THE NATURE OF THE STRUCTURAL CHANGES IN NERVE ENDINGS IN STARVATION AND IN BERI-BERI

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Most authors who have considered the relation of beri-beri to inanition record their opinion that inanition alone does not produce beri-beri. In this inability to reproduce the disease by starvation alone those who see a close parallel between beri-beri and the various forms of toxic neuritis find their best ground for believing that beri-beri is a similar disease.

That inanition does reproduce in birds a condition indistinguishable during life from beri-beri and with a similar pathology of the nervous system has been claimed by Chamberlain, Bloombergh and Kilbourne (1) and also by Eijkman and van Hoogenhuyze (2). The latter specifically states that the sciatic nerves show a similar myelin degeneration to that found in the polyneuritis induced by feeding the deficient diet. In addition, Eijkman claimed that if, during the food deprivation, the birds were "drenched" with water, the symptoms were produced much more rapidly.

Most of the pathological investigations of beri-beri have been carried out either in birds or in man. Kimura (5) has recently made an important and extensive investigation of the degenerative and regenerative changes occurring in rice-fed pigeons. The diet of polished rice is, however, so defective in proteins, fat and salts that one hesitates to apply in detail the results so obtained to experimentally induced beri-beri in mammals. A survey of the literature discloses that there are a number of points relating to the pathology of the disease that have not been adequately decided. The disease has been conceived as a general involvement of the nervous system (Vedder (8)); or as disease of the motor cells of the spinal cord (McCollum and Simmonds (7)). Findlay (3) sees in the chromatolytic changes in the nerve cells the basis of the pathology of the disease. Again, it has been regarded as a peripheral neuritis, but no precision has been reached as to what nerves or what portions of the nerve trunks are chiefly involved. Finally, the disease has been looked on, not as a specific affection of the nervous system, but as a condition characterised by a failure of growth and development.

The involvement of the sensory nerves has been a matter of inference or assumption rather than resting on any examination of the nerves themselves. Reference to the sympathetic nervous system are of a similar nature. Lesions are attributed to this system rather on the basis of certain symptoms than on the actual observations of the cells and fibres. The state of this system is

of some importance since it has been shown that the adrenal undergoes hypertrophy in beri-beri (McCarrison (6) and Kelleway (4)) and inanition (McCarrison (6) and Findlay (3)).

The fact is often mentioned (Vedder (8), Voegtlin and Lake (9), and others) that the myelin degeneration bears no relation to the symptomatology of the disease. The immediate recovery from the disease after the administration of the vitamin (cats 12 hours, Voegtlin and Lake (9), McCarrison (6) and others in birds) is a difficult observation to reconcile with any profound changes in the nervous system.

The objects of the present investigation were to determine, if possible, the point at which the degeneration began in the nervous system; to find out what changes occurred in the sympathetic portion of the autonomic nervous system; to study as well the changes, if any, in the peripheral nervous system that occur in animals deprived of food. At the same time the histology of the ductless glands was observed.

MATERIAL AND METHODS

This communication is part of an investigation that is being carried out by Professor Drummond[1] of the Department of Biochemistry of University College. He has very kindly provided me with all the material used in this report. The observations presented here are confined to rats. Professor Drummond placed at my disposal three series of rats. The first were animals that had been fed on a deficiency diet and had developed beri-beri. The diet consisted of: caseinogen 20 parts, rice starch 65 parts, salt mixture 5 parts, cod-liver oil 2 parts, hardened oil 10 parts. Some of the animals were only slightly affected, others were moribund. A second series were rats that had been allowed to drink at will, but had been deprived of all food for a period varying from four to seven days. A third series comprised rats that had been kept under similar conditions to the second group but had been allowed to nibble a yeast extract at will.

A considerable number of histological methods were used. Some were investigated by the Marchi method. These were first injected through the aorta with equal parts of 10 per cent. formalin and Muller's fluid. The brain, cord and peripheral nerves were then prepared in the usual way. The preliminary fixation is a point of some importance, for handling of the tissues before fixation will give rise to a positive Marchi reaction and in beri-beri, where the changes are slight, this may lead to an error of interpretation. In addition to Marchi preparations of the brain and spinal cord, other brains were stained for Nissl granules by toluidin blue, and others were prepared by the Bielschowsky method.

The peripheral nerves studied were the sciatic and its branches. These were dissected out and fixed on glass slides. Some were stained with silver nitrate, counterstained with safranin and teased in oil of turpentine. Others were

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stained with osmic acid and teased. Others were stained by the Marchi method as already described.

The smaller nerve trunks, the intermuscular portions of the nerves and the nerve endings were stained with methylene blue *intra vitam*, with the Bielschowsky method and with gold chloride. The *intra vitam* staining with methylene blue was especially useful, for by it were obtained excellent preparations of the gut plexus and of the distribution of non-medullated nerves in relation to the capillaries and small vessels in the muscles.

