Thalidomide neuropathy: a clinical, electrophysiological, and histological follow-up study

PAMELA M. FULLERTON1 AND D. J. O'SULLIVAN2

From the Middlesex Hospital and The Institute of Neurology, Queen Square, London

In 1960 it became apparent that patients who had taken thalidomide regularly as a nocturnal sedative for some months might develop peripheral neuropathy (Florence, 1960; Kuenssberg, Simpson, and Stanton, 1961; Frenkel, 1961; Fullerton and Kremer, 1961; Raffauf, 1961; Scheid, Wieck, Stammler, Kladetzky, and Gibbels, 1961). In affected subjects sensory symptoms predominated; weakness was usually mild and involved proximal rather than distal muscles. Half the cases described by Frenkel (1961) and by Fullerton and Kremer (1961) had, in addition, evidence of mild pyramidal tract damage.

In 1961, Fullerton and Kremer reported that weakness improved rapidly when patients stopped taking thalidomide, but little improvement in sensation occurred—at least, during the first six months. Kremer (1965) later expressed doubt as to whether recovery of sensation ever occurs. In a four to five year clinical follow-up of 71 patients in Sweden, Hafström (1967) also found that weakness recovered, but in 55 of the patients there was no improvement in sensation, and the remainder had some residual sensory symptoms. Simpson (1963) and Hafström (1967) have both suggested that occasionally the condition may continue to progress after withdrawal of the drug.

Eleven of the 13 patients described by Fullerton and Kremer (1961) and 11 other patients have been seen at intervals for between four and six years after stopping thalidomide. Their clinical, electrophysiological, and histological findings are presented below.

METHODS

ELECTROPHYSIOLOGY Since motor conduction velocity appeared to be unaffected in the previous study (Fullerton and Kremer, 1961), this was not re-examined at follow-up. The electrophysiological study was, therefore, restricted to sensory nerves. Digital nerve action potentials were recorded through saddle electrodes over the median nerve at the wrist, following stimulation of the nerves of the index finger with ring electrodes, as described by Dawson (1956).

HISTOLOGY Biopsies were taken from the sural nerve at the level of the lateral malleolus at the ankle in six patients between two and six years after stopping thalidomide. In most cases only a fascicular biopsy was performed. The specimen was divided into two parts and each was stretched on a card frame.

The first portion was fixed in Flemming’s solution, after which 5 μ transverse sections were stained with Kultschitsky’s haematoxylin as described by Gutmann and Sanders (1943). All sections were photographed at a magnification of x 250 and enlarged to a magnification of x 500 for the estimation of fibre density. Selected bundles from each nerve were photographed at an enlargement of x 1,000 and from these diameters of approximately 1,000 fibres were measured. Details of photography, of the method of fibre counting, and of the measurement of fascicular area were similar to those described by Swallow (1966) and by O’Sullivan and Swallow (1968). In the preparation of histograms all fibres with a diameter of 2 μ or less were grouped together. Larger fibres were subdivided into 1 μ groups. Fibre density for each size group was then calculated.

The second portion of each nerve was fixed in 10% formol-saline. Part of this was stained with 1% osmium tetroxide and single fibres were isolated by the method described by Thomas (1955). The remainder was processed and transverse sections stained with haematoxylin and eosin.

RESULTS

CLINICAL FEATURES Eleven of the patients reported here were previously described by Fullerton and Kremer (1961). A further 11 patients were seen during the second half of 1961, after the original report had been prepared. Most of the patients were seen at six monthly or yearly intervals during the follow-up period. When the patients were first seen, the most distressing symptoms were painful paraesthesiae of the feet and hands, which were present in all cases. Muscle cramps were common, but not usually as distressing as the paraesthesiae. Actual muscle weakness occurred in a minority only. When present, weakness was usually mild; it was
also later in onset than the paraesthesiae and tended to affect proximal muscles. In the most severely affected patients, examination revealed some muscle wasting in addition to proximal weakness. Ankle jerks were often depressed or absent, but in a proportion of patients other reflexes were increased and plantar responses extensor. On general examination, a number of patients were found to have developed brittle finger nails; reddening of the palms was another occasional finding. The incidence of these abnormalities in the 22 patients is shown in Table I.

