebessert waren, aber langsam und nur leicht während einer evolutiven Periode von 10 Jahren.

Das Elektroencephalogramm zeigte keine Besserung. Ein ungewöhnlicher Fall und die Befunde des EMG zeigten das Vorhandensein einer Affektion der motorischen Neuronen.

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Pathogenesis of Mental Retardation in Amino acid Disorders

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It is known that most of hereditary amino acid disorders are more or less associated with mental retardation. This fact suggests that any of amino acids, if it accumulates in excess in blood, may cause mental retardation. In other words, the cause of mental retardation should be ascribed to an extreme imbalance of amino acids in blood rather than to a toxic effect of a specific amino acid or its derivatives.

TABLE 1. — Severity of amino acid imbalance in serum in amino acid disorders.

	Amino acid elevated in serum	Deviation from the normal level
Phenylketonuria	Phenylalanine	20 — 50 times
Hyperphenylalaninemia	Phenylalanine	5 — 10 times
Histidinemia	Histidine	4 — 20 times
Tyrosinosis	Tyrosine	4 — 6 times
Maple syrup urine disease	Leucine	8 — 25 times
	Isoleucine	8 — 20 times
	Valine	6 — 10 times
Hyperglycinemia	Glycin	10 — 40 times

Table 1 shows the degree of amino acid imbalance in blood in several amino acid disorders. High incidence of mental retardation in phenylketonuria (PKU) can be understood by the findings that the amino acid imbalance is severe in degree in this particular disorder. Hyperphenylalaninemia and tyrosinosis are usually not associated with mental retardation. In these disorders, the amino acid imbalance is milder in degree. The patients with histidinemia show often speech retardation which is thought to be a mild type of mental retardation. The amino acid imbalance is of intermediate degree in histidinemia. There is the amino acid imbalance in severe degree in maple syrup urine disease or hyperglycinemia. The patients with these disorders are in most cases mentally retarded if survived longer.

The next question is how the amino acid imbalance causes mental retardation.

1. Brain protein synthesis in experimental hyperaminoacidemia

There is a possibility that the presence of an amino acid in exess in blood may dis-

turbe the transport of other amino acids into the brain and result in the decrease in protein synthesis in the brain. It has been recognized that the uptake of an amino acid from blood into the brain is carried out by active transport mechanism. Linneweh et al. [1] observed autoradiographically that an excess of phenylalanine restricted the entrance of ^{3}H-leucine into the brain in experimental PKU. In in vitro experiment by using rat brain slice, an inhibitory effect of phenylalanine on the uptake of tyrosine, arginine and ornithine into the brain was observed by Neame [2] and similarly an inhibitory effect of leucine, isoleucine and their α-keto acids on the incorporation of ^{14}C-valine into the brain or the brain protein was reported by Appel. [3].

Our study was undertaken to investigate the in vivo effects of several amino acids on both the uptake of ^{14}C-leucine into the brain and the incorporation of ^{14}C-leucine into the brain protein by using 17-day old rats. The rats with hyperaminoacidemia, previously produced by intraperitoneal loading of 0.15 or 0.30 m-moles of a single amin acid, were intraperitoneally injected with ^{14}C-leucine and then sacrificed one hour following the ^{14}C-leucine injection. Control rats were treated in the same way except for using saline instead of the previous loading of amino acid. Radioactivity was assayed in the free amino acid fraction and protein fraction in the brain. Free amino pattern in the brain was quantitatively determined by automatic amino acid analyzer. A detailed description of the method has been given elsewhere [4, 5].

In these experiments, an amino acid imbalance in blood was definitely induced as shown in Table 2. Except for the amino acid loaded, all the amino acid levels remained within normal range. In case of phenylalanine loading, levels of tyrosine as well as phenylalanine in serum were elevated. Phenylpyruvic acid or 0-hydroxyphenylacetic acid was not detectable in the brain from rats with phenylalanine loading by paperchromatography.

TABLE 2. — **Serum amino acid levels of the rats one hour after the intra-peritoneal injection of a single amino acid (mg/100ml).** (Loaded dosis of each amino acid is shown in Tables 2-8).

	Phenylalanine		Valine		Histidine		Methionine	
	Loaded	**Control**	**Loaded**	**Control**	**Loaded**	**Control**	**Loaded**	**Control**
Aspartic acid			1.38	0.48	4.03	3.79	4.39	4.25
Threonine	2.07	2.03	1.71	1.65	5.65	2.86	4.09	3.78
Serine+glutamine	12.00	11.30	2.73	6.30	11.90	10.62	7.07	9.30
Proline	3.51	2.73	1.47	1.40	3.84	2.25	2.73	1.89
Glutamic acid	10.06	8.68	2.70	2.29	6.35	3.14	2.44	2.51
Glycine	3.26	4.00	0.84	1.50	5.04	3.44	2.78	2.90
Alanine	9.24	8.56	2.59	3.56	8.38	7.64	9.50	8.51
Valine	4.59	3.19	64.70	2.62	4.14	3.60	2.48	2.02
Methionine	1.69	1.31	0.42	1.19	2.50	1.44	124.40	1.15
Isoleucine	1.55	1.22	1.05	1.26	1.49	1.32	1.31	1.20
Leucine	2.57	2.11	2.09	2.09	3.07	2.60	1.97	1.15
Tyrosine	55.00	2.67	0.51	2.03	1.51	1.28	0.91	1.10
Phenylalanine	57.00	1.41	0.39	1.12	1.12	0.97	0.69	1.05
Lysine					3.39	3.67	7.21	3.48
Histidine					166.90	3.65	2.67	2.60
Arginine					2.16	1.88	3.34	2.54

* Values represent the averages from 2 to 4 rats.

Both the uptake of ^{14}C-leucine into the brain and the incorporation of ^{14}C-leucine into the brain protein were significantly diminished, when phenylalanine, valine, histidine or methionine had been loaded (cf. Table 3). It was also found that the loading of single amino acid in excess caused a

TABLE 3. — **The uptake of ^{14}C-leucine into the brain and the incorporation of ^{14}C-leucine into the brain protein in experimental hyperaminoacidemia.**

	Uptake of ^{14}C-leucine into the brain	Incorporation of ^{14}C-leucine into the brain protein
Control group	100,0 %	100,0 %
Phenylalanine loading group	50,2 %	51,8 %
Control group	100,0 %	100,0 %
Valine loading group	55,3 %	77,8 %
Control group	100,0 %	100,0 %
Histidine loading group	31,0 %	41,7 %
Control group	100,0 %	100,0 %
Methionine loading group	73,8 %	60,8 %

NOTE: VALUES REPRESENT THE AVERAGES FROM 2 TO 4 RATS.

significant alternation of the free amino acid pattern in the brain (Fig. 1-4). Amino acid loaded was found to be elevated in the brain whereas most of the other amino acids tended to decrease; as a whole total moles of free amino acid in the brain re-

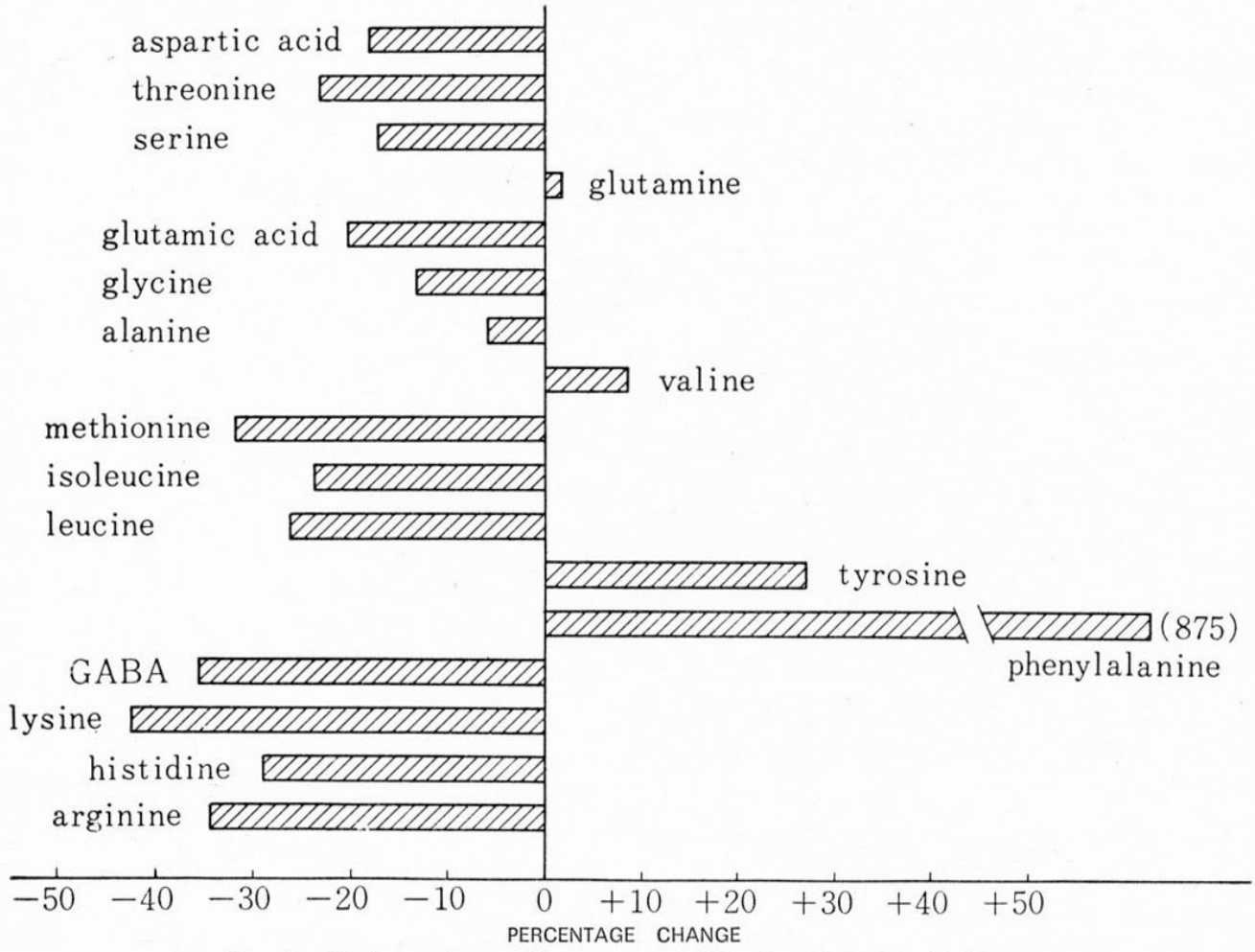

Fig. 1. Brain amino acids in rats with phenylalanine loading. Note: control values set at zero:

