VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

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VOLUME X

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EDITORS' PREFACE

The Editors of *Vitamins and Hormones* heartily welcome Dr. G. F. Marrian as a member of the editorial team. They have long felt that these volumes would be strengthened by addition of a third editor with a different scientific and geographic viewpoint. The successive issues of *Vitamins and Hormones* continue to meet with acceptance and the Editors hope that the almost complete absence of adverse comment indicates that these volumes are filling the need for critical reviews in the vitamin and hormone field adequately.

It will be noted that a majority of the articles in the present volume are concerned with hormones. This is a reflection of the greater amount of research being conducted at present in the hormone than in the vitamin field. The articles come from the fields of biochemistry, endocrinology, experimental zoology, animal husbandry and clinical nutrition. In this respect they typify the breadth of scientific interest in these substances and their functions.

THE EDITORS

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I. Introduction

Neuropathy in its strictest sense means pathological change in the nervous tissue. Nowadays however, the term is used to describe damage to nervous tissue when the primary lesion is a degeneration of the nerve cell or its processes with little or no evidence of true inflammatory reaction. The cause of the degeneration is disturbance of the normal metabolism of the cell. This may result from (1) toxic factors, extrinsic or intrinsic, including the "allergies," or (2) deficiency of substances necessary for normal cell metabolism. Such a deficiency can occur when adequate amounts of one or more of these substances are not available to the cell whether from (a) interference with the blood supply, (b) failure of the body to produce them either by synthesis or from store, or (c) insufficient amounts being present in the gastrointestinal tract or absorbed therefrom.

The term "dietary neuropathy" is applied to a variety of clinical syndromes when there is good evidence that lack of a factor or factors essential for normal nerve cell metabolism plays an important part in the cause of the disorder. In the great majority inadequate absorption is responsible but toxic agents, a poor blood supply, deficient synthesis, deficient storage by the body, antienzymes or analogues may be precipitating or aggravating factors.

Our knowledge of these syndromes has been greatly extended by observations made on prisoners-of-war and undernourished European populations during World War II. The majority, however, had been recognized and accurately described before that time. Major advances in neurocellular chemistry and the development of biochemical methods of vitamin assay and of synthesis or extraction have thrown much light on the cause and nature of the lesions. There are, however, still many aspects of the nutritional neuropathies to be explained. Why are only certain persons affected in a large group, when the food intake of each member is often roughly the same? Defective absorption, inadequate

bacterial syntheses, disturbed liver function, inherent deficiencies of, or excessive metabolic demands on the cells affected, are all attractive theories, but proof as yet is lacking. Why are some of the features so prevalent only in the tropics or subtropics, although the food and vitamin intake may be much less in certain groups in temperate climates? This difference was emphasized by the high incidence of specific deficiency syndromes among prisoners-of-war in the Far East and the very low incidence in grossly undernourished persons in occupied countries and concentration camps in Europe. Abnormal carbohydrate, protein, fat, or vitamin ratios in the diet are a possible explanation, as are high malarial, dysenteric or worm infestation rates, but the evidence is still inconclusive. There are undoubtedly other factors as yet unknown or purely speculative.

There is, therefore, still much scope for investigation of these problems, both by accurate observation with controlled experiments in the field and by detailed biochemical or biophysical methods.

A clinical classification of the dietary neuropathies is still the most satisfactory, since the cause of some of them is not yet definitely established and they are only believed to be due to a deficiency state. In the patient it is usually easy to show that there is an insufficiency of a number of substances in the diet. It is therefore difficult to attribute the condition to lack of a specific substance. The therapeutic test is valuable when a clear-cut and immediate response is obtained, but when this is not the case, deficiency of the substance used cannot be ruled out as the causative factor. In human nervous system lesions, a prolonged therapeutic test is rarely justifiable without an over-all improvement in the diet so that the results are difficult to assess.

Damage to nervous tissue is seldom quickly reparable. Three grades of damage can be postulated. (a) The structure of the cell and its processes may appear intact but its function is grossly impaired, either from physicochemical disturbances or from pressure of adjacent edema. (b) Demyelinization of the axis cylinder may be present. (c) There may be demyelinization, axon destruction, and even cell death. In the first instance specific therapy may rapidly restore normal function. In the other two the response will depend on whether the nerve processes concerned have a neurilemmal sheath or not. When this is present, as in a peripheral nerve, remyelinization may gradually occur, or there may be a slow re-growth of the axis cylinder; but where a central neuron is involved the damage may be permanent in spite of intensive specific therapy.

