Latent neuropathy in diabetes and alcoholism

Electromyographic and histological study

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THE PRACTICE of muscle biopsies has revealed that both contractile and nervous elements of the motor apparatus were very sensitive to various aggressions.[1,2] This sensitivity is compensated by a remarkable power of regeneration. As far as the motor innervation is concerned, this reactive process consists in a collateral sprouting of the spared nerve fibers which results in an increase of the terminal innervation ratio and, therefore, in an extension of the motor unit territory.[3,4]

Thus, it may be assumed that the clinical manifestations of a neural aggression are the result of a conflict between regressive and regenerative forces. In acute and massive injuries, the degenerative process obviously dominates the clinical picture and results in a marked reduction of motor power. In diseases leading to chronic and partial involvement of motor neuron or peripheral nerves, the occurrence of muscular weakness can be delayed and partially overcome by the compensatory process of collateral ramification and also by the hypertrophy of the muscle fibers. This is probably the case in motor neuron diseases. It may even happen, provided the disease process is slight enough, that a satisfactory balance between regressive and regenerative changes makes the illness clinically inapparent.[2] Such a condition may be qualified as subclinical or latent neuropathy.

In fact, a good number of biopsies taken in various conditions, including febrile illness of long duration, systemic diseases, malignant tumors, and uremia, have revealed conspicuous changes of the terminal innervation pattern in patients having no symptoms of neural involvement and even in the absence of neurogenic atrophy of the muscle tissue. These changes are a mixture of mild regressive or dystrophic alterations of the terminal arborizations and intramuscular nerve fibers and increased collateral ramification of motor axons. Such a histological picture, giving a clear morphological evidence of the opposite forces acting on the nervous structures, has been considered to represent the very first stage of the latent neuropathies occasionally preceding the fascicular atrophy of the muscle fibers, which itself may precede the clinical evidence of a reduction of the motor power.[2]

The finding of subclinical alterations of intramuscular nerve fibers led us to undertake a systematic investigation of the neuromuscular function in various conditions known to involve the peripheral nervous system, with special interest to the cases in which the symptoms of polyneuropathy were very slight or absent.

The purpose of the present study is to describe the histological changes occurring in muscle tissue and terminal motor innervation in diabetic and alcoholic patients and to analyze the evolution of these changes in connection with the clinical picture. The histological features, quantitatively estimated, have been compared with the electromyographic behavior of the neuromuscular territories submitted to a biopsy.

It was expected that such a broad approach

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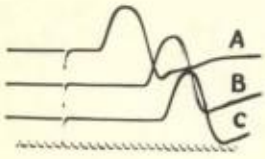


Fig. 1. Latency in evoked potentials in abductor pollicis brevis by stimulation of median nerve above wrist (A), above elbow (B), in axilla (C). Time base: 0.001 second. Case 522, group 1

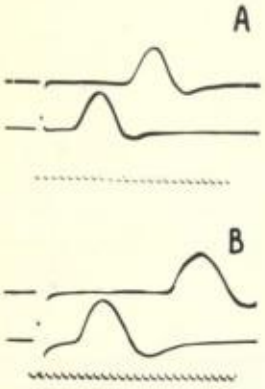


Fig. 2. Latency in evoked potentials in extensor digitorum brevis by stimulation of lateral popliteal nerve at head of fibula and above ankle. Time base: 0.001 second. Conduction velocity within normal range in case 505, group 2 (A), reduced in case 486, group 3 (B)

should provide useful information about the mechanism of the neural involvement in two very common causes of chronic polyneuropathy.

METHODS

The patients, submitted to a general clinical examination, were investigated by stimulation electromyography in a selected nervous territory, either median nerve or lateral popliteal nerve. A neuromuscular biopsy was then taken within this territory in palmaris longus or flexor carpi radialis for the median nerve and tibialis anticus for the lateral popliteal nerve. The intensity-duration curve and detection electromyography were performed in this muscle at the time of the biopsy. The contractile power of the biopsied muscle was graded according to the system proposed by the medical research council.[5]

Electrophysiological study. The nerve conduction velocity was measured by the usual method.[6] A square electrical stimulus, usually