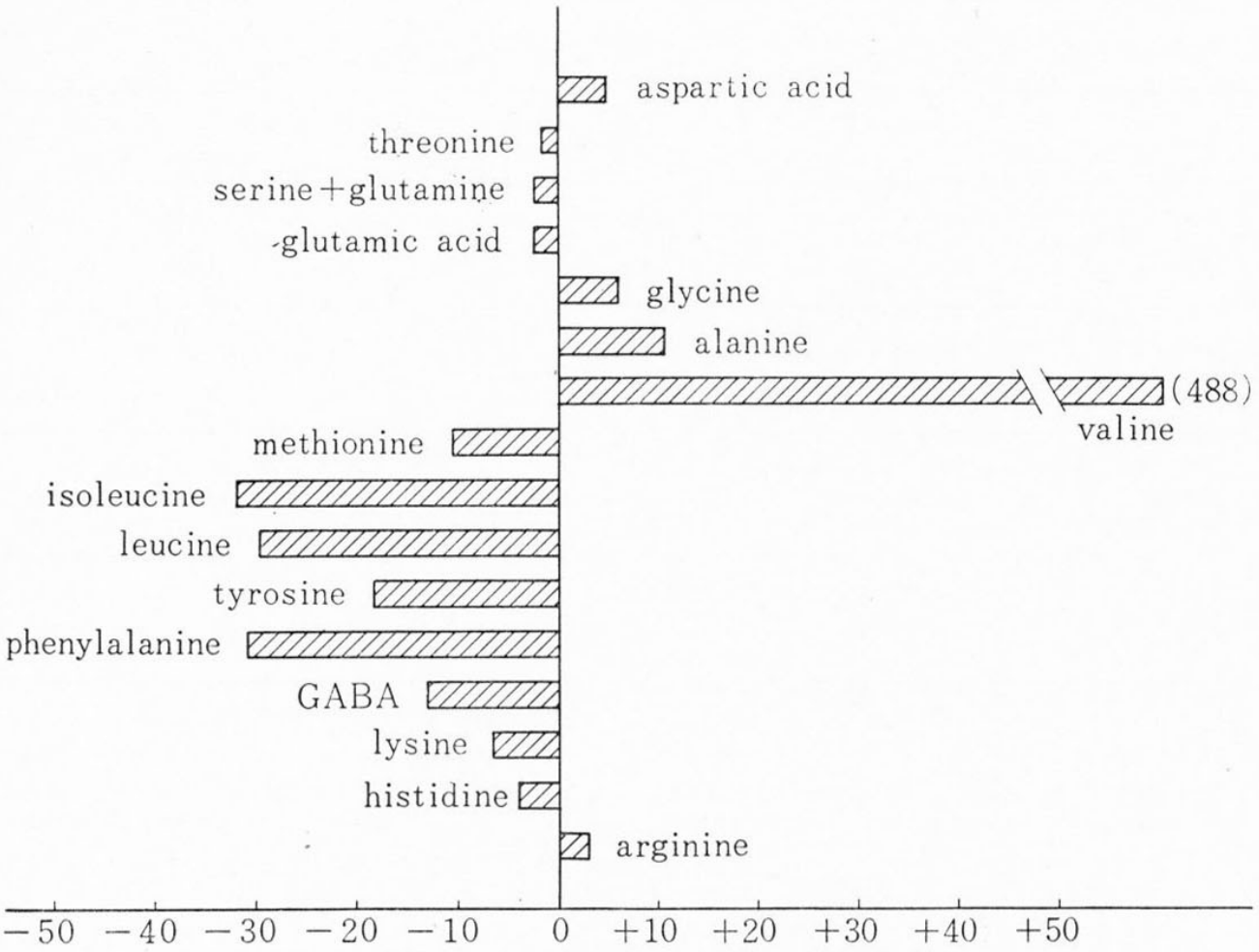

Fig. 2. Brain amino acids in rats with valine loading.

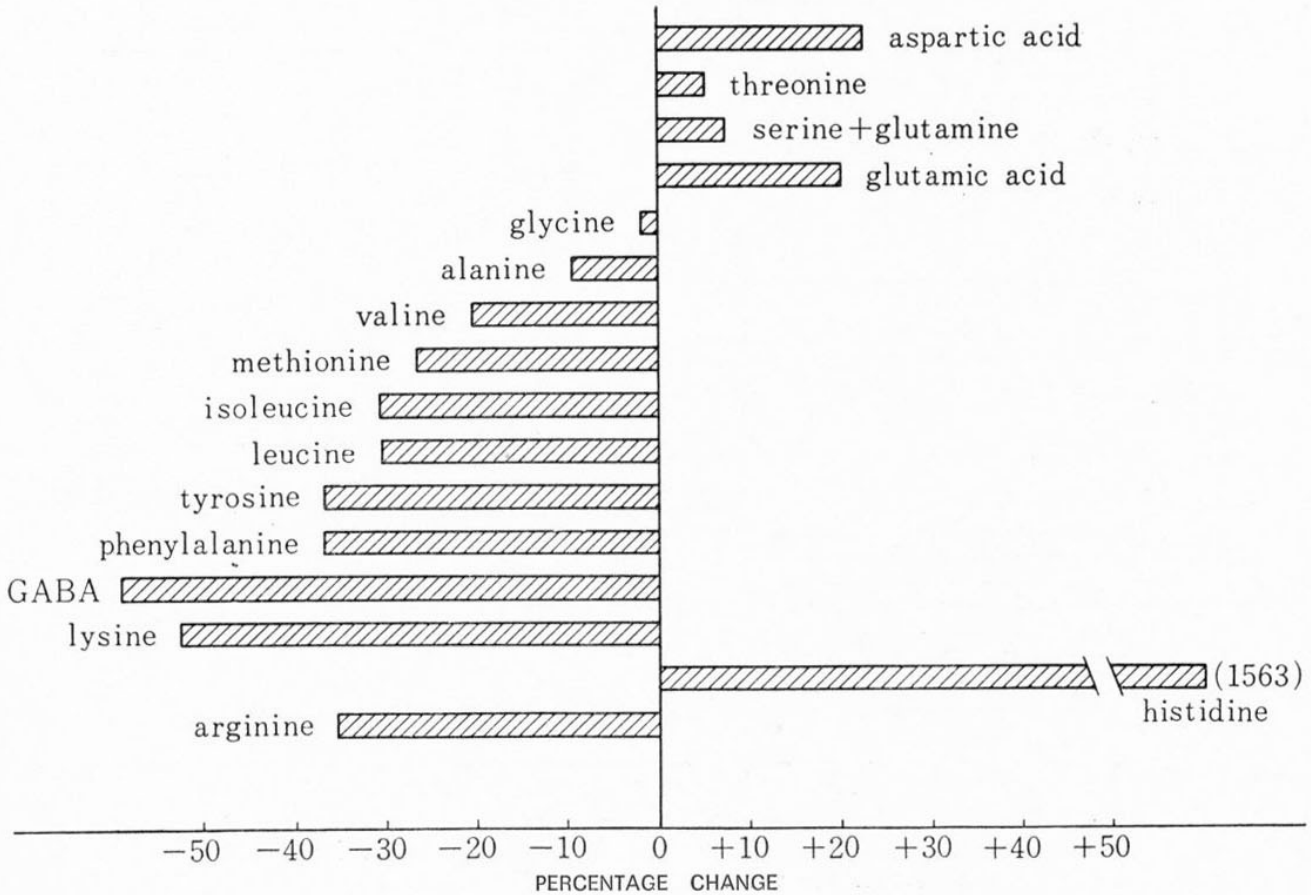

Fig. 3. Brain amino acids in rats with histidine loading.

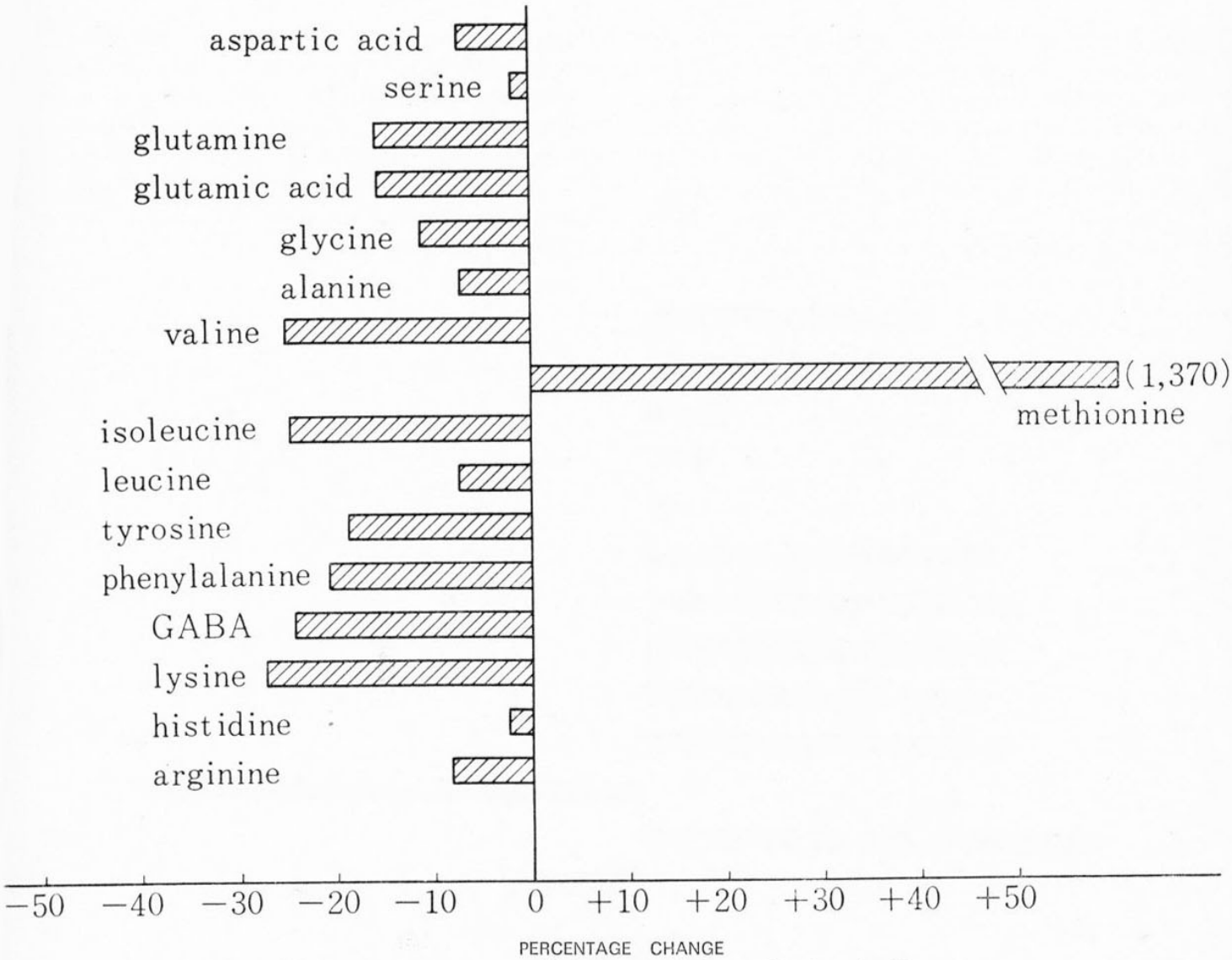

Fig. 4. Brain amino acids in rats with methionine loading.

mained almost unchanged before and after the loading of single amino acid. This implies that free amino acid pool in the brain is a constant capacity. Therefore, if a certain amino acid enter excessively into the brain due to the increased level of the amino acide in blood, the uptake of other amino acids into the brain may diminish. This may be the reason why the alternation of free amino acid pattern in the brain takes place by the loading of single amino acid.

