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QUANTITATIVE AUTOMATED ANALYSIS OF SURAL NERVE BIOPSIES FROM PATIENTS WITH URAEMIC POLYNEUROPATHY

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ABSTRACT

A controlled automatic method of human cross section analysis was used to study myelinated fibres in sural nerves. The biopsies were performed on 8 uraemic patients with polyneuropathy and 3 controls. The microscopic image was transmitted by a Plumbicon television camera to a Cambridge Instruments image analyser (QTT 720) connected to a digital computer PDP 11/34. The measured variables included fibre density, the histograms of fibre diameters and myelin sheath thickness. Rapid and precise results were obtained by this method. They demonstrated axonal loss in uraemic patients and suggested demyelination in one patient. The method used in association with single fibre studies allowed us to demonstrate different clinico-pathologic patterns of uraemic polyneuropathy, including an acute axonal degeneration with secondary demyelination or a predominantly demyelinating neuropathy associated with slight axonal pathology.

INTRODUCTION

In order to facilitate morphometric studies of myelinated fibres (MF), different techniques of automation were performed (SCHROEDER, 1975 ; ZIMMERMAN et all., 1980 ; ELLIS et all., 1980 ; SAVY et all., 1981). A quick automated method of human nerve fibre cross section analysis was proposed in a previous study (SELVA et all., 1981).

This program was used to study morphological abnormalities of sural nerve biopsy specimens from 7 uraemic patients who had developed polyneuropathy.

PATIENTS AND METHODS

Patients. Nerve biopsy was performed in 7 uraemic patients

and 3 controls. Patients 1-2-3 presented with an acute polyneuropathy at the beginning of the dialysis. Patients 4-5-6-7-8 developed a subacute or chronic polyneuropathy in spite of the dialysis (hemodialysis for patients 4-5-6-8 and ambulatory peritoneal dialysis for patient 7). The controls were 3 adult patients with muscular dystrophy. More controls are presently under study.

Methods. The nerve specimens were taken under local anesthesia and fixed in a 3.6 % solution of glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 and an osmolarity of 360 mmol. They were then dehydrated in ethanol and embedded in Epon. One micron thick cross sections were stained with 1 % thionine. The cross sections of embedded fascicles were studied using a controlled automatic method.

The microscopic image (objective x 100 under oil) was transmitted by a Plumbicon television camera (720 lines) to a Cambridge Instruments image analyser (QTM 720) which was connected to a digital computer (PDP 11/34). The image was systematically scanned. The density of MF per mm^2 of endoneurial area, the fiber diameters and the myelin sheaths thickness were determined automatically. The image digitized and then binarized by thresholding. The hardware analysis was based on the fact that myelin sheaths were rings centered by a "hole" (the axon) which was always larger than 3 picture points in our system. The software analysis was essentially a parametric selection according to the fibre diameter. The mean diameter, Dm was calculated from the Feret diameters : $Dm = (D_0 + D_{90})/2$. Myelin sheath thickness was calculated according to the formula $E = Dm/2 - \sqrt{Dm^2/4 - A/\pi}$. Where A is the myelin area. Each fibre selected was automatically marked by a cross on the visual display to aid the observer in identifying errors.

There were 3 types of errors :

Fibre omission. A fibre on the specimen was not selected by the program.

Fibre construction. Non fibre on the specimen but the program selected something else as a fibre.

Fibre clustering. 2 or sometimes 3 individual fibres were in close contact and the program analysed the cluster as a single fibre. The first two factors were due to a problem of thresholding. Some small fibres were too lightly stained to be detected while more deeply stained elements such as nuclei were picked up. The improvement of preparation techniques is expected to solve this problem. The segmentation of clusters will need a sofware analysis. When the number of errors was too high (patients 2-3), the observer could cancel the first

trial and do manual corrections of the image with the light pen before another automated analysis. It was possible to separate clustered fibers in this way and to delete some elements (nuclei for example). All the measurements were performed on samples of 300 to 400 fibres.

RESULTS

Fig. 1 shows the histograms of fibre diameters corresponding to the 7 patients and 3 cumulated controls. Axonal loss was demonstrated in patients 1-2-3-4-5-7. It predominated on large MF in patients 1-2-3-4. In patients with an acute neuropathy (1-2-3) the fibre density was very low (30 ± 18 % of controls). It was higher in the other patients (74 ± 18 % of controls). In patient 8 the overall density of myelinated fibres was found to be normal but the incidence of small diameter (less than 8 microns) fibres was much higher (73.3 %) than in controls (60.4 %) while the iregenerating fibres have a small caliber, this finding suggests a regenerating procers after degeneration.

