Globular neuropathy¹

A disorder of axons and Schwann cells

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There are at least two distinct patterns of reaction in the peripheral nerves in cases of peripheral neuropathy. Axonal degeneration or segmental demyelination (or both) may occur and their histological appearances are now well known. Disorders associated with primary destruction of axis cylinders include the classical example of Wallerian degeneration, and the latter, sometimes termed 'Schwann cell diseases', includes the neuropathies of diabetes mellitus (Thomas and Lascelles, 1966) and metachromatic leucodystrophy (Dayan, 1967).

We have recently studied one family in which peripheral neuropathy has occurred in members of three generations, and another unrelated family only one member of which was definitely affected. Nerve biopsies from both families have shown both axonal destruction and segmental demyelination associated with unique features of Schwann cell dysfunction.

MATERIAL AND METHODS

Under local anaesthesia, a sural nerve and peroneus brevis muscle were biopsied. For light microscopy they were fixed in buffered formol saline and paraffin and frozen sections cut and stained by conventional methods. Isolated fibres were 'teased' from the nerve by freehand dissection after post-fixation in 10% osmic acid and maceration in 60% glycerine (Dayan, 1967). The internodal lengths and diameters of such fibres were measured directly with a micrometer eyepiece at magnifications of $\times 100$ and $\times 950$ respectively. Part of the sural nerve of Case 1 was post-fixed in 1% osmic acid and the number of myelinated fibres and their diameters were measured directly in transverse sections at a magnification of $\times 950$, with the aid of a Fleming Particle Size Micrometer and Analyser, Type 526 (Fleming Instrument Co. Ltd., Stevenage, U.K.).

For electron microscopy, small pieces of nerve were post-fixed in buffered osmic acid and embedded in Epikote. Ultra-thin sections stained with lead citrate and uranyl acetate were examined in an AE1 'EM6' electron microscope.

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CASE REPORTS

FAMILY A: CASE 1

A male clerical worker (Neurological Centre No. 2515) aged 52 years had for 15 years noticed progressive weakness of his left foot. The right foot and both hands had been affected for about five years.

On examination, the patient had normal facies, moderate thoracic kyphosis, marked wasting of all distal muscle groups in the lower forearms, calves and feet, and relatively good preservation of proximal limb and girdle muscles. He had mild pes cavus. His hand grip and small hand muscles were weak, and he had difficulty in walking both on the level and up stairs. He had considerable weakness of dorsiflexion and eversion of the feet and of dorsiflexion of the toes. Distal to the elbow and knees there was severe diminution of all modalities of sensation, including joint position and vibration senses and perception of pain and temperature. The tendon reflexes and plantar responses were absent. Peripheral nerves did not feel thickened. There was no nystagmus or cerebellar ataxia. The feet were not ulcerated. General physical examination and the ECG were normal. Conduction velocities in the right median and left ulnar nerves were reduced to 18 m/sec.

Laboratory investigations were all normal including blood count, liver function tests and electrophoresis of serum proteins. Phytanic acid was not detected in his serum (Professor J. N. Cumings).

FAMILY HISTORY This is shown in Figure 1. The index patient (III.9), of our study, had three live children, one of whom (IV.7), was also affected and forms Case 2 of this report.

The mother (II.8) of the proband was 85 years old when examined and considered herself to be in good health, although for many years her feet 'turned in' when she walked.

On examination of the cranial nerves she had bilateral nerve deafness. There was severe wasting and deformity distally in all four limbs, particularly marked in the hands and lower legs, which were thin and showed a severe inversion deformity of the feet. Dorsiflexion and eversion of the feet were very weak, but proximally power was good. The deep reflexes and plantar responses were absent. Cutaneous sensation and joint position sense

FIG. 1. Kinship of Family A with globular neuropathy. The index patient is III.9. $\square$ = male. $\circ$ = female. ∇ = sex unknown. $\square$ = still births. $\odot$ = pes cavus.

appeared normal. Peripheral nerves did not feel thickened.

Other members of the family examined clinically were III.6, III.7, III.13, and IV.8 all of whom seemed normal. Conduction velocity in the right lateral popliteal nerve of III.7 was normal.

The right sural nerve and peroneus brevis muscle of Case 1 were biopsied.