Although the brain tissue contains essential amino acid in relatively low levels as compared with nonessential amino acid such as glutamic acid, aspartic acid or GABA, the former levels are important for protein synthesis. The optimal balance of concentration of amino acids is quite important in protein synthesis because the rate of protein synthesis is limited by the least amino acid. The levels of essential amino acid in the brain are dependent on uptake from blood, since these amino acid are unable to be synthetized within the brain. It is, therefore, reasonable to presume that the inhibition of amino acid transport by excess of a single amino acid may result in essential amino acide in the brain and in reduced rate brain protein synthesis. Decreased protein synthesis in the brain may play an important role on development of mental retardation seen in most of inborn errors of amino acid metabolism.

2. Free amino acid pattern in the brain from the patient with phenylketonuria

At autopsy of an untreated phenylketonuric patient who had mental retardation

(IQ; 30) and died of pneumonia at the age of ten years, the brain tissue was analyzed for free amino acids. The results are shown in Fig. 5. As compared with control brain of the same age, the patient's brain showed a marked increase in phenylalanine and a decrease in aspartic acid, threonine, valine, methionine, isoleucine, leucine, tyrosine and GABA. There is a similarity in the free amino acid pattern in the brain between the phenylketorunic patient and rats with experimental hyperphenylalaninemia. Neither phenylpyruvic acid nor its derivatives was detected (below 1.0 x 10^{-4}M.) by paper-chromatographic analysis in the brain tissue from the patient.

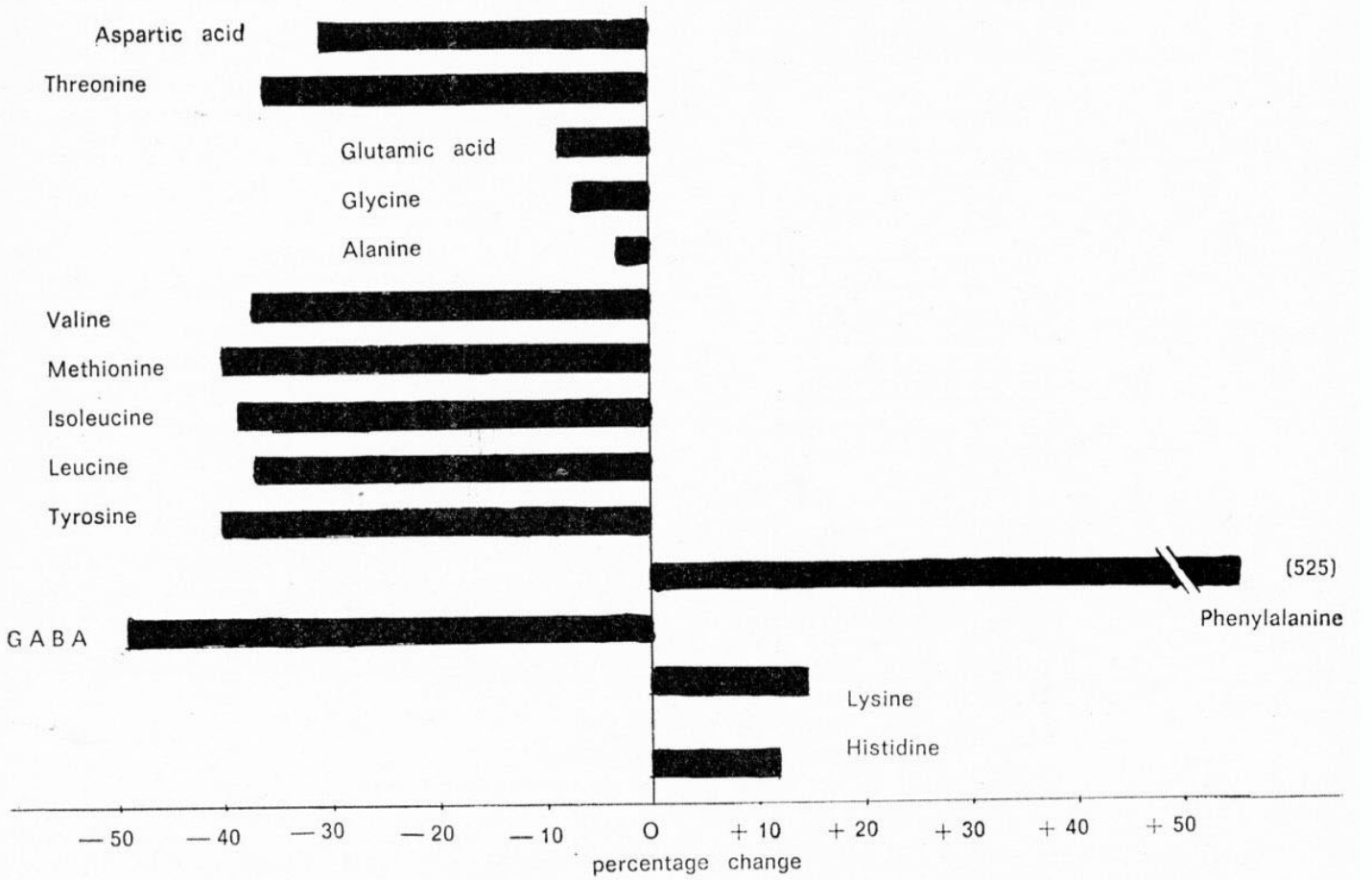

Fig. 5. Free amino acid pattern in the brain from the patient with phenylketonuria. Note: Values in the control brain set at zero.

COMMENT

There has been postulated a possibility that abnormal metabolites such as phenylpyruvic acid or O-hydroxyphenylacetic acid may have a toxic effect on the brain development. These metabolites were thought to lead to disturbance of brain metabolism, through their inhibitory effect upon oxygen consumption [6], 5-hydroxytryptophan decarboxylase [7] or glutamic acid decarboxylase [8]. There has, however, been no report of accumulation of these metabolites in the brain of phenylketonuric patients. Our results demonstrated no detectable amounts of phenylpyruvic acid or its derivatives in the brain from a phenylketonuric patient. Although these metabolites excrete in large amounts in urine, their blood levels are extremely low because of high renal clearance. In addition, such metabolites are hard to pass through the blood-brain-barrier. Thus it is unlikely that these metabolites accumulate in the brain to toxic level.

It may be more reasonable to pay attention to phenylalanine itself as a cause of brain damage in PKU [9, 10] which was found to be present at the very high level (about 20 to 50 folds as high as normal) in blood of the patient. The experimental

results described above showed that both the uptake of ^{14}C-leucine from blood into the brain and its incorporation into the brain protein were definitely inhibited when hyperaminoacidemia was induced by the loading of a single amino acid and that a disruption of free amino acid pattern in the brain occurred in the experimental condition.

It is well known that the brain shows rapid development in infancy and protein synthesis is very active in the growing brain. The fundamental construction of the brain related to "intelligence" may be established during this period. In other words, the brain in the growing period may be vulnerable by environmental changes. So, the impaired brain protein synthesis during the vulnerable period may result in an irreversible damage of brain function. This assumption seems to be supported by the fact that the patient with PKU received an appropriate dietary treatment in early infancy show normal development of the brain function and show no more mental deterioration in spite of the reversion of metabolic disturbance by abolishment of the therapy in the late childhood [11].

Recently an increasing attention has been paid to the ill effects on brain development which may result from malnutrition in utero and in early infancy [12–14]. A number of investigators have reported that undernutrition from birth to 21 days of life (weaning period) in the rat produces a persistent and permanent reduction in brain weight. In contrast, undernutrition after 3 weeks of age in the rat results in smaller effects on brain weight and complete return to normal weight when the animal is rehabilitated. These data indicate that the earlier the malnutrition, the more severe the effect and the less likely recovery. They suggest that there is a critical period of brain growth in the rat during which the brain is most susceptible to the effects of malnutrition. Platt et al. [15] have observed degenerative changes in both neurons and glia in the central nervous system of rats, pigs and dogs raised from weaning on protein deficient diets. These changes persisted even after 3 months of rehabilitation on a protein-rich diet. The severity of these change could be increased by lowering the age at which the restriction was imposed or by increasing the duration of the deficient diet.

Cabak and Najdavic [16] demonstrated in a retrospective study that Serbian children with a history of marasnus had significantly lower IQ than Serbian children in general. Stoch and Smyth [17] reported in a prospective study that South African children malnourished early in life had smaller head circumferences and lower IQ than control population. Recent studies by Hertzig et al. [18] also demonstrated that school children of 74 cases in Jamaica who had been severely malnutrished during the first 2 years of life showed a significantly lower IQ as compared with control children. Winick [14] examined biochemically the brain of infants in chilie who died of severe malnutrition during the first two years of life and found a significant resuction in content of protein, RNA and DNA in the brain.

These studies both in animals and in humans suggest that the brain development may be retarded by malnutrition and that the earlier the nutritional deprivation, the more severe the retardation.

Hereditary amino acid disorders may be regarded as a sort of malnutrition endogenously occurred, in respect of "amino acid imbalance" in body fluid.