Only one patient had taken thalidomide regularly for less than a year. This patient, who reported that he had taken 100 mg nightly for five months, was only mildly affected when first seen. The remaining 21 patients reported taking thalidomide regularly for periods of 12 to 36 months, the mean duration being 22 months. In the previous report by Fullerton and Kremer (1961) it was suggested that the total duration of dosage was related to the severity of the neuropathy. However, in the present larger series of 21 patients who took thalidomide for 12 months or more, no such correlation was apparent. Thus, the mean duration for which the drug had been taken in the four severely affected patients was 24 months; in the five mildly affected patients the corresponding duration was 22 months. But the size of the nocturnal dose—and thus the total dosage taken—did appear to be a factor in determining the severity of the resulting neuropathy (Table II). For example, all four patients who were severely affected had taken more than the usual dose of 100 mg nightly, and the patient with the most extensive sensory loss had taken 600 mg nightly. By contrast, four of the five mildly affected patients who had taken thalidomide for a year or more had received 100 mg nightly and the fifth patient had taken only 50 mg nightly.

The incidence of abnormal clinical features when the patients were first examined compared with those at the time of the final follow-up examination four to six years later is shown in Table I. It can be seen that in 10 out of the 22 patients sensory loss accompanied by distressing paraesthesiae continued unchanged. The sensory disturbance improved in approximately a quarter of the patients and the remaining quarter recovered completely. The patient who had taken thalidomide for only five months and had very mild symptoms recovered three months after stopping the drug. In the remaining patients a notable feature has been the slow tempo of recovery. Six patients improved gradually over a period of four years from the time of stopping thalidomide. Two other patients were still improving after six years. Three patients did not begin to improve until three years after drug withdrawal, and two of them were still improving two years later.

During the first few years after drug withdrawal a number of patients complained of an increase in severity of their sensory symptoms which led to the impression that the underlying neuropathy might have worsened. However, the distribution of paraesthesiae did not alter and there was no objective increase in sensory loss. The complaint of increase in paraesthesiae in the early part of the follow-up period did not appear to affect the outcome, the final state of these patients being similar to those who did not complain of such symptoms.

In five patients with slight muscle weakness, power had recovered completely after one to three years. The most severely paralysed patient (Case 2 of Fullerton and Kremer, 1961) initially improved rapidly. She had been unable to walk at the time the drug was stopped and had marked wasting and weakness of proximal muscles. Within a few months

## Table I

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Previously abnormal (no.)</th>
<th>Recovered</th>
<th>Improved</th>
<th>Unchanged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory disturbance</td>
<td>22</td>
<td>5</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Cramp</td>
<td>16</td>
<td>5</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Weakness</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Pyramidal signs</td>
<td>7</td>
<td>6</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Depressed or absent</td>
<td>9</td>
<td>3</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>ankle jerks</td>
<td>9</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Brittle nails</td>
<td>6</td>
<td>3</td>
<td>—</td>
<td>3</td>
</tr>
</tbody>
</table>

## Table II

<table>
<thead>
<tr>
<th>Severity of initial sensory loss</th>
<th>Patients (no.)</th>
<th>Mean duration of dosage (months)</th>
<th>Mean total dose (g)</th>
<th>Recovered</th>
<th>Improved</th>
<th>Unchanged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>6</td>
<td>19</td>
<td>54</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>12</td>
<td>23</td>
<td>88</td>
<td>1</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Severe</td>
<td>4</td>
<td>24</td>
<td>182</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Mild: sensory loss affecting fingers and feet only. Moderate: sensory loss extending to wrists or above ankle but not above knee. Severe: sensory loss above wrists or knees.