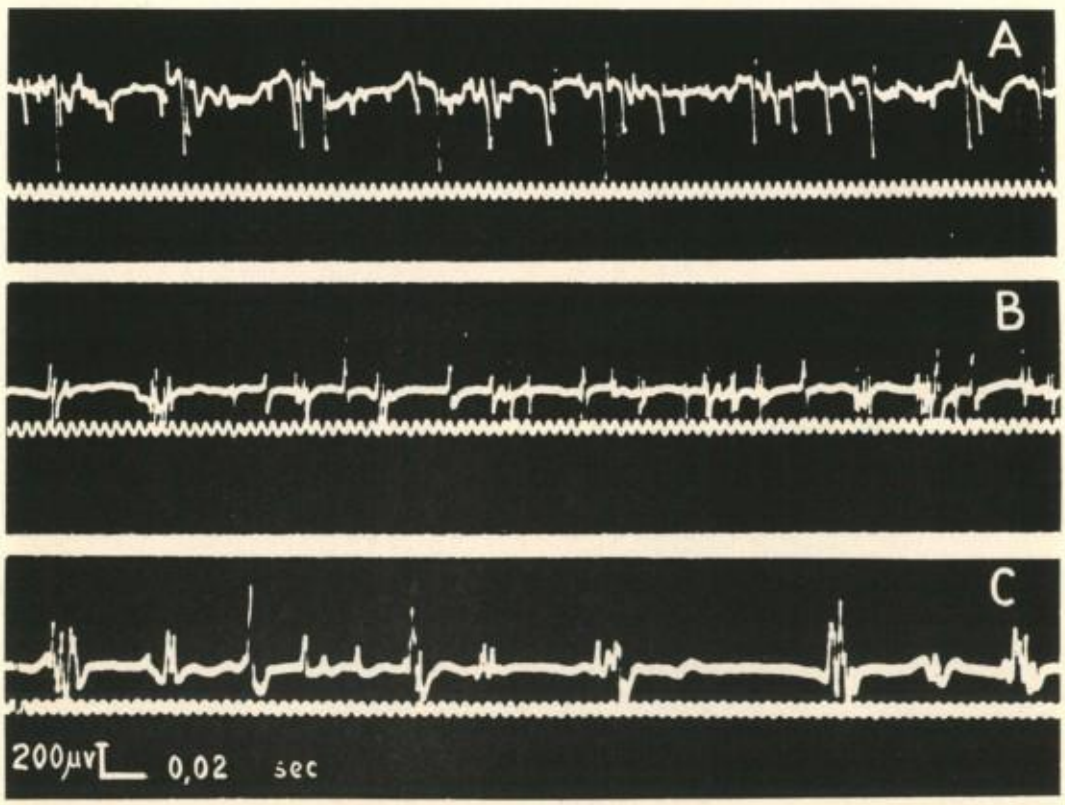


Fig. 3. Electromyographic records in moderate voluntary contraction. Normal pattern in case 550, group 2 (A). Increased number of polyphasic potentials, case 509, group 2 (B). High proportion of grouped polyphasic potentials and long-duration potentials, case 438, group 2 (C)

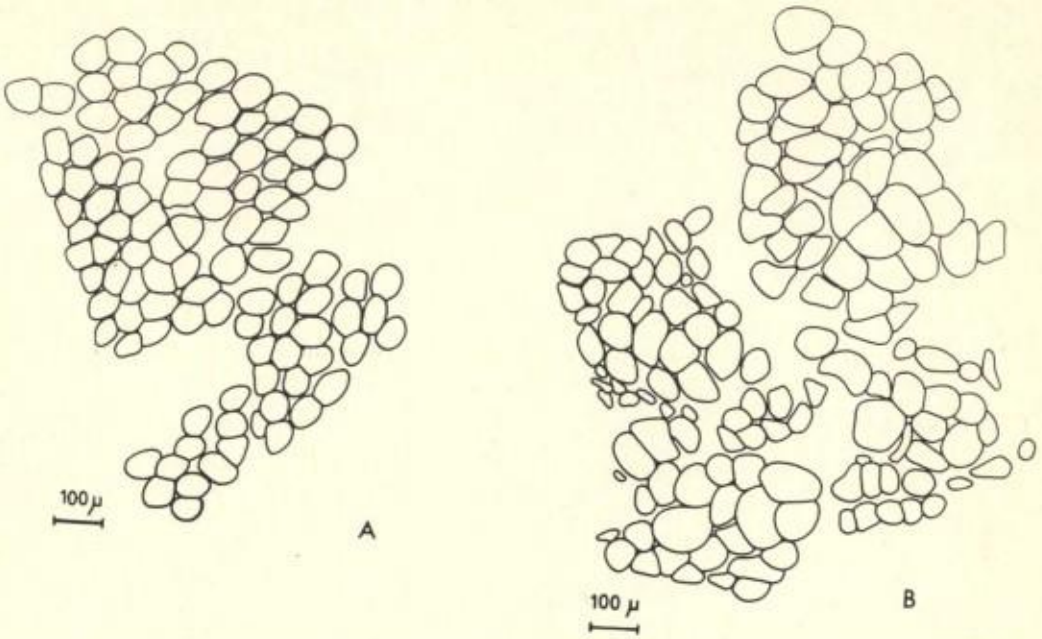


Fig. 4. Lanametric drawing of muscle fibers in transverse section used for planimetric measurements. Normal pattern (A). Single fiber atrophy and hypertrophy pattern, case 343, group 3 (B)