It has been reported that children born from mothers with PKU represents inevitably mental retardation and microcephaly in spite of no disturbance in phenylalanine metabolism [19–22]. It is presumed that high levels of phenylalanine in the mother's blood during the pregnancy may affect the developing brain of the fetus. Since the metabolites such as phenylpyruvic acid can not pass through the placenta, it is improbable that these metabolites excert influese upon the fetus. Pathogenesis of "maternal PKU" can be understood by the above-mentioned view that high level of phenylalanine in mothers blood may disturb the transport of the other amino acids by the placenta and cause an imbalance of free amino acid pattern in the fetus and thereby protein synthesis in the brain may be impaired. In general ,the placenta has a function to maintain higher levels of each of the free

amino acids in the fetus than in the mother. Accordingly, the imbalance of amino acid in the mother's blood may reflect exaggeratedly in the fetus. Kerr et al. [23] produced maternal hyperphenylalaninemia by feeding excess phenylalanine to pregnant rhesus monkeys and found that the levels of phenylalanine in umbilical cord serum were greater than those in the maternal serum. Infant monkeys born from mothers with hyperphenylalaninemia represented a low birth weight and significant reduction in learning behavior. Allan and Brown [24] reported a successful treatment of maternal PKU. The patient was a 27 year-old woman with phenylketonuria who had three mentally retarded children previously. At her fourth pregnancy, she was given low phenylalanine diet during the later five months of pregnancy and two weeks after the delivery. Serum phenylalanine of the patient was maintained at about 3.5 mg/dl throughout the treatment period. As a result, the patient was delivered of a full-term infant who exhibited normal development and 108 of DQ at the age of 9 months.

These findings from maternal PKU also give us the evidence that an amino acid imbalance affects brain development.

On the other hand, an imbalance in amino acid pattern in blood was less remarkable in homocystinuria or cystathioninuria because of high renal clearance of homocystine or cystathionine. But the patients with these disorders have sometimes mental retardation. In these disorders, it is a matter of great concern that cystine become an essential amino acid because the in vivo conversion of methionine to cystine is blocked by the enzyme defect.

Therefore, the patients with homocystinuria or cystathioninuria are apt to fall in cystine deficiency according to nutritional condition. In fact, we found a decrease of cystine content in the liver from a patient with homocystinuria [25]. It is reported that cystine content in hair from the patients with homocystinuria or cystathioninuria is often diminished [26, 27]. Wright [28] reported that the homocystinuric patient with mental retardation received only cow's milk during infancy, while the cousin who was not mentally retarded received human milk during the first six weeks of life. This report would be of interest since the casein from cow's milk is a relatively poor source of cystine while the casein from human milk is a considerably richer source of cystine.

There is a possibility that deficiency in cystine may be a rate-limiting factor of brain protein synthesis. Therefore, the development of mental retardation in homocystinuria or cystathioninuria may be dependent on nutritional condition in the early period of life in addition to underlying genetic defect. This may be the reason why some patients are mentally retarded and some mentally normal in these disorders.

The same situation will be applied to the disorder of tryptophan metabolism such as congenital tryptophanuria [29], hydroxykynureninuria [30] or B_6 dependent xanthurenic aciduria [31, 32] Triptophan plays a physiologically important role on its conversion to NAD which is essential in energy producing system. NAD is formed from dietary nicotinic acid as well as in vivo conversion from tryptophan. The supply of NAD derived from tryptophan may be more significant in infancy which represents a rapid development. In the disorder which has a block in the pathway from tryptophan to NAD, there is a tendency to be apt to be deficient in NAD according to nutritional condition. Probably the incomplete supply of NAD might be a handicap for the brain development in early stage of life. In our sibling patients with B_6 dependent xanthurenic aciduria [32] the older sister who had been given a vitamin preparation containing 2 mg of B_6 and 20 mg of nicotinic acid throughout the infancy showed a mild retardation of speech development (IQ being 98) and a younger sister who had not been given any vitamin preparation was mentally retarde (IQ being below 50).

In the disorders involved in urea cycle such as congenital hyperammonenia, citrullinemia, argininosuccinic aciduria of hyperargininemia, the elevation of blood ammonia may be responsible for the brain damage, because ammonia is known to pass easily through the blood-brain-barrier and excert toxic effect on the central nervous system.

Summarizing above, at least three type of mechanism may be postulated for development of mental retardation in inborn errors of amino acid metabolism (Fig. 6).

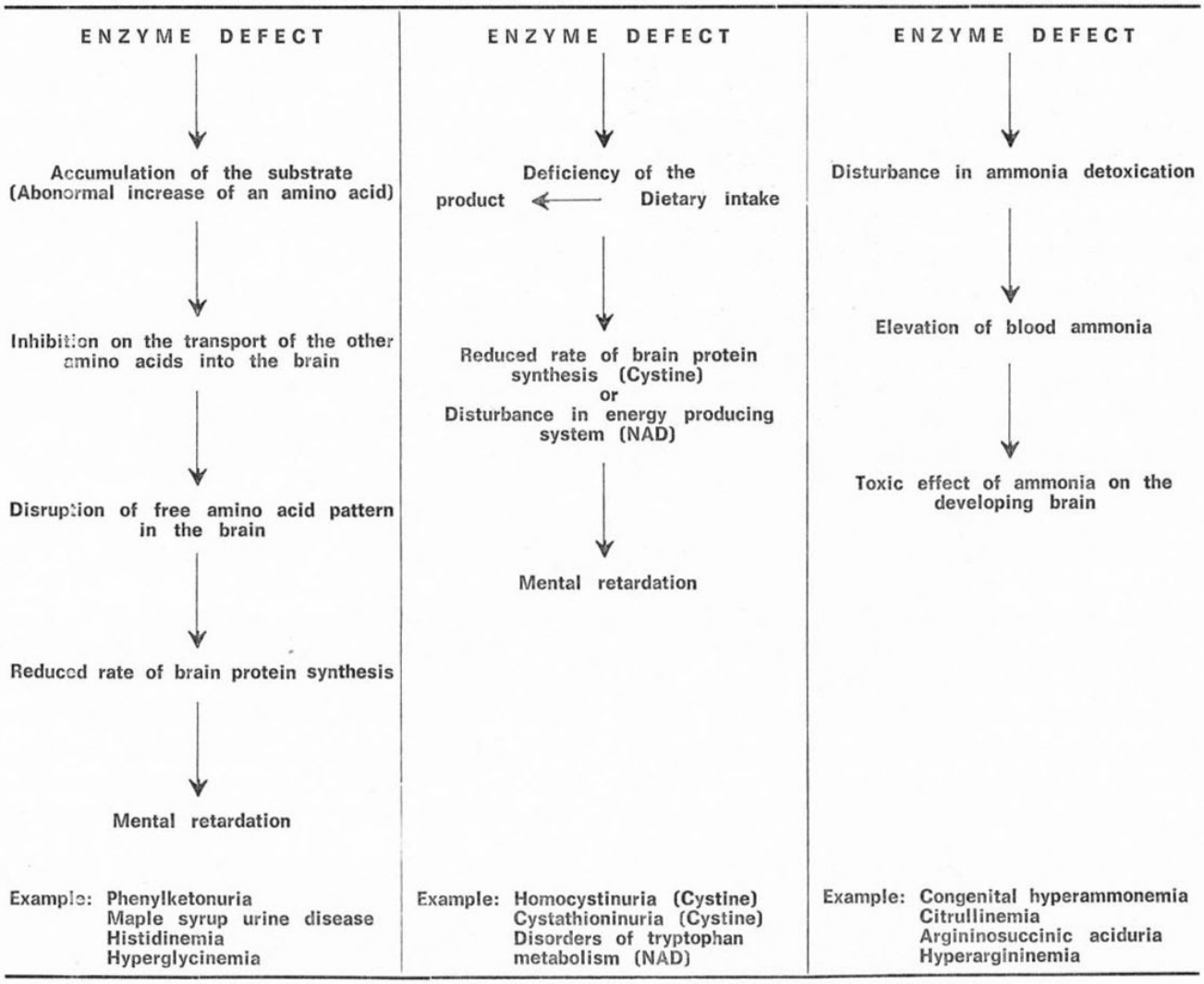

FIG. 6. — Pathogenesis of mental retardation in amino acid disorders.

SUMMARY

It is known that most of inborn errors of amino acid metabolism are more or less associated with mental retardation. The results of experimental hyperaminoacidemia showed that both the uptake of ^{14}C-leucine from blood into the brain and its incorporation into brain protein were definitely diminished when hyperaminoacidemia was induced by the loading of a single amino acid to the rat. Furthermore, the disruption of free amino acid pattern in the brain was found in these experiments. These findings suggest that an amino acid in excess, in other words an extreme imbalance in amino acid in blood, may result in decrease of brain protein synthesis.

The reduction of brain protein synthesis in the early period of life which represents a rapid development of the brain may play an important role on pathogenesis of mental retardation in the disorders accompanied with a remarkable increase in certain amino acid in blood.

In the disorders of methionine or tryptophan metabolism, the nutritional factor such as the intake of cystine or nicotinic acid may participate in the development of mental retardation, in addition to underlying genetic defect.

Thus, hereditary amino acid disorders might be regarded as a sort of malnutrition occurred endogenously.

RESUMEN

Es sabido que la mayoría de los errores innatos del metabolismo de amino ácidos están más o menos asociados a retardo mental. Los resultados de la hiperaminoacidemia experimental mostraron que tanto la absorción de 14C-Leucina de la sangre al cerebro como su incorporación a la proteína del cerebro, estaban definidamente disminuidas cuando fue inducida la hiperaminoacidemia con la carga a la rata de un único aminoácido. Además en este experimento fue hallada desorganizada la forma del aminoácido libre. Estos hallazgos sugieren que un aminoácido en exceso, en otras palabras, un desequilibrio extremo de amino ácidos en la sangre, puede resultar en una disminución de la síntesis de la proteína cerebral.

La reducción de la síntesis de la proteína cerebral en edad temprana, la cual implica un desarrollo rápido del cerebro, puede jugar un papel importante en la patogenia del retardo mental en los desórdenes acompañados con un aumento notable de cierto aminoácido en la sangre.

En los desórdenes de metionina del metabolismo de triptofano, el factor nutricional tal como la incorporación de cistina o ácido nicotínico puede participar en el desarrollo de retardo mental, agregado al defecto genético de base.

De este modo desórdenes hereditarios de aminoácidos pueden considerarse como un modo de desnutrición que ocurre endogenicamente.

RÉSUMÉ

Bien est connu que défauts innés de metabolism amine- acide sont plus ou moins unis a retardation mental. Les résultats avec hiperaminoacidemie expérimental ont prouvé que tant l'absortion de 14C-Leucine de la sang au cerveau, comme l'incorporation a la proteine du cerveau, se disminuirent définitivement quand on induit hiperaminoacidemie chargeant au souris un seul amine acide.

Encore, on a trouvé dans ces expériments le rupture de traces de libre amine acide, dans le cerveaux. Ces découverte suggèrent qu'un excès d'amine acide, c'est-a-dire un extrême imbalance d'amino acide dans la sang peut avoir une diminution de la synthèse de la proteine du cerveau.