The various clinical syndromes can be divided into two main groups. Group I—where the lesion is predominantly in the peripheral nerve. This group comprises (a) beriberi neuropathy, including "alcoholic polyneuritis" and "polyneuritis gravidarum"; (b) the painful feet

syndrome. Group II—where the lesion is predominantly in the central nervous tissue. This group includes (a) Wernicke's encephalopathy; (b) niacin (nicotinic acid) deficiency encephalopathy, if such a condition exists; (c) retrobulbar neuropathy; (d) the cord syndromes, namely "spinal ataxia" and spastic paraplegia, or a combination of both.

There is a third group of diseases of the nervous system in which deficiency of specific food factors *may* play a part as precipitating or aggravating elements, but they cannot properly be classified as "deficiency neuropathies." Examples in this category are: the relationship of vitamin E to progressive muscular atrophy, and the relation of the B complex vitamins to Korsakow's psychosis and depressive states. In the same way subacute combined degeneration of the spinal cord in pernicious anemia cannot be regarded as a "dietary neuropathy" although the mechanism of its production may be closely related to or identical with that of the cord syndromes. The lesion may result from a defect in the enzyme systems concerned with the metabolism of nerve tissue, and this defect may have a variety of causes as already mentioned. This latter group, however, will not be reviewed here.

II. Beriberi—Peripheral Nerve Lesions Including "Alcoholic Peripheral Neuritis"

1. Pathology

It was only during the latter half of the last century that the study of morbid neuroanatomy became the *sine qua non* of clinical investigation in this field and the earliest reliable reports of pathological changes observed in the nervous system in beriberi came from Bälz (1882). He termed the disease "pan-neuritis endemica" and stated that "its nature is that of a true neuritis with degeneration of the fibers quite analogous to that observed in peripheral paralysis or that produced by cutting the nerves." Scheube (1884) found few changes in the spinal cord, but noted swelling and later degeneration of the medullary sheath into droplets and finally disintegration and complete absorption of medullary sheath and axis cylinder; in the peripheral nerves from cases of paralytic beriberi the changes were most marked in the muscular branches and there was marked atrophy of the associated muscle fibers. Pekelharing and Winkler (1887) gave the first detailed account of the histological findings in a large series of 85 cases. These confirmed Scheube's findings, but in cases of long duration degenerative changes were found in the posterior root ganglia and these extended centrally into the columns of Goll. The anterior roots showed minimal changes. Some of the anterior and posterior cells of the spinal cord showed degeneration. These were regarded

as secondary to damage to the peripheral processes. They also pointed out that these changes were not specific to beriberi, but occurred in all forms of peripheral neuritis. Wright (1901) found changes in the posterior spinal ganglion cells and anterior horn cells, and in the nuclei of the medulla, in cases where the fibers originating from these cells were atrophied.

Further detailed examination of the peripheral nerves and spinal root ganglia by Yamagiwa (1899) and Wright (1902) confirmed these earlier observations. Dürck (1908) and other workers noted circumscribed recent hemorrhages as almost constant findings in the gray matter. They were regarded by them as inflammatory, but Tsunoda (1909) held that they were purely degenerative in origin and he was thus the first protagonist of what is now the generally accepted view.

2. Etiology and Experimental Pathology

At the turn of the century there was considerable controversy and speculation as to the causes of beriberi. As a result of the pioneer experimental work of Eijkman (1897) the theory that there was a deficiency of some essential substance in the diet was rapidly gaining ground. Eijkman showed that fowls fed on polished rice developed polyneuritis that could be cured or prevented by the administration of a watery or alcoholic extract of rice bran. These experiments were repeated and the results confirmed by subsequent workers, and a detailed description of the histological changes in the nervous system of fowls with polyneuritis induced in this way was published by Vedder and Clark in 1912. In 1938 Vedder summarized the changes as follows. Every nerve fiber showed some evidence of degeneration though the extent of the lesion varied greatly in different fibers of the same nerve. The myelin was degenerated and there was swelling of the nerve sheath with disintegration of the axis cylinder in 10 to 15% of the cases. The earliest change was slight swelling of the medullary sheath with a tendency towards fragmentation at the circumference. Then large fatty globules that distended the nerve were seen. These became much smaller and finally the neurilemma contained only a few scattered droplets of fat. These changes did not commence in the peripheral nerves but were generalized, affecting both dorsal and ventral nerve roots, all tracts of the spinal cord, medulla, pons, midbrain, and internal capsule.