BRAIN AND SPINAL CORD

The Marchi preparations were entirely negative. No trace of degeneration was found in the brain or cord. The silver staining of the brain and cord was followed in serial sections. No changes were found. Particular attention was paid to the paraventricular areas and the hypothalamus because of their supposed association with temperature regulation and functions of the sympathetic nervous system. The cranial nerve nuclei and the issuing nerve roots were also examined with care. No lesions could be discovered. The nuclei of the vagus were quite normal in appearance. The Nissl sections disclosed no chromatolytic changes in the central nervous system. Particular observations were made on the motor nuclei of the ventral horn without revealing any changes in these cells.

These observations lend no support to the hypothesis that the essential lesion of beri-beri is a disorder of lipoid metabolism as shown by the chromatolytic changes in the nerve cells. This was put forward by Findlay (3). His observations were made on birds.

CHANGES IN THE NERVE TRUNKS

The Marchi examination of the sciatic nerve was negative. In a few sections an occasional black dot was observed but these were so rare that a negative interpretation of the results needs no qualification. The teased preparations disclosed normal fibres abundantly. An occasional doubtful fibre could be found but this was extremely rare. However, as the nerves were examined more distally changed fibres were more frequently met with. Here and there the myelin appeared to be aggregated into globular masses leaving the axis cylinder uncovered. The axis cylinder appeared to be always intact despite these changes in the medulla.

Kimura (5) states that it is generally believed that the disease starts peripherally but that this has not been adequately demonstrated. It remains uncertain whether the disease begins in the final termination of the nerve, in the naked axis cylinder, in the smallest intermuscular bundles, or at the entrance to the muscle. Various observers claim to have seen degeneration in the larger nerves, such as the femoral, etc. All are agreed that only a small proportion of the fibres are affected. McCarrison (6) estimates that never more than 15 per cent. of the fibres are involved but that a far greater proportion

have their function impaired. Vedder and Clark (8) estimate that the number of fibres affected is about 10 per cent. They believe beri-beri to be a disease of the whole nervous system. The conclusion that the affection begins in the intermuscular portion of the nerve fibres is supported by the above observations.

THE STATE OF THE NERVE ENDINGS

The beri-beri rats stained with gold chloride (figs. 1 and 2) showed that the changes in the endings were considerable. The normal ending is a fine branching complicated net-like ending of the axis cylinder beneath the sarcolemma of the muscle fibre. In the deficiency disease, the rich complication becomes lost. In some cases the medullated fibre appears to end blindly and to bear no nerve ending at all. In other cases the fibre ends in a bulbous manner, indicating the position of what was once an end organ. More commonly the fibre forms an ending, but this differs from the normal in that the finer branching is no longer visible and the ending is coarse and has lost its delicate tracery. The individual elements are swollen and expanded and give the ending a gross and deformed appearance. The fibre connected with the ending may be much altered, but in general, the alteration in the nerve fibre is much less than that of the ending. In the nerve fibre the change usually takes the form of a swelling of the myelin, which would appear to be more fluid since the nerve fibres become irregular in outline. The changes are most marked where the nerve fibre pursues an intramuscular course, and becomes progressively less marked as the individual fibres are followed back to where they join the nerve bundles. The changes appear to affect first the myelin. Fragmentation of the myelin, the formation of droplets in the myelin and disappearance of the myelin can be observed. The axis cylinder may have disappeared or become fragmented, but as a rule it persists even when the myelin has been much altered. From a study of the gold-chloride preparations it would appear that the changes are most marked in the nerve endings and in the myelin and then in the axis cylinder where it is covered by myelin, and that these changes are best seen in the intermuscular portions of the nerves where they have separated into groups each of a few fibres running towards their own terminal plates.

The silver preparations (figs. 3 and 4) are the least satisfactory for study, and Kimura (5) also records his dissatisfaction with the method. The mode of ending is not demonstrable, for silver preparations have to be studied in sections, whereas the others are spread out so as to permit the examination of the whole fibre, in continuity with its ending. The silver specimens show a similar irregularity in the outline of the nerve fibre, which appears to be swollen and expanded right to its ending. The endings have lost much of their finer branching. The changes are seen best in the intermuscular part of the nerve.