La diminution de la synthèse de la proteine cérébrale dans le premier période de la vie, le quel représente un développement rapide du cerveau, peut jouer un important rôle au pathogenie de retardation mental, dans les defauts unis avec une augmentation remarquable dans certains amino acides dans le sang.

Dans les défauts de metionine du métabolisme de tryptophan, le facteur nutritif comme l'admission de cystine ou acide nicotinic peut participer dans le dévelopment de la retardation mentale, en outre de défaut genetic fondamentals.

De cette manière, défauts héréditaire de amino acids peuvent être consideré comme un cas de mauvaise nutrition passé endogène.

ZUSAMMENFASSUNG

Es ist bekannt, dass die meisten angeborenen Mängel des Aminosäuremetobolismus mehr oder weniger mit mentaler Verzögerung verbunden sind. Das Resultat von experimentalen Hyperaminosäuren zeigten, dass die Einführung von ^{14}C-Leucin des Blutes ims Hirn und sein Einschluss in das Gehirnprotein gemindert wurden, wenn die Hyperaminosäure vermittels der Anwendung einer einzigen Aminosäure der Kette zugeführt wurde. Noch mehr, fand sich der Bruch des Musters der freien Aminosäure bei diesen Experimenten. Diese Befunde suggerieren, dass eine Aminosäure im Überschuss, oder mit anderen Worten, eine extreme störung des Gleichgewichtes von Aminosäuren im Blut, eine Verminderung der Synthese des Gehirnproteins bewirken kann.

Die Reduktion der Synthese des Gehirnproteins in der frühen Lebensperiode, die eine schnelle Entwicklung des Gehirns darstellt, Kann eine wichtige Rolle spielen in der Pathogenese der mentalen Beschränkung bei den Mängeln, die von einer bedeutenden Vermehrung einer gewissen Aminosäure im Blut begleitet sind.

Bei Mängeln im Metabolismus vom Tryptophan oder Metionin, kann der Ernährungsfaktor wie der Zutritt von Cystin oder Nikotinsäure bei der Entwicklung der mentalen Beschränkung als Addition des unterliegenden genetischen Defektes teilhaben.

Daher können die vererbten Irregularitäten der Aminosäuren als eine Folge der Mangelernährung im Endogenium betrachtet werden.

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HISTORY OF MEDICINE

Historical Reflections on the Backgrounds of Neurophysiology:

Inhibition, Excitation, and Integration of Activity

JOHN F. FULTON*

Since this is the centenary year of the *birth* of one of the most influential physiologists of our generation and the *ter*centenary of the *death* of another man who left a greater mark on the evolution of medical science than any individual in our annals, it is highly appropriate that in 1957 we should concern ourselves with the development of the major concepts of physiology. Between Charles Scott Sherrington, born in 1857, and William Harvey, who died in 1657, there was no one of comparable stature in the field of physiology — the subject which the College of Medicine of the State University of New York has so wisely chosen to commemorate in its centenary yeat. I consider it a special honor to be invited to participate in these timely observances, and I propose to speak on inhibition, excitation, and integration of activity in the nervous system, with particular reference to inhibition.

Charles Scott Sherrington

Charles Sherrington, who devoted the best years of his life to the study of the nervous system, first saw the light of day in Yarmouth, a seaside town in the county of Norfolk. He had a good primary education in the classics and chose poetry as an avocation. Later, during his continental travels as a student, he came under the influence of men such as Fritsch and Hitzig, Goltz, Koch, Pflüger, Helmholtz, Cohnheim, Waldeyer, and du Bois-Reymond, all well-known figures — physiologists, bacteriologissts, and clinicians — in the hey-day of German scientific medicine.

When he returned to England he came strongly to admire the experimental neurologist, David Ferrier, who discovered the motor areas of the cerebral hemispheres, and also Michael Foster of Cambridge University, the first to hold a full-time chair of physiology in England. Foster was a great teacher and while he published almost nothing he nevertheless trained a generation of men of high destiny in physiology and medicine — and perhaps his most distinguished pupil was Sherrington himself.

Another prominent figure at Cambridge in those days, but one much less colorful than Foster, was John Newport Langley, a neuroanatomist who flirted from time to time with physiology and who introduced the nicotine method of locating synapses in the ganglia of the "autonomic nervous system" (a term which Langley introduced).[1] It was in collaboration with Langley that Sherrington in 1884 published his first scientific paper, one describing the spinal degenerations which follow injury to David Ferrier's newly discovered motor cortex. This paper set a pattern for nearly all of Sherrington's subsequent scientific publications, for throughout his life he always sought to turn anatomical facts into physiological language. His interest in the phenomenon of central inhibition began at this time and it continued to engross his mind until a short time before he died in 1952.

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Réné Descartes and reciprocal innervation

We may now turn to our main theme, the early history of the concept of inhibition in the "Integrative action of the nervous system," the term coined so appropriately by Sherrington exactly fifty years ago.[2]

Nowhere in the writings of Galen or in the later publications of Vesalius, Paré, Fernel, or Harvey is there a clear statement of the concept of the reflex, and it remained for the renowned French philosopher, mathematician, and experimentalist, Réné Descartes (1596-1650), to be the first to grasp the idea of reflex action and to describe it in clear language that no one could misunderstand; but while Descartes carried out many fundamental experiments in the field of physics, his beliefs concerning the reflex were not, so far as is known, put to the test of experiment. His diagrams of the reflex, however, were clear and convincing and they have been oftentimes cited in the literature of the history of psychology and neurophysiology — most recently by Dr. Horace Magoun in one of the earlier symposia of this series.

Descartes was aware of the existence of the pineal body and he postulated that all incoming sensory impulses impinged on the pineal and from there were reflected' back into appropriate motor nerve channels which in turn caused muscles to contract. He concerned himself not only with the reactions to painful stimuli, but also with visual reactions to light as indicated in the well-known Cartesian diagrams. There is also some basis for the suggestion that Descartes had a glimmering of the law of specific nerve energies later to be adumbrated by Johannes Müller.

The notion of active inhibition of movement is less clearly ser forth by Descartes than is the concept of the reflex. In his celebrated book, *De homine*, first published posthumously by his friends in 1662 (in Latin, although the text was originally written in lucid, idiomatic French), he discusses the problem of reciprocal innervation of antagonistic muscles, devoting special attention to the extraocular muscles of the eye. In a lateral willed movement, he argued, one external ocular muscle is contracted while its antagonist is actively inhibited. His explanation of the nerve impulse was, however, still essentially Galenical. He insisted that during a lateral movement of the eye, 'vital spirits' were *conducted into* the external rectus muscle by valve channels in nerves leading to that muscle, whereas simultaneously 'vital spirits' *emerged* from the internal rectus (by valve channels leading *away* from the muscle in question) causing the muscle to cease contraction. The necessity of reciprocal innervation was thus clear to him, but he seems to have trought that the essential mechanisms were peripheral and not central, as we know them to be. In another book, *Passions of the soul*, there occurs the oft-quoted passage in which the word "reflected" is used.

> For in certain persons that [previous associations] disposes the brain in such a way that the spirits reflected from the image thus formed in the gland proceed thence to take their places partly in the nerves which serve to turn the back and dispose the legs for flight, and partly in those which so increase or diminish the orifices of the heart, or at least which so agitate the other parts from whence the blood is sent to it, that this blood being there rarefied in a different manner from usual, sends to the brain the spirits which are adapted for the maintenance and strengthening of the passion of fear, *i.e.*, which are adapted to the holding open, or at least re-opening, of the pores of the brain which conduct them into the same nerves (Article XXXVI).

During his wanderings through Europe Descartes had come to know many of the crowned heads and nearly all the great philosophers and schoolmen of Europe, and during the last five years of his life he chose to settle in Stockholm where the gift ed Queen Christina had befriended him even as she had long been his patron. He died in Stockholm on 2 February 1650 at the early age of fifty-four, and was buried in a corner of the great cemetery at Stockholm in a spot reserved for distinguished aliens. As a footnote to history, one may refer to the scholarly note of Dr. E. Weil telling the macabre story of the peregrinations of his remains which were soon disturbed by his well-meaning friends both in

France and Sweden, and how his skull came eventually to be placed in the Musée de l'Homme in the Palais de Chaillot next to another glass case containing the skull of a criminal from the Collection of the phrenologist, Gall. In 1666, in accordance with the wish of his friends in Paris, his remains were exhumed to be returned to France. The sentimental Chevalier de Terlon, French ambassador to Sweden at the time of the exhumation, cut off the forefinger of the right hand which had served as the instrument of the deceased responsible for his immortal writings. Terlon prepared a copper coffin, unfortunately only two feet and a half long, and in order to close the coffin he was obliged to detach the skull. Its small size enabled the coffin to be disguised, and after many delays en route to Paris, including a three-month hold-up at Copenhagen, it finally arrived in Paris early in January, 1667. It was later taken with great pomp to the Abbey of St. Etienne-du-Mont where a tomb with an elaborate epitaph was erected. The skull, however, had a more sordid fate; a devoted Cartesian follower, named Hanström, a captain in the Swedish Guards, ran off with the original skull and exchanged it for another. Between then and 1821 it was in and out of the libraries of many Swedish collectors, and eventually, in 1821, it fell into the hands of Berzelius who wrote to Cuvier, the foremost academician of France, offering the skull. The previous owner, Arngreen, had paid for it Fr. 37, a small sum for the head that had created *Discours de la méthode pour bien conduire sa raison.* Berzelius' offer was accepted by the Académie des Sciences where it was received with *déférence religieuse.* By 1913 some doubt had arisen concerning the authenticity of the skull, and finally Dr. Paul Richer, the anatomist, was asked to clear the question; by comparing the skull with different portraits of Descartes, he felt he had established its authenticity without question. Dr. Weil quite naturally raises the question of whether the skull should not now be properly reinterred with the body in the Descartes tomb in St. Germain-des-Prés where it was moved in 1819.

This unusual story that is scarcely known outside of France takes me back to the year 1923 when Lady Osler, who shared Sir William's great interest in Sir Thomas Browne, took me to the ceremony of the reinterment of Sir Thomas Browne's skull which had been stolen many years before from his tomb and which was finally ordered reinterred by the Archbishop of Canterbury who had not previously knwno of the sacrilege of its theft — not, however, before Sir Arthur Keith and Miss Tildesley had written a monograph on the skull, recording its measurements, with innumerable photographic reproductions, a volume that has become a great desideratum of all the more earthy admirers of Sir Thomas Browne. The young minister at St. Peter's Mancroft was hard put to it trying to decide upon a suitable service, but, if I recall correctly, he ended with an abbreviated version of the usual burial service of the Church of England, and it met with Lady Osler's complete approval.