Similar changes in the peripheral nerves have been found in thiamine-deficient animals by other workers since that time (McCarrison, 1919; Findlay, 1921; Voegtlin and Lake, 1919; Woollard, 1927; Zimmerman and Burack, 1932). These findings resemble very closely those already noted in the peripheral nerve in naturally-occurring beriberi in man, and Kimura

(1920), in a detailed study of the peripheral nerves from beriberi patients and in polyneuritis gallinarum, could find no recognizable difference between the two lesions.

Although it is now generally agreed that deficiency of thiamine is an important factor in the production of peripheral neuritis in beriberi, there have been conflicting reports as to whether pure thiamine deficiency under experimental conditions can cause peripheral neuritis in man and animals. That starvation alone can produce changes in peripheral nerves very similar to those resulting from thiamine deficiency was first demonstrated by Chamberlain and his co-workers in 1911 and subsequently by other workers. Vedder and Chinn (1938) found that rats that were starved and given 3 mg. of thiamine daily developed changes in the peripheral nerves identical with those in rats that were starved and given no thiamine, but they were less extensive. The suggestion is made that anorexia —an early symptom of thiamine deficiency—leads to a deficient calorie intake and that the resulting starvation is responsible for the nerve changes. This, however, is unlikely, as signs of peripheral neuritis have been produced experimentally with a diet deficient in thiamine but with an adequate number of calories, and severe polyneuritis often occurs in apparently well-nourished persons. On the other hand, some workers (Engel and Phillips, 1938; Gildea *et al.*, 1930; Prickett, 1934) found no, or few, degenerating nerve fibers in thiamine-deficient rats and expressed the opinion that the symptoms of thiamine deficiency could not be explained on the basis of observable pathological lesions. In a later paper Prickett *et al.* (1939) point out that, when rats are rendered acutely thiamine deficient by a thiamine-free diet, no marked changes are found in the peripheral nerves, but when inadequate amounts of thiamine are present in the diet, marked changes in the myelin sheath and axis cylinder appear, particularly when studied with polarized light. Aring and his colleagues (1941) have demonstrated a marked reduction in the number of myelin sheaths per square millimeter, particularly those of larger size, in biopsy specimens of peripheral nerves from pellagrins presenting mild signs of peripheral neuritis. In longitudinal section along with the myelin degeneration there was a considerable amount of beading, fragmentation, and corkscrew formation of the axis cylinders. The signs rapidly disappeared on thiamine administration alone. These histological findings are almost identical with those that Greenfield and Carmichael (1935) described in the peripheral nerves of patients with subacute combined degeneration of the spinal cord. Walshe (1941) points out that, clinically and pathologically, beriberi is a typical polyneuritis and Kinnier Wilson (1940) states "that by whatever diverse and heterogeneous factors nerve substance sustains injury its means of reaction are

strictly limited and well defined. A strict division between parenchymatous neuritis and an interstitial neuritis cannot be made as, where parenchymatous degeneration occurs, there is reaction of the interstitial cells and *vice versa.*"