A study of the methylene-blue preparations (fig. 5) shows that in some fibres the axis cylinder may be intact but in others it may be irregular and its ending become a large bulbous swelling. The naked terminal network is

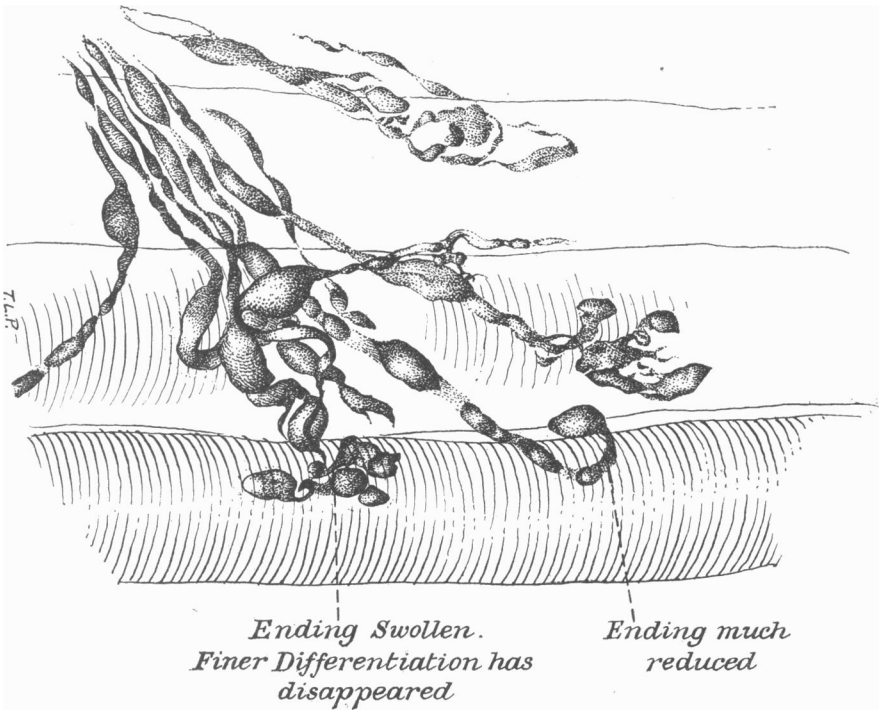

Fig. 1. Terminal motor nerves and endings. Stained with gold chloride. Vastus medialis of rat with severe beri-beri. × 1000. For detailed description see text.

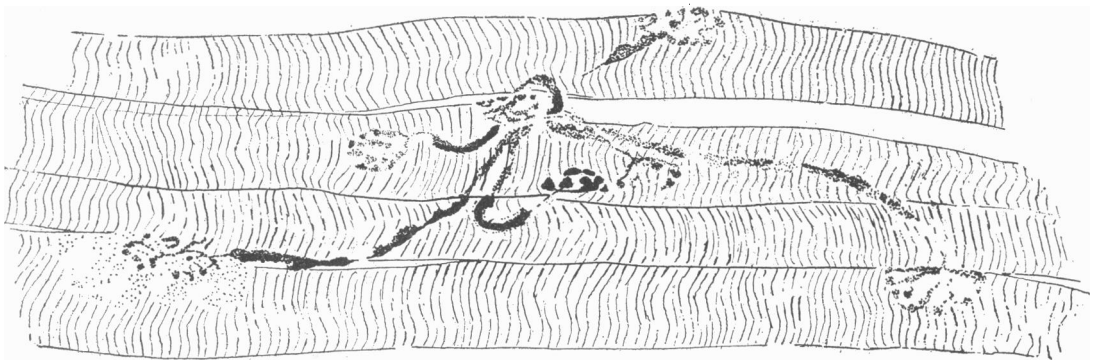

Fig. 2. Terminal motor nerves and endings of the muscles of front of thigh from rat with moderately severe beri-beri. Gold chloride. × 500.

swollen. The normal thickenings in the terminal apparatus have become large bulbous discs. The finer branches have disappeared or perhaps fused to give rise to the larger bulbs that are present. The total size of the ending is reduced, though the few elements present are much swollen. The axis cylinder beyond the terminal apparatus may be affected, but on the whole it is the part of the nerves which has undergone least change. The myelin may have disappeared

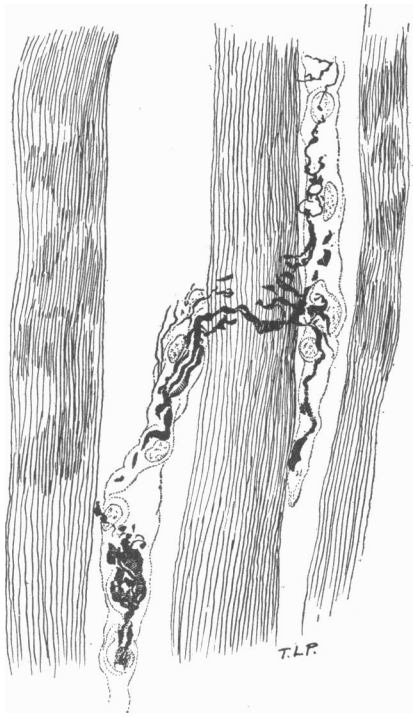

Fig. 3. Terminal fibres and endings in the thigh muscle of rat with moderately severe beri-beri. Terminal fibres irregular in outline and the endings confused and reduced. Bielschowsky method. × 500.