Sir Kenelm Digby

In 1937 I had occasion to write a short biographical monograph entitled, *Sir Kenelm Digby. Writer, bibliophile and protagonist of William Harvey.* Digby, who was born in 1603, on the 11th of June to be precise, was a swashbuckling adventurer whose father had been hanged for treason because of his part in the Gunpowder Plot. At the age of fifteen we find Digby in Gloucester Hall at Oxford; he left in 1620 without taking a degree because he had fallen in love with the beautiful Venetia Stanley. At the age of twenty-four he went off on a privateering expedition, one, as it turned out, more of piracy than privateering. He had two small ships, the "Eagle" of four hundred tons, the "George and Elizabeth" of 250, and in January they captured off Gibraltar several Flemish and Spanish ships, and a few days later they took over a Dutch ship near Majorca. Following this, he proceeded with his loot to the island of Zante where he encountered heavy storms, but where he fared better than had Vesalius sixty years earlier. On reaching the neutral Turkish port of Scanderoon on the coast of Syria, he attacked without warning the French and Venetian ships in the harbor which surrendered after three hours of fighting. On his way back he wrote his famous commentary on a

stanza of Spenser's *Faerie Queene.* Apart from these adventures in his youth and a truly great book published in English in Paris in 1644 under the title, *Souls and bodies,* Digby is probably best known for his famous epitaph:

Under this Tomb the Matchless Digby lies;
Digby the Great, the Valient, and the Wise;
This Ages Wonder for His Noble Parts;
Skill'd in Six Tongues, and Learn'd in All [the Arts.
Born on the Day He Dy'd, Th'Eleventh [of June,
And that Day Bravely Fought at [Scanderoun.
'Tis Rare, that one and the same Day [should be
His Day of Birth, of Death and Victory.

In my account of Digby I pointed out that he was the first Englishman to appreciate the true significance of Harvey's discovery of the circulation. The fact that he wrote of it in English in his *Souls and bodies* helped convince the doubting British medical public. When I prepared this monograph I had not realized that Digby also had described reflex action and particularly the conditioned reflex. Let me quote several telling passages from *Souls and bodies*:

> And not only such aversions... do cause these effects of feare, and of trembling, and of flyng from those that do make such impressions; but even the seeing them angry and in fury doth the like...
> And thus you see, how they are strong impressions upon sense, and not any discourse of reason, that do governe beastes in their actions: for if their avoiding men, did proceed from any sagacity in their nature, surely they would exercise it...

Other contributions of the early 19th century

After Descartes and Digby and prior to Sherrington the most important development concerning the central inhibitory process came in the nineteenth century. Ideas concerning reflex action were greatly clarified in the mid-eighteenth century by Robert Whytt in his celebrated monograph, *An essay on the vital and other involuntary motions of animals* — a book which did much to stimulate investigation of the workings of the nervous system. Whytt's emphasis on 'involuntary motions' laid the groundwork for all subsequent studies on the so-called 'autonomic nervous system' of Langley.

The versatile Scottish surgeon, Charles Bell, envisaged the idea of reciprocal mechanisms during the execution of willed movements. Thus in paper in the *Philosophical Transactions* of the Royal Society, published in 1823, Bell wrote: "The nerves have been considered so generally as instruments for stimulating the muscles, without thought of their acting in the opposite capacity, that some additional illustration may be necessary here. Through the nerves is established the connection between the muscles, not only that connection by which muscles combine to one effort, but also that relation between the classes of muscles by which the one relaxes while the other contracts." He then goes on to cite the need for reciprocal innervation of the eye muscles, but, like Descartes, he believed that the machanism was probably peripheral rather than central.

The next important advance came with the discovery by the Weber brothers — Eduard (1806-1871) and Ernst Heinrich (1795-1878) — of the inhibitory action of the vagus nerve on the heart. Their first experiments were begun in 1845 during which they found that the heart could be brought to a complete standstill if the peripheral end of the vagus nerve were stimulated with sufficient strength. It was later disclosed that the vagus contained both inhibitory and acceleratory fibers. The Webers' observation was soon followed by reports of nerve-induced inhibition of other visceral structures. Thus Pflüger in 1867 found an inhibitory nerve to the intestinal wall and a short time later Claude Bernard came upon an inhibitory nerve to the submaxillary artery. In one of Pavlov's earliest papers (1885) he described the inhibitory nerve to the adductor muscles of *Anodon,* the bivalvedmollusc, and in the following year Biedermann found the inhibi-

tory nerve to the claw muscles of *Astacus*.

Highly significant also were studies of the Russian physiologist, Setchenov, who first postulated the existence of an inhibitory center in the central nervous system.[3]

Ivan Mihailovich Setchenov (1829-1905)

The foremost student of the central nervous system prior to Pavlov was a man who until recently was little known outside of Russia except to a few German neurophysiologists who had early recognized his genius. His name was Ivan Mihailovich Setchenov (1829-1905) and he was born in a small town on the Volga in the province of Simbirsk At the age of fourteen, he entered the school for military engineers, but after graduation from that institution he decided to study medicine at the University of Moscow, and after receiving his doctorate in 1856 he, like Pavlov, left for Germany and studied under many of the men who later were sought out by Pavlov and Michael Foster, and also by Sherrington, i.e., Helmholtz, Du Bois-Reymond, Hoppe-Seyler and Claude Bernard. Following a stay in Paris, Setchenov returned to Russia in 1860 and defended a dissertation on the physiology of alcoholic intoxication. He received an appointment as Professor of Physiology at the Imperial Medico Chirurgical Academy of St. Petersburg. In 1870 he joined the faculty of the University of Odessa, returning six years later to St. Petersburg. In 1888 he and the Minister of Education had a falling out, and instead of continuing as a professor at St. Petersburg he was reduced to the rank of "Privatdozent" in Moscow where he lived until the end of his life.

Setchenov's scientific thought as a physiologist had been profoundly influenced by his contacts with Claude Bernard where he had turned his attention first to reflex activities and then to the nature of voluntary movements. Like Descartes and Johannes Müller, he believed in the law of specific nerve energies, and he decided that all movements were probably reflex in nature. Absence of all sensory stimuli, he insisted, would make psychic life impossible. Unfortunately for Setchenov, such views ran counter to those held by the Czarist government; indeed they were regarded as revolutionary and dangerous to society, and for a time the Russian government considered destroying his monograph and prosecuting the author who did not have the prestige of his student, Pavlov, whom the Soviet masters dared not touch. Setchenov also had the courage to oppose Virchow's teachings that the cell is chiefly responsible for disease, and again, this brought added hostility from his government; as a result, he was often obliged to change his address and even his name. Wherever he was, however, the ablest young physiologists flocked to his laboratory, and it soon became recognized, even by the authorities of his reactionary government, that he had established a great Russian school of physiology that was accepted by the foremost Western European schools, and Setchenov thus, in a very real sense, laid the way for Pavlov.

The nature of the central inhibitory process

David P. C. Lloyd in 1946, writing on excitation and inhibition in two-neuron reflex arcs, distinguishes between two categories of central inhibition, namely, indirect inhibition and dirrect inhibition. Eccles and Sherrington had shown that the flexor reflex to the second of two single shock stimuli in successive combination is depressed for a matter of 120 msec. Hughes and Gasser, on the basis of a study of the negative cord potentials signalling the activity of spinal interneurons, found them also to be decreased for like intervals. Hence one need look for no explanation of indirect inhibition other than the known recovery curves of central neurons following their activation.

Direct inhibition can be demonstrated when two volleys, one excitatory and the other inhibitory, are delivered simultaneously, in which case the inhibitory volley immediately suppresses the excitatory volley. This can be readily shown in the tendon reflexes.

But to return to Sherrington. He began to interest himself in the phenomenon of central inhibition shortly after the discovery of the motor area, but his personal interest was further stirred when in 1893 he published his first paper on the reciprocal innervation of the knee jerk. This led him to study eye movements and there followed a series of fifteen papers on reciprocal in-

nervation, the last one of which appeared in 1914.

Following World War I he took up the problem anew and his attention now came to be focused on time factors concerned with central inhibition in an attempt to elucidate the nature of the central inhibitory process. It became clear at once that the inhibitory process is not a momentary thing, but that the effect of a single strong inhibitory stimulus is enduring and cannot therefore be looked upon as due to a brief interference with other excitatory impulses reaching the center; indeed, the state of subdued excitability may last for a matter of several msec., i.e., two or three, or even as long as 40 msec. Hence it was necessary to envisage a specific central inhibitory process even as previously a central excitatory process had been postulated.

In a series of remarkable papers in collaboration with E. G. T. Liddell beginning in 1923 and a later series in collaboration with J. C. Eccles, he was led to conclude that in view of the long-lasting character of central inhibition one must recognize not only a 'central excitatory state' which develops from excitatory stimuli but also a 'central inhibitory state' resulting from the summation of separate inhibitory stimuli.

Pavlov and the conditioned reflex

In February 1916 there died in St. Peterrsburg a man bearing the name of Pavlov. The brief announcement which came over the wires from the one-time capital of Russia was misinterpreted for it was actually E. V. Pavlov the surgeon who had died, and not the great physiologist. On 19 February *The Lancet*, *British Medical Journal* and the *Journal of the American Medical Association* each carried obituaries of Ivan Petrovich Pavlov, all written by distinguished physiologists. *The Lancet* notice made no reference to his work on conditioned reflexes, the other two made brief but confused allusions to his work in this field, but each one stressed his historic investigations on gastric physiology. Pavlov had described his conditioned reflex studies in London in 1906 and again at Gröningen in 1913, but little attention had been paid to these first reports. It was not until 1917 when W. M. Bayliss's *General physiology* appeared that the conditioned reflex was described in an English text, and the English-speaking world had to await until 1927-28 for the Gleb von Anrep and Gantt translations of Pavlov's lectures for adequate information concerning Pavlov's work.

From this episode it is clear that knowledge of Pavlov's studies did not spread outside Russia until 1928, a fact which should be remembered in any historical account of the subject. As in every field of learning an important contributor always had his forerunners (see section on Digby above). Over the years nearly all those who have written of animal training and animal behaviour have referred to the sudden eagerness for food at the ringing of the feeding bell or the whistle of the master; books on falconry contain many such allusions.