0.1 to 1 msec. in duration, and more than twice the threshold voltage, was delivered through the stimulating cathode at 2 or 3 points of the skin along the course of the nerve. The evoked action potential was recorded over distal muscles by silver disk electrodes. Care was taken to locate the negative electrode at the motor point of the muscle, which corresponds to the end-plate region[7] and, therefore, to the point of spreading of the muscle action potentials.[8,9] The evoked potential was displayed on a cathode ray oscilloscope triggered by the stimulator, and the latency between the onset of the stimulus artifact and the onset of the response was measured on the photographic record of the oscillographic trace (Fig. 1). The median nerve was stimulated in the axilla above the elbow and above the wrist and recorded from the extensor pollicis brevis. The conduction velocities reported in the tables concern only the elbow-wrist segment. The lateral popliteal nerve was stimulated at the head of the fibula and above the ankle and recorded from the extensor digitorum brevis (Fig. 2). According to Thomas and associates,[6] the normal values are 57.2 meters per second ± 4.2 in the median nerve and 49.7 meters per second ± 7.1 in the lateral popliteal nerve. The range of normal values obtained in our laboratory are 51.6 to 67.3 meters per second in the median nerve and 46.6 to 56.3 meters per second in the lateral popliteal nerve.[10]

The detection electromyography has been performed during the biopsy on the exposed muscle and within the fasciculi selected for

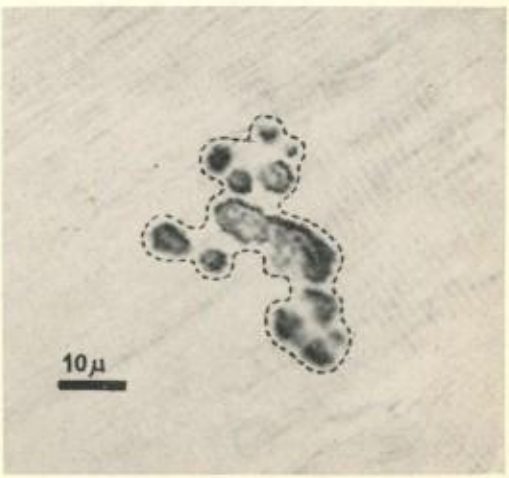


Fig. 5. Outlining of subneural apparatus. Used in measurements of synaptic surface. Case 515, group 1

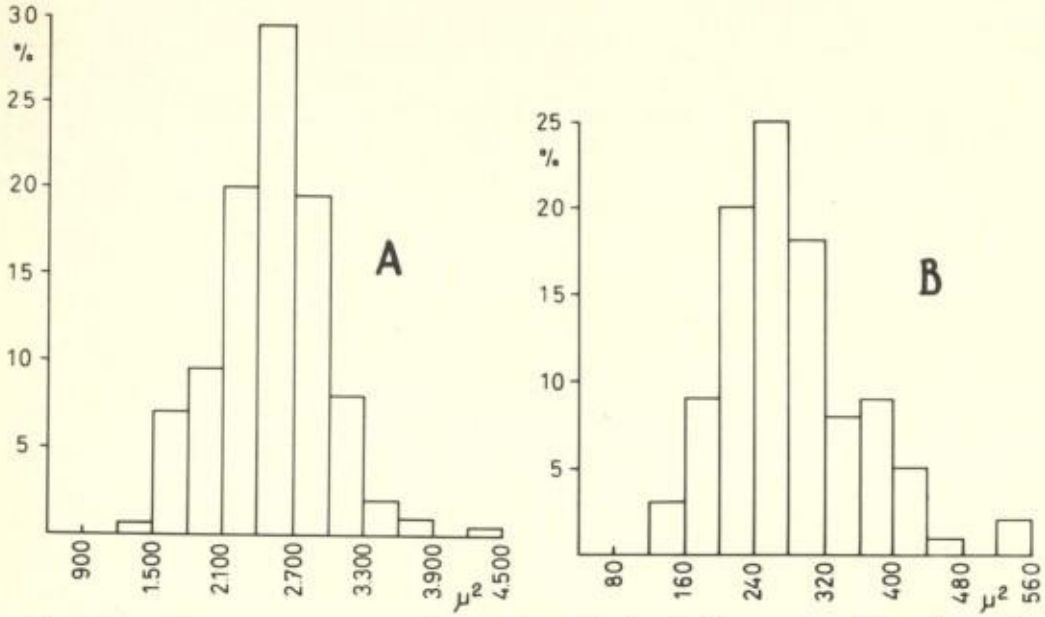


Fig. 6. Normal histograms in palmaris longus. Case 453. Muscle fiber surfaces (A); subneural apparatus surfaces (B)

sampling. A bipolar needle electrode was inserted in these fasciculi outside the region containing the end-plates zone which was to be excised. Records were taken from at least 2 regions. The analysis was performed on traces obtained at rest and during moderate voluntary contraction. We have mainly estimated the occurrence of spontaneous activity and the proportion of polyphasic potentials (more than 4 phases) and long-duration potentials (more than 12 msec.). According to Buchthal and Pinelli[11] we have considered as abnormal a proportion higher than 4%. The intensity-duration curve was established on the muscle selected for biopsy through the skin motor point, with a constant current stimulator.