Fig. 4. Transverse section of a nerve trunk lying embedded in the muscle. From thigh muscle of a rat with moderately severe beri-beri. The axis cylinders in section are irregular in outline. × 500.

in part or become much expanded. The nerve outline is grossly irregular. The regular succession of the nodes of Ranvier have disappeared. The staining varies considerably and is much less efficient than in the healthy animal. The nerve ending is stained rather palely.

The changes that have been described for the motor terminal plate apply equally well to the muscle spindles (fig. 6). The apparatus in both methylene-blue and gold-chloride methods has lost its definition, many of the individual loops are wanting or they are swollen or confused in outline. The changes are not restricted to the ending but are also apparent in the approaching nerves

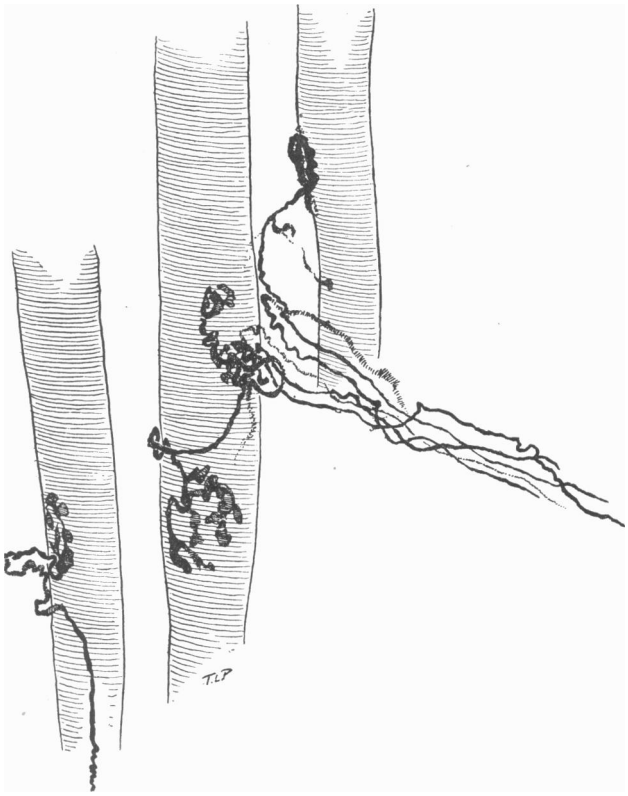

Fig. 5. Thigh muscle of a rat with mild beri-beri. Methylene blue *intra vitam* staining. Slight changes in the nerve endings. × 500.

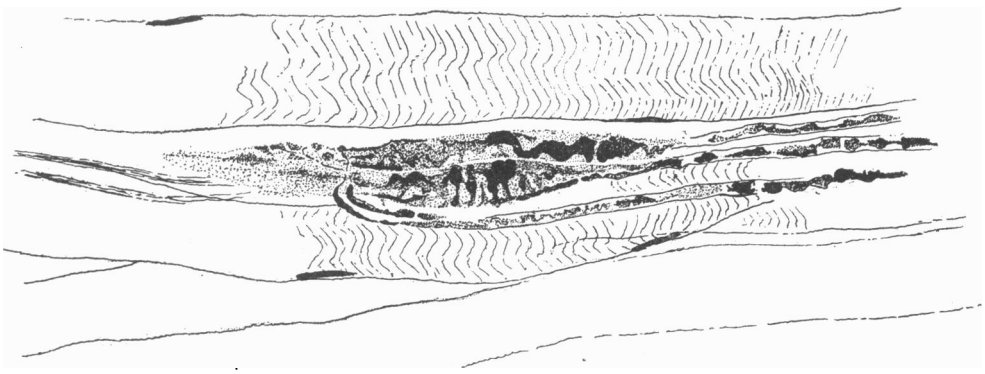

Fig. 6. Neuro-muscular spindle from thigh muscle of rat with severe beri-beri. The morphological details of the end organ have become much obscured and lost. × 500

and these too show changes mainly in the myelin, but also to a varying extent in the axis cylinder.

The observations on the terminal apparatus of the motor and sensory nerves in the muscle may be summed up as follows. In some cases there has been a disappearance of the endings. This is rare. In more cases the endings are swollen and have lost their finer differentiation. Others are only slightly changed or not at all. The myelin appears to have suffered more than the axis cylinders. It appears to have assumed a more fluid consistence, as shown by the irregularity in outline and in the spreading of the sheath from even such slight pressure as the crossing of two nerve fibres. These changes are most pronounced near the ending of the nerve and become less marked as one approaches the larger nerve bundles entering the muscle.