*These figures are based on the patients' own accounts and must be regarded as approximations only.
there was only mild residual weakness but full motor recovery had not occurred six years later.

It can be seen from Table I that pyramidal signs disappeared in all except one patient, who continued to have extensor plantar responses. Ankle jerks were initially absent in three patients and did not return in any of them. However, the ankle jerks did return to normal in three of the six patients in whom they were previously sluggish.

Nine patients had brittle finger nails and in six of them their nails returned to normal. Three patients who initially had red palms lost this sign. One patient developed diabetes. Her neuropathy failed to improve during the follow-up period, but it is not possible to say whether her diabetes might be responsible for this.

Table III shows the prognosis of the neuropathy in patients of different age groups. The numbers are too small to show any definite correlation with age, but there is a suggestion that the over 60 age-group did worse than patients aged less than 60 years. Three patients were aged 70 years or over and none of them improved. From Table II it can be seen that the initial severity of sensory loss did not appear to affect the likelihood of recovery.

NERVE CONDUCTION STUDIES The commonest abnormality to be found when patients were first investigated was a reduction in amplitude or absence of ascending nerve action potentials. Potentials with a reduced amplitude did not show any increase in latency. This suggests that conduction was normal in surviving fibres. Motor conduction velocity was also normal, even in nerves supplying weak muscles (Fullerton and Kremer, 1961).

As a standard examination at follow-up, the sensory action potential was recorded from the median nerve at the wrist after stimulation of the index finger. The measurement was made in 19 of the 22 patients at the time of the last follow-up examination. This had been done previously in eight of the patients at their first examination. In four of them, the amplitude of the action potential had been reduced and in the other four it had been absent. In only two patients did amplitude increase during the follow-up period, one of them having recovered clinically and the other having improved. The action potential was unchanged in two patients who recovered clinically and in four patients who did not.

In Table IV, the action potential amplitude at the final examination in 19 patients is compared with the severity of residual symptoms and signs. It can be seen that the action potential was markedly reduced or absent in nine patients with moderate or severe persistent clinical abnormalities. Mean action potential amplitude was higher in six patients with mild residual signs, but values below the normal range were obtained in four out of six cases. In four patients who had recovered clinically, mean action potential amplitude was slightly higher than in the previous group, but in only one patient did the value fall within the normal range.

### TABLE IV

SENSORY NERVE ACTION POTENTIAL AMPLITUDE RECORDED FROM MEDIAN NERVE AT FOLLOW-UP EXAMINATION IN 19 PATIENTS

<table>
<thead>
<tr>
<th>Sensory signs at follow-up examination*</th>
<th>Nil</th>
<th>Mild</th>
<th>Moderate and Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>4</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Average action potential amplitude ($\mu$V)$^\dagger$</td>
<td>8.2</td>
<td>6.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Individual action</td>
<td>15, 7, 7, 13, 10, 5</td>
<td>5, 4, 2, 2, 0, 0</td>
<td></td>
</tr>
<tr>
<td>potential amplitude ($\mu$V)</td>
<td>4.3</td>
<td>3.3</td>
<td>0.0, 0.0, 0.0</td>
</tr>
</tbody>
</table>

*Graded as in Table III.

$^\dagger$Normal range: 9 to 45 $\mu$V (Gilliatt and Sears, 1958).

PATHOLOGICAL FINDINGS Single nerve fibres from each of the six sural nerves were isolated and examined. Although quantitative assessment of the distribution of fibre size is difficult by this method, it was obvious during the preparation of single fibres that there were very few large diameter fibres in the specimens. No ovoids of degenerating myelin were seen, but this is not surprising since none of the patients had taken thalidomide for at least two years before the nerves were examined. Evidence of previous selective myelin damage was sought. This can be recognized by the presence of short remyelinated segments interspersed with longer normal segments. Occasional single short intercalated segments were seen in four of the six nerves and, less frequently, remyelination occurring over the length of a complete internode, indicating previous demyelination of a whole segment. However, these findings were rare and similar mild changes have been described in nerves from control subjects of similar ages (Lascelles and Thomas, 1966). There was, thus, no evidence that thalidomide had produced segmental demyelination to a significant extent.
Further information was obtained from transverse sections of nerves stained for myelin. Fibre density and fibre diameter measurements were made and compared with control values for healthy subjects studied by O'Sullivan and Swallow (1968) using the same technique. Figure 1 shows a transverse section of the nerve of Mrs. A. compared with a nerve from a control subject of the same age. There are obviously fewer large diameter fibres in the pathological nerve, although the total myelinated fibre density (5·23 thousand fibres/sq. mm) is within the control range.