Histological study. The sampling of neuromuscular biopsies was performed by electrical localization of the terminal innervation area on the exposed muscle. The intramuscular nerve fibers and endings were selectively stained by intravital methylene blue.[12] The synaptic region of the motor end plates was demonstrated by a modification of the thiocholine method of Koelle and Friedenwald for cholinesterase.[13,14] Muscle tissue was examined on longitudinal and transverse sections, after staining by hematoxylin and eosin, Masson's trichrome, and Mallory phosphotungstic acid hematoxylin.

Our material has been submitted to a quantitative histological investigation of the size of the muscle fibers and the number and situation of muscle nuclei. The modifications of the innervation pattern have been quantitatively estimated by the mean terminal innervation ratio and the measurement of the surface of the myoneural junction.

In order to obtain an objective estimation of the volumetric modifications of the muscle fibers, their size was estimated by planimetric measurement of the fiber surfaces in cross sections. According to the great variety of shape of the muscle fibers in transverse sections, the measurement of the surface is obviously more reliable than the measurement of the diameter generally used.[15] In each case the surfaces of 100 to 200 muscle fibers were measured, and the mean value and the standard deviation of the mean were established (Fig. 4). According to the asymmetry of the histograms in normal muscles (Fig. 6), the standard deviation does not accurately figure the distribution curve. However, the comparison between the arithmetic and geometric values of the mean and of the standard deviation shows that the asym-

TABLE 1
CONTROL GROUP

Case	Muscle	Muscle fibers: Mean surface μ2	σ	Range μ2	Peripheral nuclei (per fiber)	Central nuclei (%)	Innervation: TIR:1*	SNA** mean surface μ2	σ	Range μ2
90	Palm.	—	—	—	—	–	1,12	314	84	160-520
357	Palm.	2,182	517	1,200-4,200	2,7	2	—	292	87	160-620
296	Palm.	2,171	492	900-3,300	4,0	4	1,14	—	—	—
359	Palm.	2,552	554	1,200-3,900	2,4	2	1,09	—	—	—
300	Palm.	2,096	644	900-3,000	3,1	3	—	256	85	80-560
453	Palm.	2,378	595	1,200-4,500	1,1	3	1,10	269	81	120-540
321	Tib. ant.	2,201	589	1,200-3,600	2,6	3	1,08	261	90	80-520
430	Tib. ant.	2,325	662	1,200-4,500	3,0	3	1,06	397	97	80-600
428	Tib. ant.	2,459	664	1,200-3,900	2,5	4	1,26	—	—	—
185	Tib. ant.	2,338	482	1,050-4,050	3,4	2	—	276	105	100-680

*TIR = Terminal innervation ratio **SNA = subneural apparatuses σ = Standard deviation

metry of distribution is slight and that arithmetic values comprised between $\pm 1\sigma$ and $\pm 2\sigma$ give a satisfactory indication of the dispersion area.[3] It may be assumed that $\bar{x} - 2\sigma$ and $\bar{x} + 3\sigma$ in arithmetic values covers approximately 95% of the normal distribution curve. In pathological cases, we found it useful to take the standard deviation as an indication of the dispersion of individual values and, therefore, of the process of atrophy and hypertrophy of the muscle fibers. The relative importance of both processes has been estimated by the proportion of fibers, the surface of which was outside the limits of the normal values. The increased number of muscle nuclei and their central displacement are often reported in muscle biopsies and seem to represent an unspecific reaction of the muscle tissue to many kinds of aggressions. However, in the case of neural atrophy, increased density of nuclei may be more apparent than real and requires a quantitative estimation. So we found it necessary to make a careful count of peripheral and central nuclei in 100 fibers in each case on strict transverse sections, according to Greenfield and associates.[15]

The terminal innervation ratio gives an indication of the collateral ramification of the nerve fibers. It corresponds to the number of motor end plates which originate from a given number of terminal axis cylinders. The normal mean value averages 1.1 per 1 and does not exceed 1.27 per 1.[3] The surface of the myoneural junction is easily estimated by the size of the subneural apparatus[16] which in man is formed by a series of separate elements or units.[17] To figure the true synaptic area, it would have been necessary to measure the sum of the surfaces of individual units for each subneural apparatus. Now, such a figure should not have given an indication of the scatter of the units on the surface of the muscle fiber, which is an important feature in neuromuscular pathology. Thus, we have preferred to measure the area containing all the units of the subneural apparatus (Fig. 5). This value, although giving an approximate figure of the synaptic surface, represents accurately the degree of dispersion of the units and the size of the myoneural junction area. The subneural apparatuses selected for planimetric measurement were those clearly seen on frontal view