When these observations are compared with those of other observers, it is to be remarked that no paper dealing with the changes in the nerve endings has been found. Some importance should be attached to the state of nerve endings as the neuro-muscular apparatus offers a highly differentiated field in which slight morphological changes might be easily observed. The changes are conspicuous and of varying degree. I believe they offer a better basis than has hitherto been suggested for the quick recovery that occurs when the animal is given the vitamin.

The degree of the changes in the intermuscular portion of the nerve fibres and the nerve endings as compared with the paucity or absence of changes elsewhere suggest that the disease exerts its effects at this point.

THE SYMPATHETIC NERVOUS SYSTEM

It would seem that this system has never been adequately investigated. McCarrison (6) has reported changes in the cell of coeliac ganglion and in Auerbach's plexus. Kimura (5) expresses the opinion that this system must be affected, but apparently made no observations on it.

Coeliac ganglion. In several rats these ganglia were removed and after fixation in alcohol were stained with toluidin blue and the cells investigated. No changes of any sort were found in these cells. The Nissl granules were unchanged and the absence of any sort of chromatolytic changes was beyond doubt.

By means of methylene blue the state of the gut plexus was investigated and here again I was able to find no definite changes in the cells. There seemed to be a considerable reduction in the richness of the nerve fibres of this plexus. There is much atrophy and wasting of the muscle and glandular coat, but nothing definite in the way of affection of the plexuses was ascertained except this reduction in the abundance of nerve fibres.

Voegtlin and Lake (9) fed dogs, cats and rats on alkali-treated meat. The rats never developed beri-beri. Some dogs escaped the disease; others died without nervous symptoms; others developed incoordination, spasticity, and