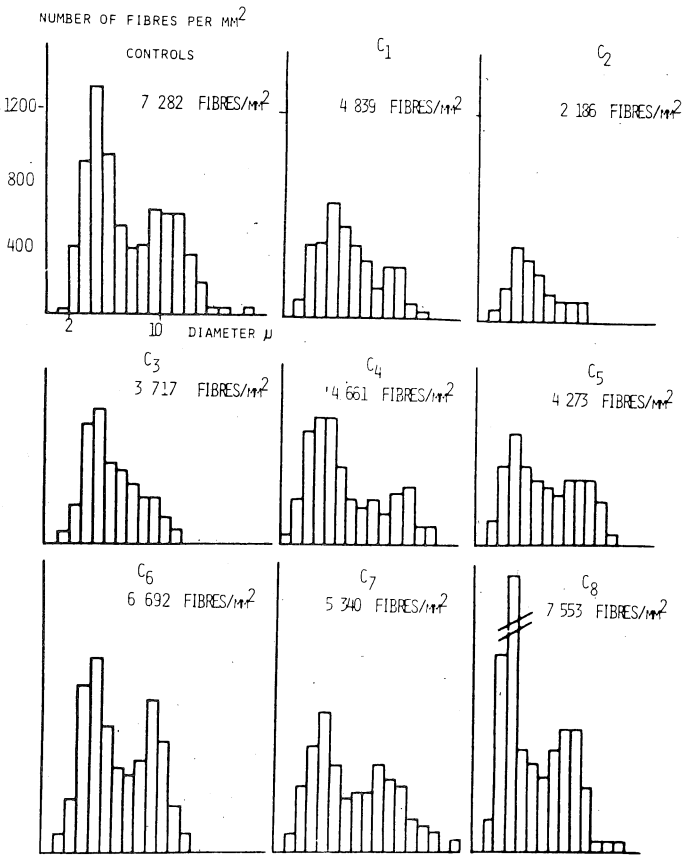

Fig. 1 : Histograms of external diameters.

Fig. 2 shows histograms of the thickness of the myelin sheaths. Their profiles seem similar to the diameter histograms. The coefficient of correlation between diameter and myelin thickness was very high (extreme values of 0.84 and 0.91 for the controls ; 0.63 and 0.89 for the patients). For patient 1 the comparison of the myelin thickness histograms with the histograms of the fibre external diameters shows that the loss of myelin sheaths which are thicker than 1.75 μ is not explained by the same loss of fibres of large caliber. These results suggest demyelination for patient 1.

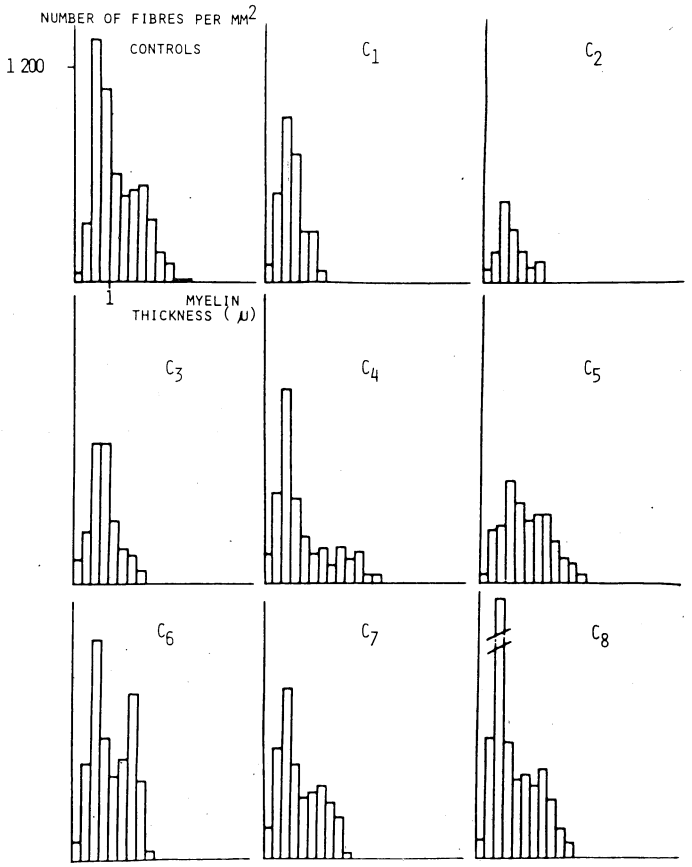

Fig. 2 : Histograms of the thickness of the myelin sheaths.

DISCUSSION

Our observations demonstrate pathological changes consistent with an acute axonal neuropathy in patients 1-2-3. They also suggest a demyelinating process for patient 1. An axonal loss is also demonstrated in most of our patients with a chronic neuropathy. All these results are consistent with the literature (DYCK et all., 1971 ; THOMAS et all., 1971).

Single fibre studies performed in our patients confirmed severe demyelination of fibres in patient 1. These two methods must be used in association. We could in this way demonstrate different clinico-pathologic patterns of uraemic polyneuropathy (SAID et all., 1982) including an acute axonal degeneration, a progressive axonal degeneration with secondary demyelination or a predominantly demyelinating neuropathy associated with slight axonal pathology. It is of interest to note that in patient 1 automated cross section analysis might predict demyelination. For further information we need a larger control group to be able to use statistical methods for estimation of variation between controls and for comparisons between patients and controls.

It is our conclusion that the use of the automated method showed to be helpful in giving us rapid and precise results in demonstrating axonal pathology in patients and suggesting a demyelinating process in one of them. This method however fouled to confirm remyelination of fibres demonstrated by single nerve fibre studies.

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