In Fig. 2 histograms of fibre diameter are shown for the six thalidomide nerves and compared with the mean histograms for eight control subjects under the age of 60 and eight control subjects aged 60 years and over. The two control histograms were constructed from the data given by O'Sullivan and Swallow (1968). In both control groups the distribution of fibre diameter is bimodal, but it can be seen that the large fibre peak has been lost in all the pathological nerves and that there is a marked reduction in the number of fibres greater than 6μ. In contrast to this, the small fibre peak is preserved and in two cases apparently increased (Mrs. A and Mrs. F.).

A change in fibre density could reflect either an alteration in fibre number or a change in fascicular area. The area of each fascicle for the control and thalidomide nerves are, therefore, shown in Fig. 3 in relation to the subject's age. There is no obvious difference between the two groups. The mean fascicular area for the control nerves is 0·099 sq. mm (S.D. ± 0·069), and for the thalidomide nerves is 0·089 sq. mm (S.D. ± 0·063). The difference between the two means is not statistically significant.

In Fig. 4 fibre density, expressed as thousand fibres/sq. mm is plotted against the subject's age. In Fig. 4a it can be seen that, for the pathological nerves, total fibre density falls in the lower part of the control range or below it. There is, however, a marked reduction in density in only one nerve (Mrs. S.). On the other hand the density of 2·6μ fibres is not decreased in any of the nerves (Fig. 4b), but is actually increased in two of them. In the absence of a significant change in fascicular area, the marked increase in small fibre density in these two cases (Mrs. A. and Mrs. F.) strongly suggests regeneration.

These pathological findings will now be considered in relation to the clinical and electrical follow-up on the individual patients (Table V). Mrs. S. had taken large doses of thalidomide (600 mg nightly) for 16 months and was one of the most severely affected patients in the series. At the

FIG. 1. T. S. sural nerve stained for myelin. a. From control subject. b. From thalidomide patient (Mrs. A.). Scale 100μ.
Thalidomide neuropathy: a clinical, electrophysiological, and histological follow-up study

FIG. 2. Histograms of fibre diameter for the sural nerves of six thalidomide patients. Control histograms are for eight sural nerves in each age group calculated from the data of O’Sullivan and Swallow (1968).

At the time of stopping the drug she had extensive sensory changes affecting the whole of both legs, and the fingers of both hands, and also some proximal limb weakness. The weakness gradually recovered, but there was no change in sensation for three years. However, during the 18 months before biopsy she had begun to improve slightly. Unfortunately, the patient’s paraesthesiae were such that sensory nerve conduction tests were unsatisfactory. Histological examination demonstrated that total fibre density was reduced to a greater extent than in any of the other nerves examined. This may be related to the severity of clinical involvement.

Mrs. L. was only mildly affected while taking thalidomide; despite this, she did not improve during the five year follow-up period, but continued to complain of paraesthesiae and cramps, and to show a mild sensory deficit in the hands and feet. The nerve action potentials recorded from the median nerve at the last examination were within the normal range, but it can be seen from Fig. 2 that the large diameter peak in the histogram of the sural nerve was considerably reduced, whereas the small fibre peak was preserved. The difference between the electrical and histological findings may be related to the fact that the patient’s symptoms and signs were predominantly in the lower limbs, whereas the electrical tests were done on the upper limb.