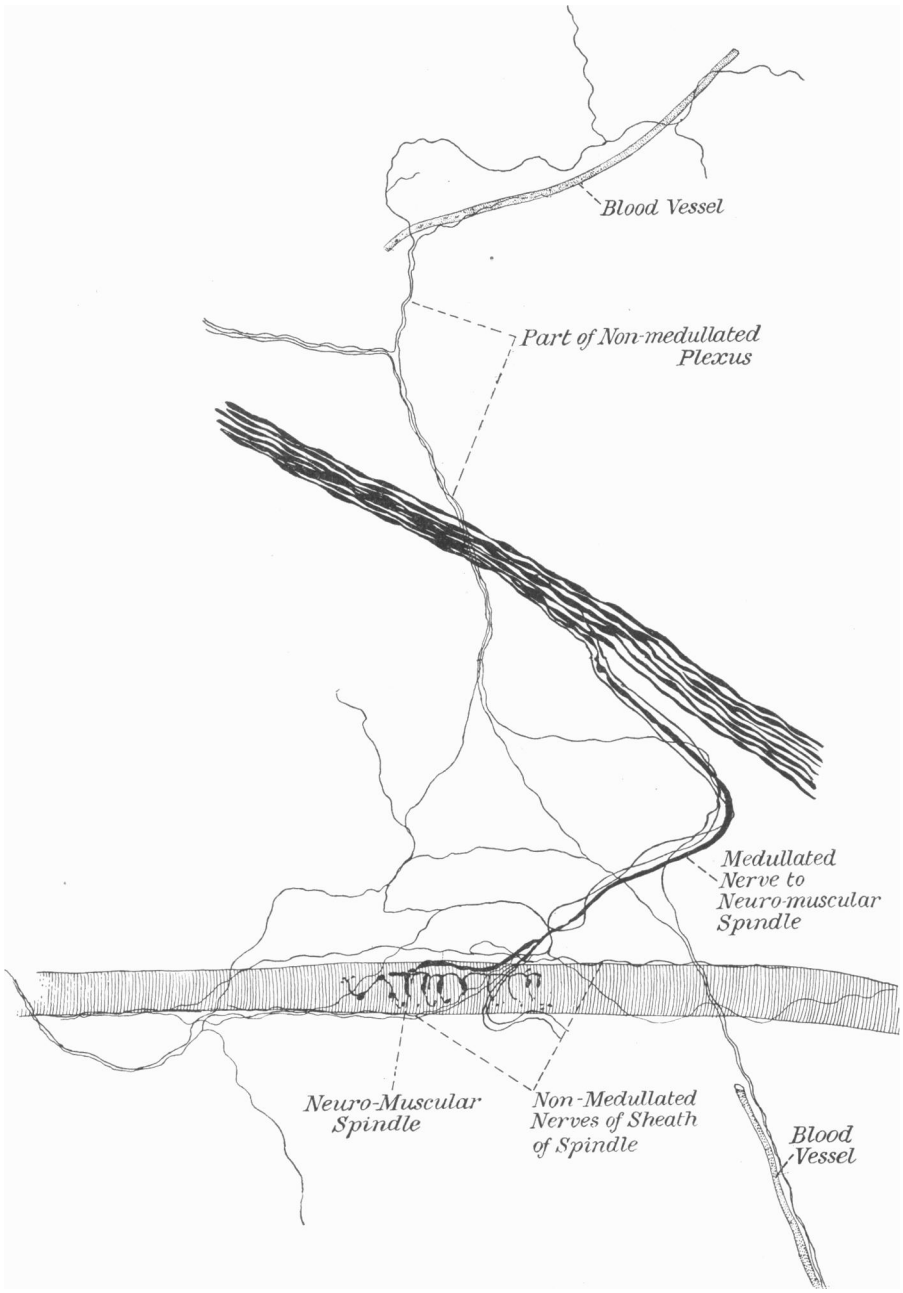

Fig. 7. Non-medullated nerves, neuro-muscular spindle and capillaries from the muscle of the thigh of a rat with severe beri-beri. The non-medullated nerves issue from the nerve trunk. Methylene-blue preparation. For description see text. × 500.

convulsions. Stimulation of the sciatic nerve gave qualitatively normal results, while stimulation of the cervical sympathetic was followed by immediate dilatation of the pupil. Marchi examination disclosed degeneration in the sciatic nerve varying from mild to severe and a scattered degeneration was also present in the cord. There was no correspondence between the degenera-

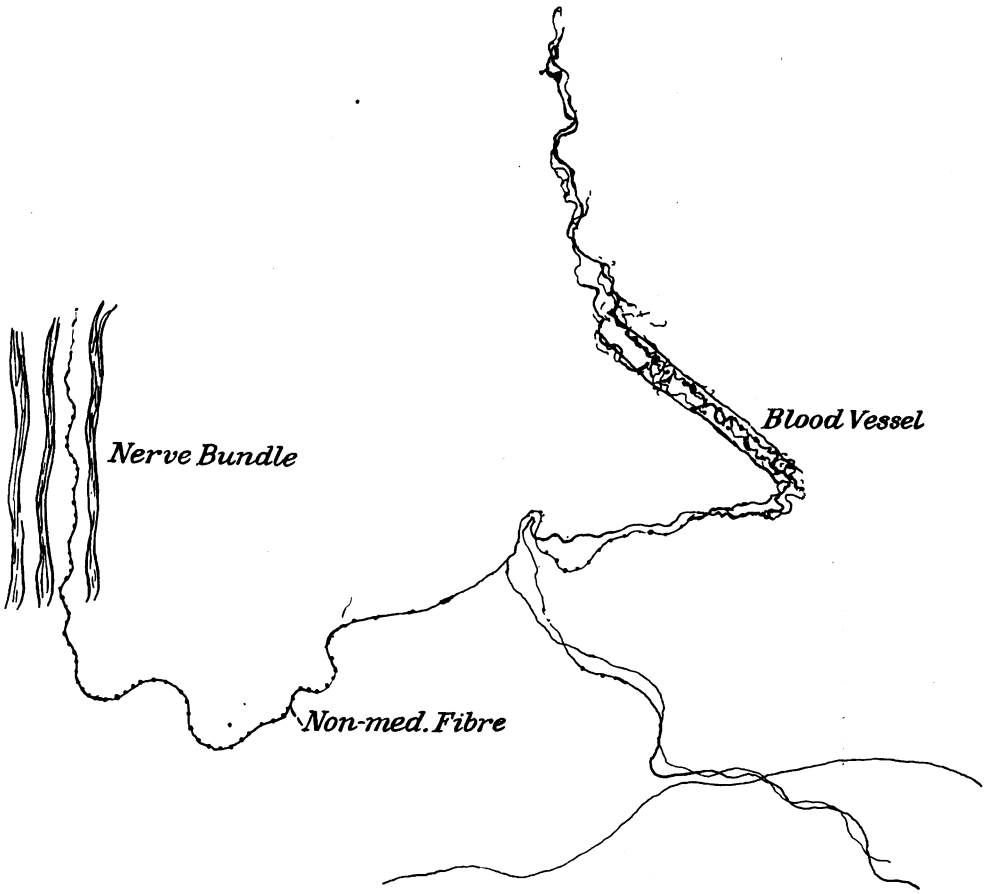

Fig. 8. Similar preparation to fig. 7 showing the distribution of non-medullated fibres from a methylene-blue preparation of a muscle. × 500.

tion and the symptoms observed. Kimura (5) says it is difficult to understand how the peripheral vascular system behaves during life in beri-beri; that it is not normal one can well believe because the sympathetic nervous system must be depressed organically or at least functionally.