HISTOLOGICAL FINDINGS (a) *Sural nerve* There was severe loss of normal myelinated axons, marked endoneurial fibrosis, and an excess of fine, unmyelinated fibres. In frozen sections there was a small number of what appeared to be ovoids of myelin-like material (Fig. 2) which stained magenta by the periodic acid-Schiff technique, black with Sudan black B, weak orange with Sudan III, and showed birefringence in polarized light. The fibre spectrum in a transverse section showed a severe loss of nerve fibres and a relative excess of small ones (Fig. 3).

Teased fibres presented a bizarre appearance. Instead of normal myelinated segments of constant thickness

FIG. 3. Case 1. Fibre diameter spectrum of sural nerve from Case 1. There is an excess of fine nerve fibres.

FIG. 2. Case 1. Sural nerve biopsy showing a small number of myelin-like ovoids in a frozen section. (Sudan black, $\times 75$.)

and length separated by nodes of Ranvier, there were frequent bare areas and poorly stained internodes of diverse lengths many bearing osmiophilic globules (Figs. 4, 5) which were rounded or fusiform in shape and occurred anywhere along individual segments. Although most lay symmetrically on fibres, a few were on one side of the myelin sheath while still being within the endoneurial tube of that fibre. The globules measured between 10-15 μ in length, and 8-12 μ in diameter.

A semi-quantitative analysis of the relationship between internodal lengths and diameter of individual fibres is shown in Fig. 6, displayed by the convention of Fullerton, Gilliatt, Lascelles, and Morgan-Hughes (1965).

FIG. 4. Case 1. Consecutive lengths of a single fibre teased from the sural nerve. There are short and long segments, bare internodes and osmiophilic globules. (Osmic acid, $\times 100$.)

FIG. 5. Case 1. Bizarre thickenings and irregularities of the myelin sheath, a 'globule', and demyelinated segments are present in a teased fibre. (Osmic acid, $\times 400$.)

It shows a wide scatter of segmental lengths most of which are much shorter than the normal value for fibres of that particular diameter. Some fibres contained some short segments and others of normal length. These changes are characteristic of segmental de- and remyelination and of regeneration after axonal degeneration (Lascelles and Thomas, 1966; Thomas and Lascelles, 1966). The presence of some de- and remyelinated segments on fibres which also bore internodes of normal length implies that Schwann cell damage and healing had occurred independently of axonal degeneration. Morphologically these processes seemed to follow a normal course, except that restricted para-nodal damage was not seen. Degenrating axons and empty Schwann cell tubes were not found.

As little cellular infiltration was seen apart from

occasional macrophages and mast cells, the overall impression was of an indolent process. There was marked proliferation of endoneurial fibroblasts. Intraneural blood vessels appeared normal.

Electron microscopy All the axons seen appeared normal and contained recognizable mitochondria, neurotubules, and occasional electron-dense, membrane bounded vesicles.

Some large axons were seen (Fig. 7a) which were surrounded by a thin sheath of Schwann cell cytoplasm and lacked a recognizable myelin sheath. A very few hemi-nodes of Ranvier were also found in which the other half of the node was lacking, although a virtually normal axis cylinder was still present. These changes were interpreted as evidence of segmental demyelination. Although in some areas normal appearing Schwann cells

FIG. 6. Case 1. Graphical analysis of fibres from the sural nerve by the method of Fullerton et al. (1965). Shaded area encloses normal mean and 95% confidence limits (Thomas and Lascelles, 1966). Each vertical line represents one nerve fibre. The very wide scatter of points shows the effects of segmental demyelination and axonal degeneration and healing.

and their associated myelin sheaths were seen, in many fields the sheaths were abnormal (Fig. 7b). They had expanded into swellings containing electron dense whorls and dense droplets of such complexity as to make their exact morphological relationships difficult to determine. Schwann cells generally had nuclei of normal appearance which were surrounded by a variable amount of cytoplasm containing the usual organelles and a small amount of endoplasmic reticulum. There was no obvious increase in lysosome-like structures. The complex globules of altered myelin impinged closely on perinuclear Schwann cell cytoplasm, axis cylinder, and the surrounding fibrous tissue. Sometimes their myelin lamellae appeared to run through an area of debris to join a normal myelin sheath on the other side.