Mrs. A. had severe sensory symptoms when she stopped taking thalidomide in 1961. She continued to complain of unremitting paraesthesiae for the next three years and there was no change in her neurological signs. In 1964, no sensory nerve action potential could be recorded from the median nerve at the wrist. At this time the sural nerve biopsy was carried out (Fig. 1). There were very few large

FIG. 3. Area of individual fascicles of sural nerves in relation to age. ○ = 27 control nerves studied by O’Sullivan and Swallow (1968). ● = six thalidomide nerves.
Mrs. F. had a relatively severe sensory disturbance while taking thalidomide. Sural nerve biopsy two years after stopping the drug showed a marked increase in the number of small diameter fibres suggesting that regeneration was occurring. However, her sensory symptoms and signs had not changed at the time of biopsy, nor was there any improvement during a further three year follow-up period. No sensory nerve action potential could be recorded from the median nerve either at the time of biopsy or at the final follow-up examination.

Mr. H. was initially severely affected and his symptoms and signs remained unchanged throughout the follow-up period of six years. No sensory nerve action potential could be recorded at the last examination. The sural nerve contained very few large diameter fibres and no increase in small diameter fibres. There was, thus, no clinical, electrical, or histological evidence of recovery in this patient.

Mrs. P. showed essentially the same features as Mr. H. She was clinically severely affected and did not improve. Only a small sensory nerve action potential could be recorded at follow-up examination. There was no histological evidence of regeneration.

Summarizing the findings in these six patients, clinical improvement occurred only in two of them (Mrs. S. and Mrs. A.), although the histological findings also suggested regeneration in a third patient (Mrs. F.). The remaining three patients showed no clinical or histological evidence of regeneration.

**DISCUSSION**

Thalidomide (γ-phthalimidoglutarimide) was recognized to have peculiar teratogenic properties in 1961, and, as a result of this, there has subsequently been considerable interest in its metabolism. Much is now known about its mode of breakdown and distribution in various animals (Schumacher, Smith, and Williams, 1965a and b; Fabro, Smith, and Williams, 1967a and b). Because part of the thalidomide molecule is related to glutamic acid, it has been suggested that its toxicity depends on interference with normal glutamate metabolism. However, results of experiments to test this hypothesis—at least as far as the embryopathy in animals is concerned—suggest that the toxicity is not primarily due to such an action (Fabro, Schumacher, Smith, and Williams, 1964; Hirschberg, Osmos, Bryant, and Ullmann, 1964; Fabro, Schumacher, Smith, Stagg, and Williams, 1965a; McColl, Globus, and Robinson, 1965). Fabro et al. (1964) have demonstrated that an N-substituted phthalimide structure is essential for the embryopathy, and later...
suggested that interaction with diamines might be biologically important (Fabro, Smith, and Williams, 1965b), but, as yet, there is no experimental evidence to support this interesting suggestion.

Even less is known about the biochemical basis for thalidomide neuropathy. Experimental work has been hampered by the difficulty in producing the neuropathy in animals. Diezel (1963) produced some morphological changes in posterior columns, spinal ganglion cells, and posterior roots of dogs given thalidomide for over a year, and Klinghardt (1966) was able to produce peripheral nerve degeneration in rats and mice by giving high doses of the drug for long periods. Other workers have been less successful in producing neuropathy in a wide variety of species (Staemmler and Lagler, 1965).

The incidence of neuropathy among patients who took thalidomide is unknown, although at one time the manufacturers of Distaval suggested an incidence of 0.5% in patients who had taken the drug for two months or more. Data from the present study suggest that individual idiosyncrasy to the drug is unlikely to be important, since the severity of the neurological disturbance appears to be related to the dose and amount of drug taken.