The methylene-blue preparations of the muscles of the beri-beri rats were particularly favourable for the study of the distribution of the fibres belonging to the sympathetic system. They were clearly stained and could be followed

for relatively great distances in such preparations. Figs. 7 and 8 are drawn from such and show clearly that there has been no change in the peripheral non-medullated fibres. These form a very considerable plexus in the sheath of the muscle which they reach mainly from the branches which run in the nerves to the muscle. They can be followed in the nerve bundles and often subdivide while still in these bundles. After leaving the nerve bundles they ramify widely and so form a considerable plexus. The distribution of the branches of this plexus often brings them into close relationship with other nerves, and in fig. 7 they are seen in relationship with a neuro-muscular spindle. They appear to wrap themselves about the sheath of the spindle. However, out of the plexus individual fibres can be traced and they come into relationship with blood vessels. On these they can be traced for long distances, gradually growing finer and finer until they pass beyond the range of visibility.

These very clear preparations of the sympathetic fibres can leave no doubt that this system is not involved in the degenerative changes that overtake the endings of the muscle nerve fibres.

A qualitative examination of the adrenals in a great number of cases has been made. In all cases the cortex appeared to be quite normal and there was no deviation from the normal in the fat staining of the cortex. Findlay (3) believes that the enlargement is cortical and correlates this with chromatolytic changes in the nerve cells. In all cases there was an abundance of chromaffin in the medulla of the suprarenal. This has been noted by McCarrison (6) and Kelleway (4). The latter suggested that though there was this abundance of adrenalin, yet it was possible that it might not be discharged into the blood stream because the normal outlet into the medullary sinuses appeared to be obstructed by an accumulation of cells belonging to the cortex. A study of the serial sections of the glands does not confirm this view of Kelleway. The disposition of the medullary substance shows no deviation from the normal and there is no reason why the normal method of discharge should not operate; at any rate this obstruction by cortical cells is not present when serial sections are examined.

From this study of the nervous system, it is concluded that the changes in this system begin in the terminations of the nerves in the muscle, that the change spreads back into the nerve bundles in the muscle and grows less the farther the nerves are followed. In the larger trunks it is difficult to find any fibres affected. The sympathetic system escapes entirely, and at the same time there is an abundance of chromaffin substance in the suprarenal with the same disposition in regard to the veins of the medulla as in the normal animal.

CHANGES IN INANITION

It is obvious that the findings in the beri-beri animal present a close parallel with the changes that occur in an animal that is deprived of all food. Wasting, muscular asthenia, hypothermia, hypertrophy of the suprarenal are common

to both (McCarrison). It is true that, save for Eijkman, observers are accustomed to confirm the idea that the polyneuritis is peculiar to beri-beri. It is to be remarked that many animals die without showing any nervous manifestations; that on the same diet many animals do not develop the clinical picture of beri-beri; that in all cases (pigeons, dogs, cats, rats) the symptoms, when they are present, disappear in a few hours, six to twelve, after the administration of the appropriate vitamin. The experimental cases are not comparable to what occurs in the chronic cases in man, as presumably there the time allows of far-reaching degeneration of the nerves.

In view of these facts it became important to determine whether the same picture occurred in animals deprived of all food.

The same methods were used as in the case of beri-beri. Preparations of the terminations of nerves in the muscle were made by the various methods and an examination of figs. 9 and 10 will show that real changes do occur in the nerves and at the same place as happens in beri-beri. The changes are decided, but they are not so pronounced as in beri-beri. Many normal endings are present. They are, of course, present in beri-beri, but more fibres are there affected. The changes are of the same kind. The arborisation of the terminal motor plate becomes reduced and the finer elements are no longer seen, or the ending becomes bulbous and confused. The changes extend into the nerve-bundles of the muscle, and an occasional altered nerve fibre can be found in the nerve trunk. The changes in animals deprived of food are of the same kind and distribution as occurs in the beri-beri animal. They are not so extensive and are more strictly localised than in beri-beri. The rats were deprived of food for four to six days. A rat on a deficient diet takes about three to four weeks to reach the same condition. This difference in duration may be a sufficient explanation for the differences that occur in the condition of the peripheral nerves in the two cases. In the animals allowed to nibble yeast extract at will, though deprived of food, changes in the terminal motor apparatus have been observed, but it is very difficult to establish differences between these and the former group. I believe that the changes are slighter in the terminal apparatus of the nerves in this series than in the series receiving no marmite.