TABLE 2

Clinical data											Physiological data				
				Tendon reflexes					Studied region						
Case	Sex	Age	Diagnosis	S.L.	Inf. L. P.	Inf. L. An.	Paresthesia	Sense	Muscle	Nerve	I/T	Conduction velocity (meters per second)	Normal potential (%)	Polypotential (%)	Long-duration potential (%)	Spontaneous potential
522	M	62	A.W.	+	+	+	0	N	P.L.	M	N	43.6	100	0	0	0
515	M	38	A.W. + T.M.	+	0	0	0	N	P.L.	M	–	43.2	—	—	–	–
525	F	55	A.W. + K.	+	0	0	0	N	P.L.	M	N	46.1	94	4	0	0
505	M	84	D.	+	+	0	0	N	P.L.	M	N	—	—	—	–	–
506	F	75	D.	+	0	0	0	N	P.L.	M	N	47.8	100	0	0	0
516	M	61	D.	+	0	0	0	N	P.L.	M	N	45.8	100	0	0	0
413	F	52	D.	+	0	0	+	N	P.L.	M	N	38.0	72	28	0	0
526	F	63	D.	+	0	0	0	N	P.L.	M	N	46.1	100	0	0	0

An. = Ankle reflex
A. = Alcoholism
D. = Diabetes
Inf. L. = Inferior limb
I/T = Intensity duration curve
K. = Korsakoff psychosis
L.P. = Lateral popliteal nerve
M. = Median nerve

and well separated from each other, in order to avoid the inclusion of units belonging to neighbor subneural apparatuses. The surface of 100 subneural apparatuses was measured in each biopsy. The mean value and standard deviation were calculated as in the muscle fibers, and the proportion of individual values exceeding the normal range was noted.

In addition to the quantitative data, qualitative histological observations were recorded concerning the structure of the muscle fibers, the state of interstitial tissue, and the changes in axis cylinders and terminal arborizations.

MATERIAL

We have studied 30 patients: 13 diabetic, 15 alcoholic, and 2 both diabetic and alcoholic in an age range of 27 to 84 years. All the diabetic patients were under insulin or hypoglycemic sulfonamide derivative treatment. The duration and severity of the illness has not been taken into account. Most of the alcoholic patients had been hospitalized for one or another complication of their addiction, mainly, Korsakoff's psychosis or Wernicke's encephalopathy. Two of them (case Nos. 402 and 403) had an active pulmonary tuberculosis, 1 had tuberculous meningitis (case No. 515), and 2 others were diabetic (case Nos. 157 and 437). As usual among alcoholic patients, it was very difficult to obtain reliable information concerning the duration of alcoholic habits and the amount of daily consumption. We have roughly estimated the minimum intake at 50 cc. daily for at least ten years in most of the patients. In some of them, a thiamine deficiency was suggested by an excess of blood pyruvate after a glucose load. Most of the patients were submitted to electromyography and histology in only one nerve territory. In 2 patients (cases 413 and 505) both median and lateral popliteal nerve territories were explored.

Only 12 of our 30 patients had symptoms of sensory neuropathy, mostly pain or paresthesia, or both, in lower limbs. Impairment of vibration and position sense was found in 9 of these patients; 4 of them had sensory ataxia. All patients but one had loss of deep reflexes, at least in lower limbs. A moderate reduction of motor power, at least in the territory of the nerve studied or in the biopsied muscle, was present in 6 alcoholic patients, 1 being also diabetic, and in 1 diabetic subject. Only one other diabetic patient (case 343) had a marked weakness in the biopsied muscle.

The histological and physiological investigations were performed in regions selected according to the following gradation: (1) Clinically normal limb: deep reflexes present, no motor weakness, no sensory symptoms. (2) Limb in which the deep reflexes are lost, but having retained a normal motor power. Sensory symptoms present in 4 of the patients of

(GROUP 1)

Histological data												
Muscle fibers							Innervation					
								SNA				
Mean surface $\mu 2$	σ	Range $\mu 2$	Percent outside normal range −	+	Peripheral nuclei (per fiber)	Central nuclei (%)	TIR:1	Mean surface $\mu 2$	σ	Range $\mu 2$	Percent outside normal range −	+
2,061	475	900-4,500	0	0	3	1	1,05	348	176	40-880	1	3
2,829	740	900-4,800	0	2	2,8	2	1,15	338	173	40-880	1	4
2,031	599	900-3,300	0	0	2,4	1	1,1	164	87	80-560	0	0
2,097	724	900-3,900	0	0	2,7	2	1,18	156	60	40-360	1	0
2,071	830	300-5,100	7	5	2,8	3	—	256	189	40-720	2	3
2,178	709	900-4,200	0	0	3,2	1	1,23	—	—	—	–	–
1,410	555	300-3,000	8	0	2,4	1	1,38	199	60	40-360	2	0
1,932	589	600-3,300	1	0	2,9	0	—	—	—	—	–	–