Swank and his associates (1941), however, claim that the changes in the peripheral nerves of experimentally starved rats differ from those seen in the thiamine-deficient animal. In starvation the changes are confined to the myelin sheath where the myelin droplets diminish in size and eventually disappear. The axis cylinders remain intact and the animals do not exhibit signs of paralysis. In further experiments Swank and Prados (1942) rendered pigeons acutely and chronically thiamine deficient by careful dietetic measures. In the acutely deficient birds that received practically no thiamine, opisthotonos rapidly became the outstanding feature. The symptoms were immediately relieved by thiamine. Birds killed when opisthotonic showed few or no degenerative changes in the central nervous system. When the diet was partially deficient in thiamine, opisthotonos did not occur and leg weakness was the characteristic feature. Degenerating nerve fibers were always found in the peripheral nerves, the changes being practically identical with Wallerian degeneration such as occurs in myelinated fibers of the divided sciatic nerve of a rat. The number of degenerated nerve fibers in the sciatic nerve corresponded closely with the degree of paralysis. Degenerative changes were seen earliest in the large and long fibers and appeared first at the point most distant from the cell body, thereafter proceeding centripetally to within a few millimeters of the dorsal root ganglia in the most severe cases. After about four days chromatolysis appeared in the dorsal root ganglion cells. On the administration of thiamine the total number of myelinated nerve fibers greatly increased and in spite of the apparent severe damage to the peripheral nerve, regeneration of the axis cylinder occurred and few neurons died. The chromatolysis in the cell body disappeared only after complete repair of the axis cylinder i.e., in an average of 55 days. These findings in the peripheral nerves of pigeons are practically the same as those Aring *et al.* (1941) had already noted in the peripheral nerves of pellagrins, where there was also a rapid response to thiamine treatment alone. Swank also found changes in the spinal cord. These were most apparent in the long and large fibers of the ascending spinocerebellar tract and were most marked at the peripheral end of the neuron, i.e., from the cervical enlargement upwards. It is of interest that the peripheral processes of the dorsal root ganglion cells degenerated whereas the central processes which enter the cord and form the fasciculus cuneatus and fasciculus gracilis were unaffected. This would indicate that the process of degeneration in these cells was selective,

the order of events being such as to preserve the neuron. Had the central processes degenerated, a permanent loss of function with ultimate death of the neuron would have resulted.

The conclusions that Swank draws from these findings are that, when a neuron is subjected to slow depletion of thiamine, its axis cylinder degenerates first at the point most distant from its trophic cell body because of the apparent difficulty with which this part of the neuron is maintained in its normal state. The myelin sheath also degenerates but this occurs *after* the axis cylinder is damaged. This observation is in agreement with the much earlier finding of Kimura (1920). This hypothesis of Swank's—namely, that the cell body is the primary site of the disordered metabolism and that it is the changes there which lead to the degeneration at the distal end of the axon—is more reasonable and more in keeping with physiopathological principles than the conception that local factors produce the damage at the periphery of the axon. It is supported by the two well established facts, (i) that the longest and the largest axons are the first to degenerate and (ii) that the earliest signs of damage to a peripheral nerve in beriberi appear in the areas most peripheral to the trophic nerve cell, i.e., in the toes and feet and in the tips of the fingers. As there is evidence that the cells of the nervous system utilize only carbohydrate for their energy and that thiamine is an essential agent in this process, it is understandable that, when thiamine is deficient, the nerve cell with its long processes is one of the earliest to show signs of damage.

Meiklejohn (1940) in a critical review of the relationship of polyneuritis to vitamin B deficiency concluded that "there is as yet no clear experimental evidence showing that true anatomic polyneuritis is curable by thiamine alone." Swank's and Aring's observations have been published since this statement was made and if their findings are confirmed, the criticism is no longer justified.

Thiamine has been administered to all forms of polyneuritis with disappointing results and Walshe (1941), although he is prepared to accept the hypothesis that a defect in carbohydrate metabolism in thiamine deficiency leads to nerve damage, states that the results of treatment with thiamine do not confirm the hypothesis. Improvement in peripheral neuritis, however, with thiamine therapy will depend on two factors, namely (i) that the neuritis is due to deficiency of thiamine and not to any other factor or factors such as a bacterial or chemical toxin and (ii) that the degenerative changes in the nerve are not too far advanced. The significance of a response or otherwise from nervous tissue to a specific therapeutic agent has already been discussed (Section I). The extent and rate of recovery will depend on the number of fibers

involved and the degree of damage in each. Such a conception may explain the varied results of treatment with thiamine alone in the neuritis of naturally occurring beriberi, in artificially induced thiamine deficiency in human beings and experimental animals, and in "alcoholic" peripheral neuritis, and polyneuritis gravidarum.