The swellings were considered to be the globules seen by light microscopy.

(b) *Peroneus brevis* muscle showed neurogenic atrophy. No definite lesion was seen in the few small intramuscular nerves present in the biopsy.

FAMILY A: CASE 2

This patient (Neurological Centre No. 3069) was the 4-year-old son of Case 1 (IV.7 of Fig. 1). After an uneventful pregnancy and birth the child seemed to

FIG. 7A. Case 1. Low power electron micrograph showing paucity of myelinated fibres in sural nerve. In the centre one fibre carries whorled osmiophilic debris. (Approximately $\times 1,850$.)

FIG. 7B. Case 1. Longitudinal section of sural nerve. There is a node of Ranvier on the left. On the right several coiled, multi-lamellated accumulations of osmiophilic, myelin-like material lie within the myelin sheath. ($\times 2,500$.)

develop normally for the first few months of his life. However, motor development was retarded and when he began to walk at the age of 13 months it was noticed that his right foot would frequently 'turn in' when he stood on it. He has needed calipers for walking since the age of 18 months.

Examination showed a young child of normal habitus except for marked wasting of the legs below the knees. His hands, arms, trunk, and girdle muscles appeared normal. He had weakness of all muscle groups in his legs which were flaccid. The motor weakness was most severe distally with, for example, foot drop and only slight weakness of the quadriceps. There was pes cavus deformity of both feet. Tendon reflexes seemed normal in the arms, but could not be elicited in the legs. Sensation appeared normal in the arms, but there was undoubted hypoaesthesia in the legs below knee level, with diminished perception of vibration, joint position, heat and cold. Peripheral nerves did not feel thickened.

Conduction velocities in the right median nerve (15 m/sec) in the forearm and in the right lateral popliteal nerve (10 m/sec) were very slow.

Laboratory investigations were all normal including full blood count, liver function tests, and estimation of serum proteins. Phytanic acid was not detected in his serum (Professor J. N. Cummings).

Biopsy was attempted of the right sural nerve but only a small fascicle of it was obtained. The peroneus brevis muscle was also biopsied.

HISTOLOGICAL FINDINGS (a) *Nerve* It was only possible to prepare paraffin sections in which the myelin sheaths appeared normal and one degenerating axis cylinder was found (Fig. 8).

(b) *Peroneus brevis muscle* This appeared normal.

FAMILY B: CASE 3

This patient (Neurological Centre No. 3692), a male engineering fitter, was first seen in 1952, aged 11 years, complaining of difficulty in walking.

FIG. 8. Case 2. Degenerating, fragmented axis cylinder (arrowed) in fascicle of sural nerve. (Glees-Marsland, $\times 600$.)

FAMILY HISTORY Both parents of the patient were said to be normal and so were his brother (his only sib), his wife, and both of his children, although the youngest, now aged 2 years, has not yet learned to walk.

PAST HISTORY His birth was difficult and he was cyanosed for a while. There appeared to be no immediate sequelae of this, but he did not learn to walk until he was 5 years old and his hands were weak and clumsy. These symptoms were unchanged when he was examined at the age of 11 years. At that time there was generalized weakness of the thighs and lower legs and he walked with a steppage gait. Hand muscles also were weak and wasted, but other muscle groups appeared normal. All tendon reflexes were absent and the plantar responses were flexor.

No sensory abnormalities were found.

PRESENT CONDITION The patient was not seen again until February 1968, aged 26 years, when he thought his condition has been unchanged for some time. Although his hands and legs were still weak, he could run, swim, ride a bicycle, and was employed as an engineering fitter. In September 1966 he had first noticed tingling and numbness of the tips of his fingers which made him drop things. The numbness had developed over a few weeks and had since persisted unchanged. There had been no sensory symptoms in the feet.

On examination in May 1967, there was impaired sensation to touch and pain in all the fingers to the level of the palms, and wasting of the small muscles of the hands. The hip flexors were weak and so were the extensors and flexors of the toes. The tendon reflexes and plantar responses could not be elicited. The calf and hand muscles were tender.

