# Biochemical Aspects of Nervous Diseases

ited by

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# BIOCHEMICAL ASPECTS OF NERVOUS DISEASES

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# BIOCHEMICAL ASPECTS OF NERVOUS DISEASES

## Preface

Within recent years basic knowledge concerning the chemistry, and the metabolic processes taking place in the brain, spinal cord, peripheral nerves and muscles has increased in a remarkable manner. As a consequence some very important books have been published both in America and in Europe in which some, at least, of this information has become available in an easily readable form to an ever increasing group of laboratory and scientific workers.

The application of such studies in a variety of neurological diseases can now be made, thus making possible an explanation of many of the clinical and pathological peculiarities that have been known for decades. Although a few small manuals have already been published, combining both biochemical and clinical aspects of such disorders and these have been studied by neurologists and chemical pathologists throughout the world, yet a volume devoted exclusively to the biochemistry of neurological diseases has not yet been readily available. The present volume is an attempt to remedy this omission in relation to just a few of the conditions. Individual writers of each of the six chapters have been chosen who are intimately concerned both with biochemistry and with its application to disease in man. Each author has been responsible for the accuracy of his chapter together with appropriate references from the literature, but the Editor does not necessarily concur with all the opinions expressed by the authors.

The Editor expresses his real thanks to each author, to the Plenum Press and to his secretary Miss S. Barkwell for their ready and most helpful assistance. In addition acknowledgement is given to Professor S. Granick, Dr. Atallah Kappas, Dr. Richard D. Levere, Dr. Daniel Steinberg, Grume & Stratton Inc., the Academic Press Inc. and the Annals of Internal Medicine for permission to use Tables and Figures in Chapter 1; to the Department of Medical Illustration, The Institute of Neurology for their help in providing the illustrations for Chapter 5, and to Blackwell Scientific Publications Ltd. for permission to use the illustrations in Chapter 6.

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CHAPTER 1

# Metabolic Aspects of Some Diseases of Peripheral Nerves

J. WILSON and R. H. S. THOMPSON



## 1.1 Introduction

One of the most exciting developments in the past two decades has been the intimate study of the correlation of form

and function at the ultrastructural level. Axonal and synaptic activities are now sufficiently well understood to allow an outline definition at molecular level, and it is possible to envisage an understanding of neuraxial function entirely in biochemical terms.

Although in clinical neurology the traditional aetiopathological classification which distinguishes between, for example, traumatic, infective, neoplastic, developmental, degenerative and metabolic causes of diseases, still dominates thinking, it is likewise possible (if still somewhat ambitious) to advance a similar holistic approach, and bring all conditions within the purview of the biochemist.

Nevertheless, for the purpose of this review we restrict ourselves to the conventional consideration of those toxic-nutritional-metabolic conditions which are of primary aetiological significance in the peripheral neuropathies.

Such factors are of special importance in relation to these diseases, and before considering in more detail specific abnormalities, it is worth while reflecting on the special structural characteristics of peripheral nerves which distinguish them from other neuraxial elements, and which may partly determine their characteristic responses to toxic-metabolic factors.

There is good evidence that not only is the structural integrity of an individual axon dependent on the vitality of the perikaryon, but also that there is nutritional dependence as well. As has been emphasized by Cavanagh [1], "The metabolic load imposed on their perikarya by the longest and largest fibres must be unequalled by that on any other type of cell in the body, for measurements have shown that the volume ratio of axon to perikaryon may be as great as a thousand to one."

Not only may this relationship confer a special susceptibility to metabolic derangements but so may also the endowment of certain axons with myelin sheaths. The intimate myelin investment of axons in segmental fashion by the specially differentiated cell membranes of Schwann cells serves to allow an increased rate of transmission of electrical impulses by virtue of "saltatory" conduction at the nodes of Ranvier. Since the rate of conduction of impulses in the absence of this segmental sheath is much slower, myelinated fibre function can be

markedly altered by those diseases which damage Schwann cells and produce so-called segmental demyelination.

It is thus possible to distinguish between peripheral neuropathy predominantly due to axonal disease (with secondary Wallerian degeneration) and that due to so-called segmental demyelination following on Schwann cell damage (Table 1.1).

TABLE 1.