There have been few opportunities for pathological study of the neurological changes in man. However, Klinghardt (1965, 1966) has been able to study necropsy material from two patients who suffered from thalidomide neuropathy. In the peripheral nerves there was degeneration of both axis cylinders and myelin sheaths. Dorsal root ganglion cells showed pathological changes and, in addition, marked degeneration of the posterior columns of the spinal cord was demonstrated. No segmental demyelination was found in the peripheral nerves. The histological changes in the sural nerves described here were similar to those reported by Klinghardt (1966), in that no segmental demyelination was seen when single teased fibres were examined; this suggests that the pathological process is a primary neuronal or axonal degeneration.

Quantitative studies have demonstrated a selective loss of large diameter fibres in the sural nerves. The small fibre population was preserved in all the nerves, and, in two of them, the number of small diameter fibres was actually increased. This is presumably associated with attempts at regeneration.

Many of our patients have failed to improve or made only slight improvement. A possible reason for this is that irreversible degeneration of fibre tracts in the central nervous system had occurred. It has been mentioned that Klinghardt found fibres in the posterior columns of the spinal cord to be severely affected. However, this seems unlikely to be the sole explanation for the failure of recovery in our patients. There is both electrophysiological and histological evidence of failure of recovery of the peripheral parts of the sensory fibres. In many cases sensory nerve action potentials remained absent throughout the follow-up period, whereas they would be expected to return if regeneration of peripheral nerves had taken place. In the six patients in whom we were able to study sural nerve biopsies, regeneration was incomplete in that the content of large myelinated fibres remained low, although the
specimens were taken two to six years after stopping thalidomide. After this interval one might have expected not only regeneration, but also maturation of the regenerated fibres with reappearance of the large fibre peak in the histogram. This would certainly be expected after Wallerian degeneration due to nerve crush in lower animals. In the rabbit, for example, Cragg and Thomas (1964) showed that the diameter of the largest fibres recovered to within 10-15% of normal one year after a crush.

If failure of clinical recovery is to be attributed at least partly to persistent peripheral nerve abnormalities, the reasons for this must be considered. In many of the toxic neuropathies in which primary axonal degeneration occurs, a dying back process has been demonstrated whereby the greatest damage occurs at the peripheral ends of the longest fibres. This has been demonstrated following tri-ortho-cresyl phosphate (Cavanagh, 1964) and acrylamide (Fullerton and Barnes, 1966) poisoning in experimental animals. When degeneration is limited to the distal ends of the fibres, regeneration with rapid functional recovery might be expected. If, on the other hand, the neurone itself is irreversibly damaged, regeneration may never occur; if retrograde degeneration reaches as far back as the cell body, recovery will be slow and perhaps incomplete. A mechanism of this sort could explain the long delay in clinical recovery that was found in some patients and particularly in Mrs. A., who recovered rapidly after failing to improve for three years.

SUMMARY

Twenty-two patients suffering from thalidomide neuropathy have been followed for four to six years after stopping the drug. Symptoms and signs are unchanged in approximately 50%; have improved in a quarter, and the remainder have recovered. Improvement was usually slow and, in some patients, did not begin for three years.

Sensory nerve action potentials were recorded and found to be closely related to the clinical state.

Sural nerve biopsies on six patients, performed two to six years after stopping thalidomide, showed selective loss of large diameter fibres. There was no segmental demyelination. In two of the nerves a marked increase in number of small fibres was taken to indicate regeneration.

We would like to thank Dr. Michael Kremer for his advice and help with following up patients; Professor R. W. Gilliatt for helpful criticism; Dr. R. G. Lascelles for preparation of single fibres from three of the patients; Mr. W. F. Hinkes and Miss G. Brann for technical assistance. Histological facilities in the University Department of Clinical Neurology at Queen Square were provided by the generosity of the National Fund for Research into Crippling Diseases.

ADDENDUM

Since this manuscript was submitted for publication, our attention has been drawn to a recently published monograph describing the clinical features and prognosis in 114 patients with thalidomide neuropathy (Gibbels, 1968).

REFERENCES


Thalidomide neuropathy: a clinical, electrophysiological, and histological follow-up study