P. = Patellar reflex
P.L. = Palmeris longus
TIR = Terminal innervation ratio
S.L. = Superior limb
SNA = Subneural apparatuses
σ = Standard deviation
T.A. = Tibialis anticus
T.M. = Tuberculous meningitis
W. = Wernicke encephalopathy

this group. (3) Limb showing a loss of deep reflexes and a reduction of motor power, at least in the biopsied muscles. Sensory symptoms were present in most of these patients.

The histological observations were compared with a normal control group of 10 biopsies, 6 taken in the palmaris muscles (palmaris longus or flexor carpi radialis) and 4 in the tibialis anticus. These controls were selected from biopsies performed for diagnostic purpose in adult ambulatory patients in good general condition whose neurological examination did not suggest any neuromuscular disorder. We have discarded the biopsies in which histological abnormalities in the muscle tissue or its innervation were found.

RESULTS

The quantitative histology of muscle tissue and innervation of normal controls is summarized in table 1.

The range of the mean values of the transverse surface of muscle fibers is 2,096 to 2,378 μ^2 in palmaris group and 2,201 to 2,459 μ^2 in tibialis anticus group. The mean of all individual values is 2,249 $\mu^2 \pm 449$ in palmaris group and 2,327 $\mu^2 \pm 599$ in tibialis anticus group. The difference between these values is not significant, and we may accept the mean and standard deviation of the pooled individual values in both groups as satisfactory figures of the normal range of muscle fiber surfaces within the studied material. This value is 2,285 $\mu^2 \pm 518$. We will consider as probably abnormal the mean values falling outside the range of 2,096 to 2,459 μ^2 for the mean diameter and of 482 to 662 μ^2 for the standard deviation. The range of measured individual values in the control group is 900 to 4,500 μ^2 and exceeds slightly the calculated range covering 95% of the distribution curve ($-2\sigma + 3\sigma$) which is 1,049 to 3,639 μ^2.

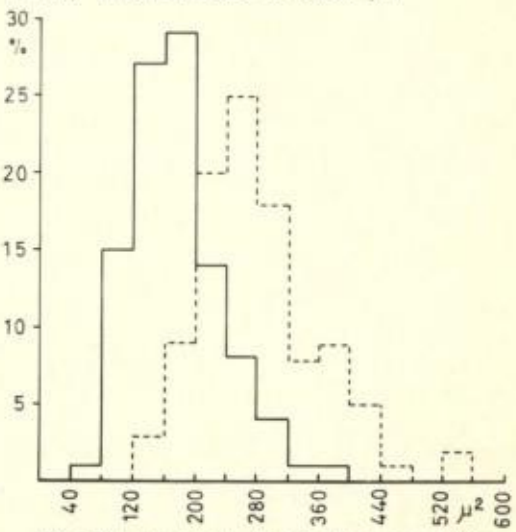


Fig. 7. Histograms of subneural apparatus surfaces in case 505, group 1, subject with diabetes (plain line), compared with normal distribution in control case 453 (dotted line). Shifting toward low values