Wallerian Degeneration and Segmental Demyelination in the Polyneuropathies

| Predominantly Wallerian degeneration | Predominantly segmental demyelination |
|---|---|
| Beri-beri | Diabetes |
| Porphyria | Leucodystrophies (metachromatic, globoid (cell)) |
| Organo-phosphorus poisoning | Hypertrophic interstitial neuritis (Déjèrine-Sottas and Refsum) |
| Alcoholism | |
| Vitamin $B_{12}$ deficiency | |

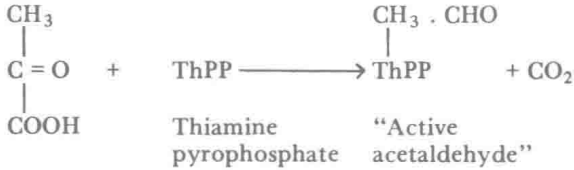
Although in the conditions now to be discussed little is known of the precise mechanism of cell damage, it seems reasonable to conclude in each case that it is due to disruption of the normal metabolic activities of either neurones or Schwann cells, resulting in one or other of the above-mentioned types of degeneration.

### *1.1.1 Thiamine deficiency*

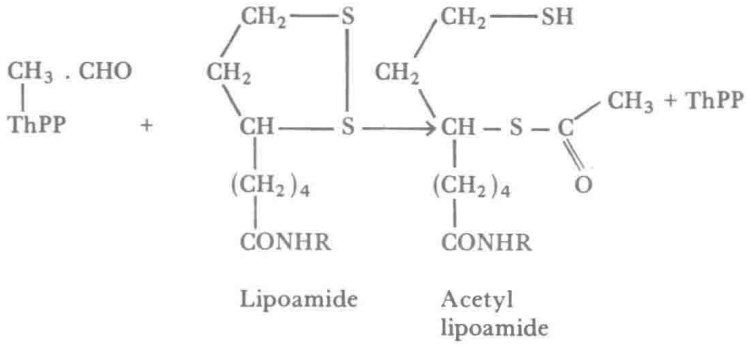
The biochemical changes that are present in the polyneuropathy which can result from a deficiency of thiamine in the diet have been well worked out in the years that have elapsed since 1885 when the Japanese naval surgeon, Takaki, first claimed that beri-beri is a nutritional disease resulting from the ingestion of excessive quantities of polished rice [2]. Eijkmann in 1897 [3] carried out a classical nutritional experiment when he showed for the first time that fowls fed on a diet of polished rice developed weakness of the legs and opisthotonus. As a result of his observations he was able to

conclude, and correctly, that the germ and the pericarp of rice contained an essential nutrient capable of protecting fowls from a disease resembling beri-beri. This nutrient, vitamin $B_1$ or thiamine, was synthesized [4], and its mode of action at the biochemical level defined in detail following the initial observations and experiments of Peters and his colleagues in Oxford on the changes in the metabolism of the brain in rice-fed pigeons. Peters was able to state that vitamin $B_1$ "is a catalyst needed for the oxidative removal of one of the lower degradation products of carbohydrate metabolism", and he added that "the biochemical lesion (in thiamine deficiency) is most closely related to the oxidation of pyruvic acid". [5]. It is now known that thiamine pyrophosphate is a part of the complex co-enzyme requirements needed for the conversion of pyruvate, by a process of oxidative decarboxylation, into acetyl-coenzyme A.

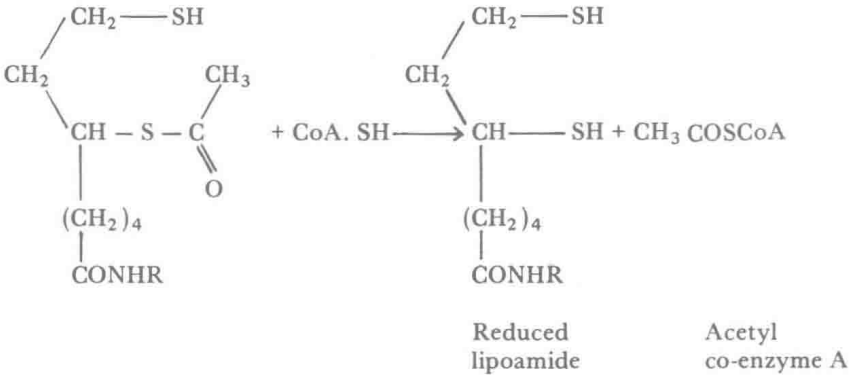
This conversion is carried out by a series of linked reactions. In the first of these pyruvate is decarboxylated by a reaction involving thiamine pyrophosphate which acts as an acceptor for the acetaldehyde which is produced