The relationship between this last condition and the neuritis of beriberi deserves further consideration. Shattuck (1928) first suggested that this latter condition was "caused chiefly by failure to take or assimilate food containing sufficient quantity of vitamin B . . . and might properly be regarded as beriberi." In 1933 Strauss and McDonald showed that the neuritis improved markedly on the administration of yeast by mouth and vitamin B concentrates and liver extract parenterally, even if the patients still consumed large quantities of alcohol. Jolliffe *et al.* (1936) produced evidence to support Strauss's findings. Brown (1941), however, did not find that thiamine hastened recovery in "alcoholic" peripheral neuritis. As already pointed out, the response to specific thiamine therapy in polyneuritis due to thiamine deficiency will depend upon the stage which the pathological process in the nerve has reached. Therefore Brown's results do not exclude thiamine deficiency as the initial cause of the polyneuritis. Chronic alcoholism is capable of producing the full clinical picture of beriberi with edema and cardiac changes that respond rapidly to thiamine therapy alone. Whether all cases of alcoholic peripheral neuritis are due to thiamine deficiency alone is uncertain but there is evidence that it is an important factor in a high proportion of cases.

Aring *et al.* (1939) found that daily doses of 100 mg. of thiamine intravenously produced rapid improvement in function in peripheral neuritis associated with pellagra, beriberi, alcoholism, tuberculosis and pregnancy. Pain disappeared in 24 hours and bedridden patients were able to walk within a few weeks. In 12 such cases biopsies of a terminal branch of the anterior tibial nerve were made both before treatment and several months after treatment with satisfactory improvement, but with residual signs of persisting nerve damage. In all cases there was extensive demyelinization, but in most the axon was intact before treatment and there was little change in the specimens taken months later. These findings suggest that the early rapid improvement in function is due to the ability of thiamine to enhance the effect of acetylcholine in the transmission of the nerve impulse. Minz (1938) had already claimed to have demonstrated such an action of thiamine and it may cause inhibition of cholinisterase. Aring *et al.* (1939) states that such inhibition was demonstrable in his cases and quotes Antopol and Glick (1939) as having made the same observations with horse and rat serum *in vitro*. Such being the case, thiamine has the same effect as physostigmine. They do not state

whether or not physostigmine produced the same rapid improvement. Moreover their theory demands an intact axon before acetylcholine can be produced at the nerve ending. A functional failure of impulse transmission resulting from a defect in nerve-cell metabolism that can be rapidly corrected by thiamine administration seems a more logical theory. Von Muralt (1948) is of the opinion that acetylcholine is liberated in parts of the nerve cell unit other than the nerve ending and reviews the experimental evidence. He also produces evidence that as well as acetylcholine, a thiamine-like substance, possibly thiamine disulfide is liberated *in* the nerve, during excitation. He suggests that these two substances may be essential for the transmission of the impulse from one node to the next in medullated nerves by providing the free energy from which the action current is derived. It is therefore an interesting speculation as to whether or not the primary nerve lesion in thiamine deficiency is lack of this thiamine-like substance, which may be necessary for node to node transmission of the impulse.

3. Summary of Pathology

The pathological findings in the peripheral nerves of patients with beriberi neuropathy range from complete destruction of the myelin sheath and axis cylinder, with the death of the parent cell, to minor changes in the myelin sheath and axis cylinder. The changes are identical with those seen in the peripheral nerves of animals rendered partly thiamine-deficient under experimental conditions. There is evidence that the administration of thiamine alone can arrest and repair these changes provided the nerve cell is not irreparably damaged. It is suggested that the primary lesion may be in the cell body and that the peripheral changes are secondary. The rate and degree of recovery will depend on the number of neurons affected and the stage which the degenerative changes in the neuron have reached.

4. Clinical Features Based on Cases in Changi Prisoner-of-War Camp, Singapore

The neurological features of beriberi are well known from the classical descriptions of the disease (Scheube, 1894; Bälz and Miura, 1905; Vedder, 1913; Shimazono, 1931). There are few detailed analyses of large series of cases to be found in the literature. As the Changi Prisoner-of-War Camp in Singapore provided an excellent opportunity for careful observation of a closed and well-organized community, where the dietary intake of the population could be calculated reasonably accurately, the author feels that some of the information from 400 patients on whom he was able to keep records is worth recording.