Electrophysiological studies showed very slow conduction velocities in both the right and left ulnar nerves (21 and 23 m/sec respectively). The left median nerve was inexcitable. In the hand muscles and anterior tibial muscles electromyography showed fibrillation potentials at rest and a reduced interference pattern on volition.

Lumbar cerebrospinal fluid contained protein 70 mg/100 ml. Glucose and pyruvate tolerance tests, serum W.R., serum B₁₂, and the serum creatine phosphokinase (32 i.u./l.) were all normal. The right sural nerve and peroneus brevis muscle were biopsied.

PROGRESS He was considered to have polyneuritis and was treated with prednisone 20 mg daily for four weeks. By the end of the course of steroid the paraesthesiae and numbness in the fingers had disappeared, but the patient thought that his hands were weaker than before. When last seen in August 1967 his condition was unchanged.

HISTOLOGICAL FINDINGS (a) *Sural nerve biopsy* The lesions observed were very similar to those seen in Case 1, with severe loss of nerve fibres, marked endoneurial fibrosis and the presence in the nerve of occasional ovoids of myelin-like material. Isolated, teased fibres had a bizarre 'beaded' appearance due to the presence on them of osmiophilic globules (Fig. 9). There was extensive segmental de- and remyelination, and osmiophilic globules of various sizes were present along the internodes.

FIG. 9. Case 3. Consecutive lengths of a single fibre from the sural nerve. There is very extensive demyelination, sometimes extending over several segments, and densely-stained globules are also present. (Osmic-acid, $\times 100$.)

There was no inflammatory infiltration in the nerve and its blood vessels appeared normal.

The relationship between fibre diameter and internodal length was measured, and the results, plotted according to the convention of Fullerton *et al.* (1965), are shown in Figure 10. Large numbers of fibres bore inappropriately short segments which are typical of regeneration after axonal degeneration, and the wide scatter of internodal lengths confirms the many observations of demyelinated and healing segments.

Electron microscopy There was severe endoneurial fibrosis and many nerve fibres were represented by thick bundles of collagen fibres with a central core of an abnormal nerve fibre. No true coils or whorls of Schwann cells were found (Fig. 11).

The majority of axis cylinders appeared normal but a few showed focal collections of mitochondria and small vesicles. Some large axons had very thin myelin sheaths and were probably undergoing remyelination. As in Case 1, Schwann cells appeared normal apart from their

enveloped multi-layered globular inclusions, apparently derived from nearby degenerating myelin. The globules appeared to lie outside the main bulk of the Schwann cell cytoplasm, but were almost certainly connected to it by continuity of plasma membrane until degeneration was well established. This was shown in a few instances by finding globules which were enclosed by a few of the outer turns of the myelin sheath. Many Schwann cells, whether bearing normal or abnormally thin myelin sheaths, contained large cytoplasmic multilaminated osmiophilic ovoids typical of 'myelin figures'.

(b) *Peroneus brevis* muscle biopsy There was marked neurogenic atrophy of the muscle. No intra-muscular nerves were found.

DISCUSSION

Members of three generations of family A and one member of the unrelated family B have suffered from a peripheral sensori-motor neuropathy. In

FIG. 10. Case 3. Graphical analysis of fibres teased from sural nerve. The very wide scatter of points is due to demyelination, axonal degeneration and healing.

family A it appeared to be inherited perhaps by an autosomal dominant gene with variable expression. It is not clear yet whether Case 3 is the only affected member of family B, or whether one of his children, whom we have not examined personally, also has a neuropathy. Although the main clinical signs were in the motor system, there were also sensory disturbances, and pathological lesions were demonstrated in both motor and sensory nerves in the muscle and nerve biopsies.

The nosological position of this disorder is not certain, so we suggest it be given the description of 'globular neuropathy' until its relationship with other neuropathies has been clarified. Clinically it appears most closely to resemble the variety of peroneal muscular atrophy with sensory loss as described, for example, by England and Denny-Brown (1952), although with more prominent hypoaesthesia. The extent of motor involvement and the lesser degree of damage to the sensory systems make globular neuropathy (GN) unlike any of the variants of hereditary sensory neuropathy including the forms with motor signs described by Alajouanine, Nick, Contamin, Cathala, Nicolle, and Penther (1962). Dyck, Gutrecht, Bastron, Karnes, and Dale (1968) have teased fibres from the sural nerve of a 51-year-old man (their Case 73-67) who had had a peripheral neuropathy for five years. The nerve fibres showed active and healed Schwann cell damage and some remnant clumps of osmiophilic debris attached to the myelin sheaths. Insufficient information is given to show definitely the relationship between their