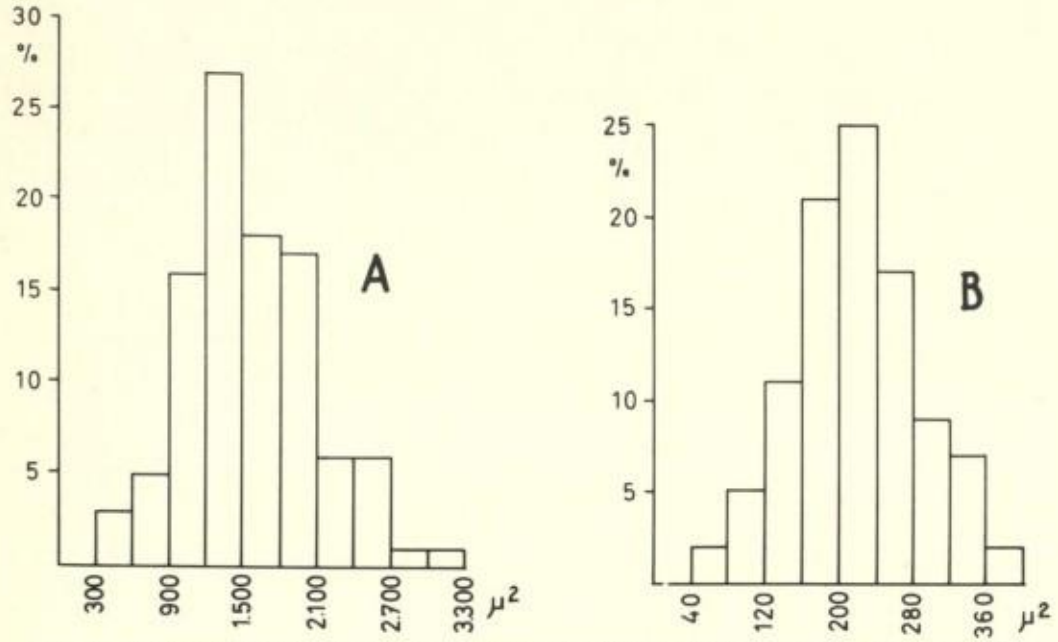


Fig. 8. Histograms of case 413, group 1, subject with diabetes. Normal distribution, muscle fiber surfaces (A). Shifting toward low values, subneural apparatus (B)

The range of mean surfaces of subneural apparatuses is 256 to 314 μ^2 in palmaris longus group (pooled mean 270 μ^2) and 261 to 397 μ^2 in tibialis anticus group (pooled mean 272 μ^2). The pooled mean of both muscles (271 $\mu^2 \pm 91$) may be accepted as representative of the normal average in the studied material, the range of normal mean values being 256 to 397 μ^2. Standard deviations falling outside the range of 81 to 105 are considered to represent an abnormal dispersion curve of the individual values. The measured range of individual value is 80 to 620 μ^2. The calculated range covering 95% of the distribution curve is 89 to 544 μ^2.

The number of peripheral muscle nuclei does not exceed 4 per fiber, and there is no more than 4 central nuclei per 100 fibers in the control group.

As previously stated, the mean terminal innervation ratio does not overcome 1.27:1 and lies within the range of 1.06:1 to 1.26:1.

Group 1. In the 3 alcoholic and 5 diabetic patients of this group (Table 2) the nerve territory investigated belonged to a limb free of neuropathic symptoms having retained its

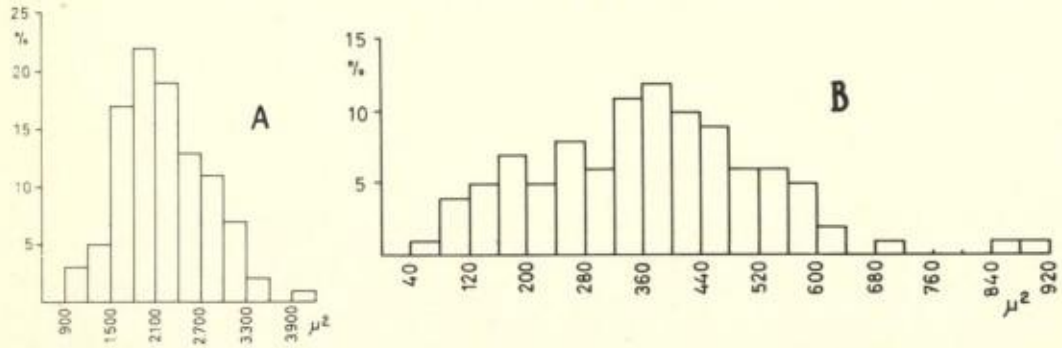


Fig. 9. Histograms of case 522, group 1, patient with alcoholism. Normal distribution, muscle fiber surfaces (A). Abnormal dispersion of individual values toward low and high values, subneural apparatus surfaces (B)

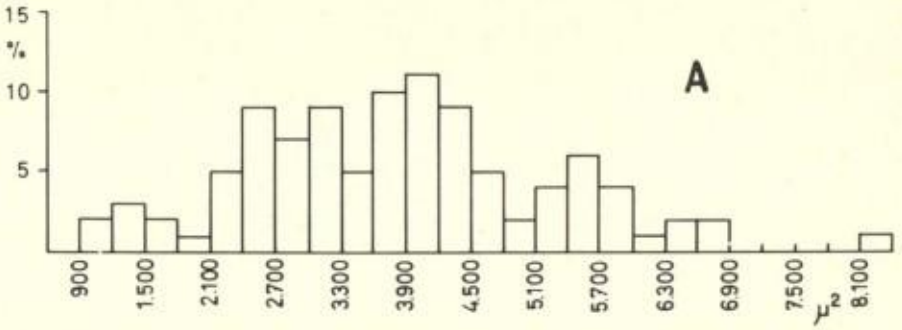
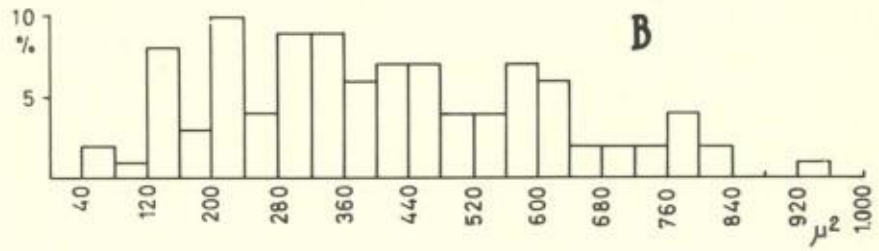


Fig. 10. Histograms of case 521, group 2, patient with alcoholism. Muscle fiber surfaces (A). Subneural apparatus surfaces (B). Abnormal dispersion toward small and high values



deep reflexes. The electromyographic investigation was carried out in the median nerve and biopsy taken in palmaris longus in all cases.