In the early nineteenth century William Beaumont gave his celebrated account of the psychic secretion of gastric juices and saliva as observed directly through a gastric fistula in his patient famous Alexis St. Martin. The smell of a beef-steak, of which St. Martin was very fond, caused a torrential outburst of gastric secretion. Beaumont's studies represent the first clear-cut instance of an experimentally studied conditioned reflex in man. Experimental study in animals under controlled conditions did not come until late in the nineteenth century, and the name which will always be connected with these studies is that of Ivan Petrovich Pavlov (1849-1936). Pavlov was born on September 14, 1849 in the district of Rjäzan in Russia, the first son of a village priest. He received his early education from a local school and, later, intending to take holy orders, he entered a neighboring theological seminary. At the age of sixteen there fell into his hands a copy of the new Russian translation of *The physiology of common life* by George Henry Lewes, that versatile character who played so large a part in the intimate life of George Eliot. Lewes's book contains several remarkable chapters entitled, "Feeling and thinking", "The mind and the brain", "Our senses and sensations", and "Sleep and dreams"; these chapters form a highly important landmark in the history of physiology, not only because they stimulated the young Pavlov (and incidentally also Williams James), but because they represent one of the earliest

objective treatises on the functions of the cerebral hemisphere. Pavlov once told me that it was under the stimulus of this book, a copy of which he kept always beside him as a '*comes viae vitaeque*' that caused him to leave theology in order to follow biological science as a career. It is thus interesting to realize that the crucial stimulus of Pavlov's life came from England.

In 1870 Pavlov was admitted to the University of St. Petersburg where he studied under the great chemist Mendeléev. On completing his work for a science degree he was admitted to the Military Medical Academy where he received his qualifications in 1879 and his M.D. degree in 1883. Meanwhile in 1880 he had become a *Privatdozent*. After this he proceeded to Germany to continue his graduate training under men such as Heidenhain and Carl Ludwig (1884-1886). On returning to St. Petersburg he assumed teaching responsibilities and in 1890 was put in charge of the physiological department of Prince Oldenburg's newly created Institute of Experimental Medicine. In 1899 he became Professor of Physiology in the Military Medical Academy, and in 1904 he received the first Nobel Prize in Physiology and Medicine for his studies in gastric physiology which had been summarized in his book *The work of the digestive glands* (1902). His gastric studies were begun in 1898 and throughout this period his fistulous animals were cared for by one Serafine Karchevokaya who in 1880 became Mme Pavlov and who outlived her husband.

That Pavlov should after 1902 have turned his attention to the nervous system was logical in view of the direction which his work on digestion had taken. To use his own words (as rendered by Gantt, p. 37) "For many yearse previously I had been working on the digestive glands. I had studied carefully and in detail all the conditions of their activity. Naturally I could not leave them without considering the so-called psychical stimulation of the salivary glands, i.e. the flow of saliva in the hungry animal or person at the sight of food or during talk about it or even at the thought of it. Furthermore, I myself had demonstrated a psychical excitation of the gastric glands." The concept of the conditioned reflex was a direct outcome of these studies. It is sometimes not appreciated that E. L. Thorndike, Franz and Yerkes in the United States had undertaken studies of a similar character at about this time, but it is characteristic of his generosity of spirit that in more than one place Pavlov referred to their priority.

Conditioning in man

Since men first made war upon one another their leaders prepared them 'to do or die' through the use of conditioning exortations. It has similarly been the primary technique of military men and political heads such as Alexander the Great, Julius Ceasar, Attila the Hun, and the modern dictators. That Pavlov himself was to become aware of this is evident from the following incident . At the opening session of the International Physiological Congress at Rome in 1932 I happened to sit beside Professor Pavlov; Government notables were on the dias and were obviously uncomfortable because Mussolini was late — 20 minutes late in fact. When he finally strode on the platform he threw his head back and raised his arm in the familiar Fascist salute, Pavlov in a loud stage whisper that could be heard all over the great meeting room of the Campodoglio exclaimed: "Eine bedingte Reflexe!"

In recent memory one does not have to go much further afield to find another unforgettable example of conditioning that brought a great nation to its knees. The slogan "Heil Hitler" will never be forgotten; and how through this and noisy harangues at huge mass meetings which were broadcast all over Germany, he stirred his people into blind conformity, his soldiers to murderous excesses, and the population in general to accept his monstrous persecution of the Jews and other minorities.

Some time ago several friends who are advisers in Washington asked me as a physiologist to what extent I thought conditioning was being used in shaping foreign policy in certain European and Asiatic states. While my information is not complete, reports which have appeared in the public press are clearly of significance. In the Soviet Union the Government is actively supporting conditioned reflex studies in every medical school within its borders, and

in Communist China at least four new medical schools are being built around conditioned reflex laboratories.

Whether this expansion of study in the conditioned reflex field stems from the respect in which their great hero Pavlov is held, or from other more basic considerations is not entirely clear. But it is having the result of filling their physiological, clinical and scientific journals with so many papers and reports, most of them repetious that there is room for little else. There is nothing comparable to this unless it be the huge volume of material which has appeared recently on "Cybernetics", that specious form of international hysteria which has spread like a contagion among those who know little about the physiology of the nervous system.

Mass conditioning is not always for evil purpose. Though music, one of the tools, most widely employed, conditioning may be used to create religious fervor or patriotic zeal — for example, the great national anthems, 'La Marcha Real', 'La Marseillaise', 'God Save the Queen', 'The Star-Spangled Banner', and many others.

Let us fervently hope that those who may use mass conditioning in the future become fully aware of its potentialities, and that such techniques be employed in the spirit and for the same high purpose that originally inspired the work of Ivan Petrovitch Pavlov, Charles S. Sherrington and others whose main purposes were to understand fundamental physiological processes such as excitation, inhibition, integration of action, learning, memory and human motivation.

NOTES AND REFERENCES

1. Later in his life some of Langley's less serious-minded students and contemporaries often poked fun at the immaculately clad old gentleman and on one ocasion Sir Joseph Barcroft sent Sherrington a student's limerick which had been discovered one day on the blackboard at the beginning of one of Langley's lectures. Sherrington, who had a fine sense of humor, was enchanted.

   There was a young man from East Anglia
   Whose nerves were a tangle of ganglia
   And the more it was seen
   He used nicotine
   The nerves grew Langlier and Langlier.

2. In his Silliman Lectures at Yale, first published in 1906.

3. It is interesting that in 1935 at the International Physiological Congress held in Leningrad and Moscow the Russian physiologists singled out for presentation to each member of the Congress a volume containing a translation of the writings of Ivan Setchenov in which particular prominence is given to his paper of 1863 on central inhibition.

• News

STATUTES OF THE INTERNATIONAL REHABILITATION MEDICINE ASSOCIATION

I. The name of the organization is the International Rehabilitation Medicine Association (IRMA).

II. The objectives of IRMA are to promote the knoledge and use of rehabilitation medicine by:

a. Supporting all organization devoted to rehabilitation and allied fields, especially the International Federation of Physical Medicine (IFPM); to form a liaison with the IFPM by the designation of the Secretary of IRMA as the liaisaon office to the IFPM if the IFPM votes to establish and maintain communication with IRMA.

b. Bringing together, through meetings, by correspondence and any other means of communication, all physicians, regardless of specialty who are interested in rehabilitation medicine and

c. Extending such aid as possible to physicians in areas where rehabilitation medicine has not reached its full potential.

III. There is only one class of membership. Membership is limited to doctors of medicine (physicians), who are members of a national medical society of their country of residence or citizenship. To accept membership from graduates of faculties of medicine who are not members of any national society. To become a member, a qualified physician must send a complete application for membership along with dues to the Secretary. Questionable applications will be resolved through the Membership Committee, composed of the Past-President, Vice-President, Secretary and Treasurer.

IV. Officers. Only members of IRMA in good standing are eligible for office. There will be the following officers: President, President-Elect, Vice-President, Secretary, Deputy Secretary, Treasurer and Deputy Treasurer. No more than two members from any one country may hold office at the same time.

a. Election. Officers shall be elected by the Council at the meeting of the Council which shall be held during the week of the international assembly, preferably on the day preceding the opening of the scientific sessions.

b. Each officer is elected for a period of four years and may be reelected for one additional term of four years. If any office is vacated between Council meeting, the President shall designate an interim replacement from the same country, whenever possible.

c. The member voted responsibility for the next world assembly is the President-Elect until he advances to the Presidency on the final day of the international assembly.

V. Council. The Council shall consist of the officers of IRMA, the Councillors and members of the Executive Committee. Councillors shall be nominated by IRMA members in their respective countries. Each country is permitted two Councillors. Names of candidates must be sent to the Secretary at least three months before the international assembly meeting.

VI. Executive Committee. The Executive Committee shall consist of the officers of IRMA, the immediate Past-President and eight Executive Members. Executive Members shall be chosen by the Council. Executive Members may be nominated only by those countries not represented among the elected officers. Councillors shall serve for four years and may be re-elected.

VII. Assemblies. IRMA will conduct an international assembly every four years, beginning with IRMA I in 1970, IRMA II in 1974, and so on. The site of the next meeting shall be determined by the Council. Members of any country may apply for the right to organize the next meeting by sending a notice of their intention to the Secretary at least three months before the meeting of the Council. The request should be accompanied by a statement of financial ability to organize such an assembly. The official language of meetings shall be English, French, Spanish and the language of the country where the meeting is held.

VIII. Dues. Each member shall pay dues of Three Dollars ($3 USA) or the equivalent in his own currency. Members are requested to pay four years' dues, Twelve Dollars ($ 12 USA), in advance. Dues must be paid within three months after receipt of invoice which will be mailed by the Treasurer in January of each year. The Treasurer will send, or will ask the Secretary to send a membership card to each member on receipt of dues, or with the next issue of NEWS & VIEWS. The Treasurer will establish an account for the funds of IRMA in a bank in a country which permits free international monetary exchange. The Treasurer will pay bills of IRMA only upon approval of the President.

IX. These rules may be amended at any meeting of the Council provided that proposed changes are mailed to the Secretary at least three months before the international assembly so that all Councillors may receive a notice at least one month before the date of the Council meeting. Any member of IRMA may propose a change by writing to the Secretary at least three months in advance of the Council meeting.

a. Since it is desired that each country be represented, if neither Councillor of a country plans to attend the Council meeting, any other member of that nation may be designated by the Councillor to represent him and he will be accorded full privileges of a Councillor at the meeting if a letter is sent to the Secretary in advance or if a letter is hand carried to the meeting by the member designated.

b. Changes in the statutes may be made by majority vote of all those present and voting.

X. NEWS & VIEWS, the bulletin of IRMA, will be published at least three times a year by the Secretary who will send a copy of each issue to every member.

Herman J. Flax, M.D., Secretary
Luhn 2 Urb. V. Braegger
Guaynabo, Puerto Rico 00657
U. S. A.