case and GN which it resembles superficially, but they may not be the same disease because of the relatively short duration of the illness in their patient and because the illustrations of teased fibres (their Fig. 7) show only a few small masses of the myelin debris such as may be found in any active demyelinating neuropathy. We have not seen histological appearances like those of GN in sural nerve biopsies from nine other cases of peripheral neuropathies which have persisted for more than three years, although occasional nerve fibres teased from every case have had a few attached osmiophilic clumps like those shown by Dyck *et al.* (1968).

The pathological features of GN appear unique and distinguish it from other hereditary neuropathies so far described. The basic pathological lesions in GN in the sural nerves of both patients from whom adequate biopsies were obtained, appeared to be axonal degeneration and regeneration as shown by occasional degenerating nerve fibres, the excessive number of fine fibres and the many axons bearing inappropriately short myelin segments, and segmental demyelination shown in 'teased' fibres as internodes devoid of any stainable myelin sheath. The majority of naked segments were very short suggesting that they were on fibres which had regenerated after axonal degeneration. Some fibres carried both internodes of normal length and the short segments of healed demyelination implying that Schwann cell damage may occur independently of lesions to the axis cylinders which they envelop.

The multi-lamellated, myelin-like structure of the globules suggests that they consist of complex phospholipids and this is supported by their staining reactions. The electron microscope evidence, which showed apparent continuity of globules and normal myelin sheath, suggests two possible ways at least in which they could arise. Firstly, as the globules appear to be breakdown products of myelin there might be a defect in the early stages of its degradation. No numerical increase was seen in structures with the morphological appearance of lysosomes which are involved in myelin degradation in other demyelinating diseases of peripheral nerves (Weller, 1965; Weller and Mellick, 1966), but it is still possible that the enzymatic degradation of myelin is abnormal. Or, perhaps the myelin being broken down has an abnormal composition which slows its removal.

The concurrence of segmental de- and remyelination in conditions of chronic axonal degeneration has been reviewed by Dayan, Graveson, Illis, and Robinson (in preparation). They concluded that in such disorders there is circumstantial evidence of an immune reaction against myelin-Schwann cell

FIG. 11. Case 3. Sural nerve. Electron micrographs.
a. Transverse section showing loss of myelinated nerve fibres, endoneurial fibrosis and one fibre partly enveloped by a globule (approximately $\times 1,200$).

b. Laminated debris are partly enclosed by the normal myelin sheath which forms decompacted loops at the node of Ranvier on the left. The axis cylinder appears normal ($\times 8,000$).

c. Globule lying within myelin sheath ($\times 7,500$). Insert shows myelin-like lamination of globule at higher magnification ($\times 50,800$).

sheaths by the chronic degeneration of nerve fibres. If this hypothesis is applicable to GN, the metabolic defect in this disease, either sporadic or inherited in its expression, has at least two effects on nerve fibres because it appears to have caused both destruction of axis cylinders and the defective removal of myelin debris which has resulted in the formation of 'globules'.

The three patients described seem to be examples of a disease of the peripheral nervous system in which both axons and their Schwann cell-myelin sheaths are damaged. Such effects of disease are perhaps more commonly recognized in the central nervous system where they sometimes appear to be due to a single genetic metabolic defect. It will be of great theoretical and practical interest if a comparable enzymatic failure can be found also in globular neuropathy.

SUMMARY

Members of three generations of one family and one member of another suffered from a progressive peripheral sensorimotor neuropathy.

Study of sural nerve biopsies from two cases showed axonal degeneration, segmental de- and remyelination and incorporation of bizarre globules of complex phospholipids into the myelin sheaths.

It is suggested tentatively that the families have a metabolic disorder, possibly inherited, which has produced chronic axonal degeneration and damage to myelin sheaths. There also appears to be either defective degradation of myelin by the Schwann cells of demyelinating segments or an abnormal myelin formed initially may resist breakdown along the usual paths.

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