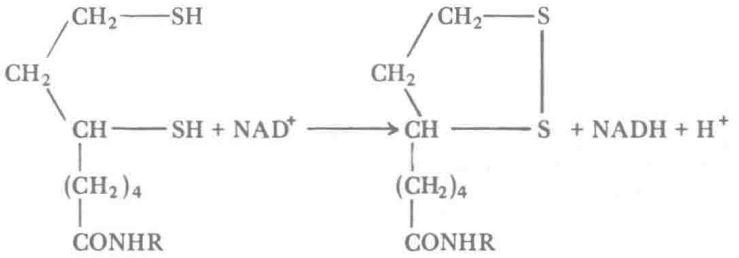

The "active acetaldehyde"-thiamine pyrophosphate complex then reacts enzymically with another co-factor lipoic acid (6,8-dithiooctanoic acid), or lipoamide, since in its active state inside the cells the acid exists in an amide linkage with a lysine residue of a specific protein



The acetyl lipoamide so formed is the immediate precursor of acetyl-coenzyme A



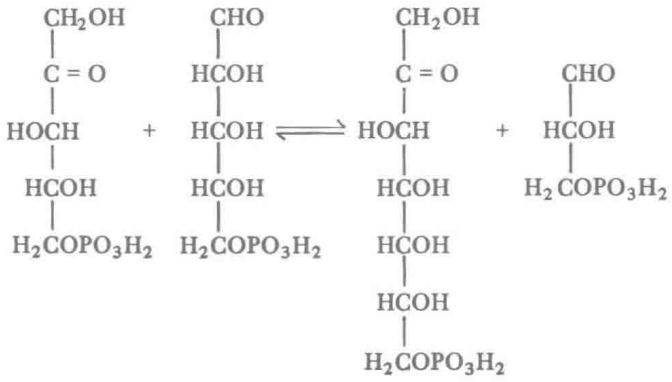

The reduced lipoamide is then re-oxidized by nicotinamide-adeninedinucleotide




In thiamine deficiency, therefore, the formation from pyruvate of acetyl-coenzyme A, which is normally followed by the oxidation of the 2-C acetyl fragment to $CO_2$ and $H_2O$ via the citric acid cycle is blocked, and the pyruvate formed by glycolysis consequently accumulates. In addition in its role in the oxidative decarboxylation of pyruvate, thiamine pyrophosphate is also needed as a co-enzyme in the similar decarboxylation of the closely related α-oxoglutarate, one of the intermediaries in the citric acid cycle.

It also plays an essential part in the alternative pathway of carbohydrate oxidation, the so-called "pentose phosphate pathway", where it is required as a co-enzyme of transketolase [6, 7]. This enzyme catalyses the interaction

between xylulose-5-phosphate and ribose-5-phosphate, one of the steps in the series of metabolic transformations resulting from the initial conversion of glucose-6-phosphate to 6-phosphogluconic acid by glucose-6-phosphate dehydrogenase.




Xylulose-5-phosphate | Ribose-5-phosphate | Sedoheptulose-7-phosphate | Glycoaldehyde 3-phosphate

In its more severe forms thiamine deficiency can present as (1) wet beri-beri; with generalized oedema and tendency to heart failure, (2) dry beri-beri, a chronic polyneuropathy with degenerative changes in the peripheral nerves, (3) infantile beri-beri, a chronic marasmic state also frequently associated with sudden heart failure, and (4) Wernicke's encephalopathy, which may be seen in chronic alcoholics and may be regarded as a "cerebral beri-beri". Less severe states of thiamine deficiency usually manifest themselves as a chronic polyneuropathy. Problems of differential diagnosis from other forms of peripheral neuritis therefore arise, and it is in this connection that studies of pyruvate metabolism may be of help.

### *1.1.2 Pyruvate metabolism in peripheral neuritis*

It has been known for many years that the block in pyruvate metabolism resulting from a deficiency of thiamine, as outlined above, results in an elevated level of pyruvate in the blood [8, 9]. A "pyruvate tolerance test" has, therefore, been devised in which a dose of glucose is given by mouth and the blood

pyruvate levels measured at 0, 30, 60 and 90 min [10, 11]. Under the conditions of the test, and it is important that the subject be fasting and at rest in bed, thiamine-deficient patients usually show a blood level greater than 1.4 mg pyruvic acid per 100 ml at 60 or 90 min.

It must be remembered, of course, that a number of factors other than a deficiency of thiamine can also result in high blood pyruvate levels. For example, there is evidence suggesting that compounds of arsenic [12] and of antimony, gold and mercury [13] owe their toxicity to their ability to react with the –SH groups in reduced lipoamide (see above), and that the therapeutic effect of dimercaprol (BAL) is due to this dithiol competing effectively with the thiol groups of lipoamide and so preventing the arsenic from interfering with the normal functioning of this co-factor [14]. In view of the fact that arsenicals inhibit pyruvate oxidation by this means it is of interest to recall the many points of clinical resemblance that have long been known to exist between arsenical neuritis and the polyneuropathy of thiamine deficiency [15]. The blood pyruvate level is also raised in patients with an untreated vitamin $B_{12}$ deficiency [16], and is rapidly returned to normal after treatment with vitamin $B_{12}$.Exercise, anoxia and fever can also cause raised levels. In order to differentiate between a thiamine deficiency and these other conditions which may also present with raised blood pyruvate levels it may be advisable to carry out a second pyruvate tolerance test after a 14-day period of parenteral thiamine therapy. If the high pyruvate level found at the first test is restored to normal following such therapy it is likely that the patient was thiamine-deficient prior to therapy.