NATIONAL MULTIPLE SCLEROSIS SOCIETY

257 Park Avenue South
New York, N.Y. 10010
Tel. (212) 674-4100

WINNERS OF SECOND ANNUAL PUBLIC EDUCATION AWARDS ANNOUNCED BY NATIONAL MULTIPLE SCLEROSIS SOCIETY

NEW YORK, Feb. 17. — A young free-lance writer's moving article, "The Misery and Mystery of MS", and a Philadelphia television station's special program on the subject were selected as the winning entries in the National Multiple Sclerosis Society's second annual MS Public Education Awards program. The winners were announced today by Sylvia Lawry, founder and Executive Director of the 29-year old organization, which is headquartered in New York City and has 178 chapters and branches throughout the United States. A $ 1,000 prize is given in each of the award competition's two categories - newspaper or magazine writing and radio or television programs.

The article, published as cover story in the June 1975 issue of *The Lion*, official magazine of the Lions Club International, a world-wide fraternal and philanthropic organization, was "thoroughly researched and sensitively written by Nancy Lee Gohla," says Miss Lawry. Miss Gohla, 25, a free-lance writer who is currently working as a Resident Dean at Bloomsburg State College in Pennsylvania, will soon resume studies for her Ph. D in Education.

"I learned an enormous amount about MS by writing this article," Miss Gohla says. "What really amazed me was the discovery that other than knowing that MS stands for multiple sclerosis, most people don't know much about this crippling neurological disease, not what it is nor how it seriously affects the lives of hundreds of Americans and their families, most of them between the ages of 20 and 40, when it is usually first diagnosed.

"That's why I hope my winning this award will encourage other free-lance writers and science editors to publish articles about this relatively little known disease which has actually long been recognized by

the scientific and medical community as a major health problem."

The television station which presented the winning program was station WPVI-TV, an ABC affiliate in Philadelphia. The station's Director of Public Affairs, John Miller of Camden, New Jersey, who produced the 30-minutes special on the station's regular weekly series, "Assignment," reports that: "We wanted to show in the tri-state area covered by the station what one local MS chapter —in this case Southern New Jersey Chapter of the National Multiple Sclerosis Society— is doing for MS patients via its patient service programs: recreational, social and rehabilitative. We also wanted to bring before the public the urgent need for public understanding of this disease and the need for the public's help as volunteers both in patient services and fund raising." The MS program was aired in October, 1975.

The panel of four distinguished media experts who served as judges for the 1975 awards program were Ruth Dudley, Director of Public Information, National Institute of Neurological and Communicative Disorders and Stroke; Gilbert Cant, Associate Science Editor, *Time* Magazine; Edward Edelson, Science Editor, *New York Daily News;* and John Troan, Editor, *The Pittsburgh Press.*

Reprints of Miss Gohla's article, "The Misery and Mystery of MS," are available from the Public Relations Department, National Multiple Sclerosis Society, 257 Park Avenue South, New York 10010. Those interested in learning more about the 1976 MS Public Education Awards may also write to that address for an award brochure, or may contact their local MS chapter.

SIR GORDON HOLMES CENTENARY SYMPOSIUM

1st - 4th March 1976

PROGRAMME

Sir Gordon Holmes Centenary Symposium

Monday 1st March

Chairman: Dr F Clifford Rose (Charing Cross)

From 10.00 am

Registration

2.00 pm
Sir Gordon Holmes at Charing Cross
Dr G Parsons-Smith (Charing Cross)

2.45 pm
Sir Gordon Holmes:
The man and the neurologist
Dr Macdonald Critchley (National Hospital)

3.30 pm
Film of Sir Gordon
Taken by the late Dr Graeme Robertson (Melbourne)

4.00 pm

Tea

4.30 pm
Memories of Sir Gordon
By those attending symposium
Opened by
Sir Charles Symonds (National Hospital)
Dr Paul C Bucy (North Carolina)
Dr Wilder Penfield (Montreal)
Dr S P Meadows (Westminster Hospital)
Dr Aldren Turner (St Bartholomew's Hospital)

6.00 pm

Reception

6.30 pm
Unveiling of bust of Sir Gordon
by Miss Kathleen Holmes

Sir Gordon Holmes Centenary Symposium

Tuesday 2nd March

Chairman: Prof G Dawson (University College)

9.00 am
Sir Gordon Holmes and neuroanatomy
Prof A Brodal (Oslo)

9.30 am
Axoplasmic transport and its neuropathological implications
Prof J Sloper (Charing Cross Hospital)

9.50 am
The pyramidal tract and the neural mechanism of the control of skeletal musculature
Dr Paul C Bucy (North Carolina)

10.10 am
The present status of the pyramidal pathway
Prof C Philips (Cambridge)

10.30 am
Discussion

10.40 am
Coffee

Sensation

11.00 am
Gordon Holmes' work on sensation and his association with Henry Head
Dr Ronald Henson (London Hospital)

11.30 am
Muscle pain: which receptors are responsible for the transmission of noxious stimuli?
Prof R F Schmidt (Kiel)

12.00 noon
The innervation of intracranial structures: a reappraisal
Dr F McNaughton and Dr. Feindel (Montreal)

12.20 pm
Vagal regulation of breathing in man
Prof A Guz (Charing Cross Hospital)

12.40 pm
Discussion

1.00 pm
Lunch

Basal Ganglia

Joint-Chairmen:
Prof G Schaltenbrand (Hamburg)
Prof R Hassler (Frankfurt)

2.00 pm
The history of Parkinson's disease and its treatment before the L-dopa era
Dr Gerald Stern (University College Hospital)

2.30 pm
Pathophysiology of Parkinsonian tremor
Prof Rondot (Paris)

2.50 pm
Biochemistry and pharmacology
Prof Hornykiewicz (Toronto)

3.15 pm
Current drug therapy
Prof Rinne (Helsinki)

4.00 pm
Tea

4.30 pm
Emerging problems of long-term L-dopa treatment
Prof Marsden (Institute of Psychiatry, London)

4.50 pm
Research into new treatments: past, present and future
Prof Max Klingler (Basle)

5.20 pm
Discussion

Sir Gordon Holmes Centenary Symposium

Wednesday 3rd March

Joint-Chairmen:
Prof H Narabayashi (Tokyo)
Prof I McDonald (Moorfields Hospital)

9.30 am
Mapping of the visual cortex by electrical methods
Prof B S Brindley (Institute of Psychiatry)

9.50 am
Vascular lesions of the occipital cortex
Dr R Ross Russell (St Thomas' Hospital)

10.10 am
Holmes' interest in visual disturbances associated with cortical damage
Dr. William Gooddy (University College Hospital)

10.30 am
Discussion

10.40 am
Coffee

11.00 am
The parietal cortex in visual orientation: the relevance of work in the monkey
Dr G Ettlinger (Institute of Psychiatry)

11.30 am
The development of binocular visual connections
Dr C Kennard (Charing Cross)

12.00 noon
Spasm of visual fixation
Prof D Denny-Brown (Boston)

12.40 pm
Discussion

1.00 pm
Lunch

Chairman: Dr. Denis Williams (National Hospital)

2.00 pm
Pathology of visual perception of space and motion
Dr M Bender (New York)

2.30 pm
A physiological classification of nystagmus
Mr E J Arnott (Charing Cross)

2.50 pm
Cerebellar control of ocular movements
Mr M Sanders (St. Thomas' Hospital)

3.10 pm
Dichotic and diotic listening: contributions to neurophysiology
Antonia and Hanna Damasio (Iowa City)

3.30 pm
Discussion

4.00 pm
Tea

Sir Gordon Holmes Centenary Symposium
Thursday 4th March

Cerebellum
Chairman: Prof K Kristiansen (Oslo)

9.30 am
A modern appreciation of Sir Gordon Holmes' study of cerebellar lesions
Sir John Eccles (Canberra)

10.15 am
Persistence of stretch reflexes following ablation of the cerebellum
Dr E G Walsh (Edinburgh)

10.40 am
Discussion

11.00 am
Coffee

11.20 am
The cerebellum and muscle spindles in man
Prof C D Marsden (Institute of Psychiatry)

11.50 am
Reconsidering the 'alpha-gamma' switch in cerebellar action
Prof R Granit (Stockholm)

12.30 pm
Panel discussion

1.00 pm
Lunch
Chairman: Dr M Charlier (Liege)

2.00 pm
Familial cerebellar degeneration
Prof B Matthews (Oxford)

2.20 pm
Hypoxic myoclonia - clinical and pathological observations
Dr Clifford Richardson (Toronto)

2.40 pm
Some observations on the pathophysiology of epilepsy of clinical interest
Prof R Aird (San Francisco)

3.10 pm
Pathophysiology of the migrainous visual aura
Prof G Baumgartner (Zurich)

3.40 pm
Discussion

4.00 pm
Tea

7.15 - 7.30 pm
Dinner
Apothecaries' Hall
In the presence of HRH, The Princess Margaret, Countess of Snowdon

PRELIMINARY LIST OF PAPERS TO BE PRESENT AT

THE 18TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR THE STUDY OF HEADACHE

Sheraton - Dallas Hotel - Dallas, Texas
June 26 - 27, 1976

★ ★ ★

Motrin. — "A new agent for the symptomatic treatment of muscle contraction headache"

Robert E. Ryan, Sr., M.D., St. Louis, Missouri.

"Clinical and therapeutic observations on a new post-traumatic headache syndrome"

N. Vijayan, M.D., Davis, California.

"Cluster headache and sphenopalatine ganglion neuralgia: comparisons, contrasts, and treatment"

R. E. Ryan, Jr., M.D., and G. W. Facer, M.D., Rochester, Minnesota.

"Conversion headache"
Russell Packard, M.D., Bethesda, Maryland.

"Migraine prophylaxis with lisuride"
Brian Sommerville, M.D., and Werner M. Herrmann, M.D.; Sydney, Australia and Berlin, West Germany, respectively.

"Computerized axial tomography in migraine"
Ninan T. Matthew, M.D., Houston, Texas.

"Drug dependency in patients with chronic headaches"
Jose L. Medina, M.D., and Seymour Diamond, M.D., Chicago, Illinois.

"Prophylactic lithium in the treatment of chronic cluster headache"
Lee Kudrow, M.D., Encino, California.

"The treatment of headache with different modalities of biofeedback therapy"
Seymour Diamond, M.D., and Jose L. Medina, M.D., Chicago, Illinois.

"Increase of plasma free tryptophan during migraine attacks"
S. Salmon, M. Fanciullacci, and F. Sicuteri, Florence, Italy.

"Functional (essential headaches) and organic (thalamic syndrome) central pain"
F. Sicuteri, Florence, Italy.