It is not my purpose to discuss the various theories of beri-beri. This much might be said, that many of the nervous symptoms occurring in animals during acute feeding experiments for the production of beri-beri cannot be confirmed. A clinical examination of a rat suffering from beri-beri is not easy, but some observations can be made. They can be tested for righting reflexes and these they respond to as does the normal animal. They are not spastic. From the nerve lesions present it is difficult to understand why spasticity should be so frequent in the description of beri-beri. Convulsions, as a final event in the disease, permit of many explanations. Occurring in the course of the disease what I have seen described as such is an alternate flexion and extension of the hind extremities in the rat without any general manifestations.

Fig. 9. Gold-chloride preparation of motor nerves and terminations from a rat that had been deprived of food for five days. ×500. Note the changes that have occurred in some of the nerve endings.

This lasts only for a brief time and may not occur again in the course of the disease. The extraordinarily rapid cure of beri-beri, which admits of no doubt, is inconsistent with any profound change in the nervous system. Moreover, it is consistent with the changes here described which are partially restricted in their distribution and confined mainly to the terminal apparatus of the

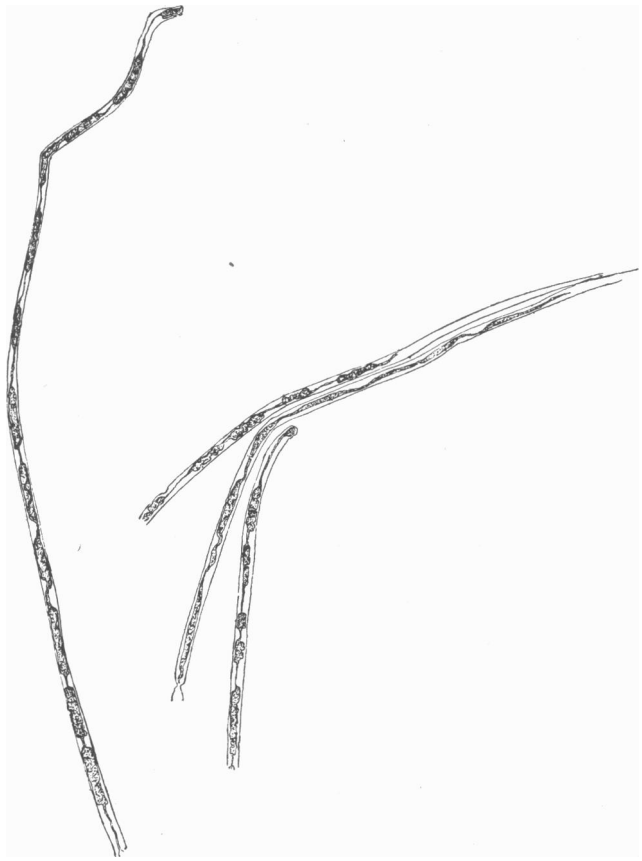

Fig. 10. Teased preparation of tibial nerve in the leg. From a rat deprived of food for five days. Osmic-acid stain. The axis cylinders intact. Alteration in the myelin.

nerve fibre. Absolute destruction is uncommon. More frequently the changes are of such a nature as would permit of imperfect function and certainly of quick recovery.

How far are they consistent with the idea that beri-beri is a disease due to the absence of a vitamin which causes the animal on such a diet to be progressively more unable to assimilate the food offered so that it gradually passes from a state of semi-starvation to complete starvation?

The agreement between the two conditions is extraordinarily close. The cardinal symptoms are the same; the wasting, the hypothermia, the hypertrophy of the suprarenal, the atrophic change of other organs and the alteration in the structure of the terminal apparatus of the nerve fibres in the muscle. The more decided nervous manifestations of beri-beri might be explained by the different duration of the two conditions. If this were so one might with some confidence put forward the notion that beri-beri as it occurs in rats under experimental conditions is really starvation arising from the inability to assimilate food in the absence of the appropriate vitamin. No observer has proclaimed that the pathological findings can be correlated with the symptoms observed during life. Kimura (5) definitely finds that head retraction in the pigeon is an early phenomenon, while affection of the cervical nerves is a very late phenomenon.

SUMMARY

(1) The morbid changes in the nervous system of the rat on a diet deficient in water soluble vitamin B affect the intermuscular medullated motor and sensory nerves and their endings. The changes rapidly subside as the nerves are examined further away from the muscles.

(2) The sympathetic nervous system presents no abnormal anatomical change.

(3) The central nervous system presents no abnormal anatomical change.

(4) Rats, deprived of food, show the same type and distribution of lesions as the rat on the deficient diet. They are not, however, quite so marked.

(5) Both series, deficient diet and no diet, show qualitatively an abundance of chromaffin substance.

(6) There is no histological evidence that the cortex of the suprarenal is affected.

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