The discovery of the function of thiamine as a co-factor for transketolase activity had led to blood transketolase estimations being used as a further test for thiamine deficiency [17, 18, 19, 20].

### *1.1.3 Alcoholic polyneuritis*

Alcoholic polyneuritis has, for long, been held to be due largely to an associated deficiency of thiamine brought about by the unbalanced deficient diet consumed by the chronic alcoholic, together with the chronic gastritis and associated

disturbances of gastro-intestinal function present in such patients.

Elevations of the blood pyruvate level in patients with alcoholic polyneuropathy were described by Beuding and Wortis in 1940 [21], and low levels of thiamine [22] and of thiamine pyrophosphate [23] in this condition were also reported. More recently Fennelly, Frank, Baker and Leevy [24] described the circulating levels of thiamine and of a number of other members of the vitamin B complex in healthy volunteers and in chronic alcoholics with and without evidence of peripheral neuropathy. Although the mean blood thiamine level was reduced in alcoholics without neuropathy, even lower levels were found in the group with neurological involvement. In a small proportion of cases some lowering of the levels of riboflavin, nicotinic acid, pyridoxine, folic acid, pantothenic acid and biotin were observed, but these levels did not, in general, appear to be correlated with the presence of neurological involvement, so that although deficiency of these other vitamins may contribute to the overall clinical picture, it is unlikely that they play a major part in the production of the neuropathy.

### 1.2 Diabetic Polyneuropathy

The underlying causes of the nervous changes that can occur in patients with diabetes mellitus present a challenge that is still with us. Symptoms of diabetic neuropathy may develop in mild, severe and controlled states of the disease, and with or without accompanying arteriosclerotic lesions.

Conflicting opinions have existed both as regards the nature of the changes that occur and as regards the aetiological factor or factors involved. The primary site of the disorder has for long been a matter of debate, some workers regarding the condition as being primarily a disturbance of the peripheral nerve fibre, and others regarding it as being primarily a neuronal degeneration affecting anterior horn and dorsal root ganglion cells, with secondary changes occurring in the peripheral nerves.

It is known that peripheral nerve conduction is slowed in patients with diabetic neuropathy, a finding which suggests that

segmental demyelination is probably occurring [25]. Thomas and Lascelles [26, 27] have made observations on isolated nerve fibres and have obtained convincing evidence of segmental demyelination from which they have concluded that diabetic neuropathy is probably the result of a metabolic disorder of Schwann cells.

Turning to the aetiology of the condition, the three main hypotheses have been those of degenerative vascular disease, thiamine deficiency and the disordered metabolism of diabetes mellitus.

Apart from some conflicting therapeutic claims there is, however, little if any, convincing evidence of an associated thiamine deficiency in this condition. Goodhart and Sinclair [28] found that the levels of cocarboxylase in the blood were normal in four out of the five patients studied by them, the fifth patient, in whom a low level was found, being also an alcohol addict. Martin [29] carried out pyruvate tolerance tests following the intravenous injection of pyruvate to a series of diabetic subjects and concluded that pyruvate metabolism was normal. Thompson, Butterfield and Kelsey-Fry [30] reported on the blood pyruvate levels in glucose-insulin tests performed on a series of diabetic patients with and without neuropathy, and found that the levels were no higher in patients with neuropathy than in the other diabetics.

The view that the nerve lesions are the outcome of degenerative atheromatous changes in the vasa nervorum has been one that has been much debated and despite the repeated reports of poor correlation between occlusive vascular disease and neuropathy it would probably be premature to exclude this as a contributory, if not a causative, factor.

The third view, that the neuropathy is the result of a metabolic disturbance occurring in the Schwann cells, is difficult to assess because of our limited knowledge of the details and quantitative aspects of Schwann cell metabolism. It has been claimed that the Schwann cells are responsible for the major part of the resting respiration of peripheral nerve [31], and that the addition of insulin increases the rate of respiration of normal resting nerve *in vitro*, presumably by increasing the rate of glucose utilization [31, 32]. There are, therefore, some slight *a priori* grounds for the view that a metabolic disorder of