The histological study revealed no qualitative changes in the muscle tissue except in one diabetic patient (case 506) in which 2 small lymphocytic foci were found. The structure of muscle fibers was well preserved. Their size did not look abnormal at routine microscopic examination. However, measurement showed a slight increase of the standard deviation in 4 cases and a small proportion of individual values falling outside the upper and lower normal range in 2 cases. There was neither increase nor central displacement of muscle nuclei. The innervation was qualitatively and quantitatively modified in the 7 cases in which it was observed. In methylene blue preparations, some axis cylinders were abnormally small and beaded in cases 522 and 505, and, in case 413, an increased collateral ramification was found (terminal innervation ratio: 138:1, figure 23). The most conspicuous changes were observed in the motor end plates. Some terminal arborizations showed a marked tendency to the reduction and fusion of the terminal arborizations with concomitant simplification of the subneural apparatus (Fig. 16, reduced end plates). Others were abnormally expanded with corresponding enlargement of subneural apparatus (Figs. 18 and 19, expanded end plates). In some end plates of the normal or expanded type, there was an irregularity of size of the telodendrial expansions, one being usually abnormally large (irregular end plates, figures 20 and 21). The presence of reduced and expanded end plates resulted in abnormal values of the mean surface and standard deviation of the subneural apparatuses in all the cases. High values of the mean and of the standard deviation indicate a predominance of expanded end plates (cases 522, figure 9, and 515, alcoholism). Low value of the mean and of the standard deviation is the expression of a general tendency to the reduction of the motor end plates (cases 505, figure 7, and 413, figure 8, diabetes). In case 506, the normal mean with high standard deviation figures an equal tendency toward both processes of expansion and reduction.

The electromyographic detection did not show deviation from the normal pattern, except in case 413, in which there was a high proportion of polyphasic potentials. It is worth mentioning that this muscle was the only one of the series presenting an increased collateral ramification of the nerve fibers. The nerve conduction velocity was moderately but definitely

TABLE 3

Clinical data											Physiological data				
				Tendon reflexes				Studied region							
Case	Sex	Age	Diagnosis	S.L.	Inf. L. P.	Inf. L. An.	Paresthesia	Sense	Muscle	Nerve	I/T	Conduction velocity (meters per second)	Normal potential (%)	Polypotential (%)	Long-duration potential (%)	Spontaneous potential
429	M	56	A.W.	0	0	0	0	V.	T.A.	L.P.	N.	36.5	92	6	0	0
509	F	67	A.K.	+	0	0	0	N.	T.A.	L.P.	N.	34.9	86	14	0	0
455	F	55	A.	0	0	0	0	N.	P.L.	M.	N.	—	—	—	–	–
403	M	55	A.+T.B.	0	0	0	0	N.	P.L.	M.	N.	37.5	81	19	0	0
521	M	81	A.	+	+	0	+	V.P.	T.A.	L.P.	N.	43.3	—	—	–	–
546	M	36	A.	0	0	0	++	V.P.	P.L.	M.	N.	48.0	95	5	0	0
438	F	56	A.K.	0	0	0	++	P.Atax.	T.A.	L.P.	N.	53.3	61	28	11	0
437	M	56	A.+D.	+	+	0	++	Atax.	T.A.	L.P.	N.	—	80	20	0	0
432	M	77	D.	0	0	0	0	N.	T.A.	L.P.	N.	34.9	86	14	0	0
475	F	69	D.	+	0	0	0	N.	T.A.	L.P.	N.	31.9	100	0	0	0
469	F	76	D.	+	0	0	0	N.	P.L.	M.	N.	50.0	57	37	6	0
465	F	76	D.	0	0	0	0	N.	P.L.	M.	N.	46.0	—	—	–	–
517	F	80	D.	0	0	0	0	N.	P.L.	M.	N.	44.4	95	5	0	0
505	M	84	D.	+	+	0	0	N.	T.A.	L.P.	N.	43.3	—	—	–	–
550	M	27	D.	0	0	0	+	N.	P.L.	M.	N.	30.8	95,5	4	0,5	0
413	F	50	D.	+	0	0	+	N.	T.A.	L.P.	N.	25.0	—	—	–	–

reduced in 6 cases. The lowest value was obtained in case 413, the only one showing an abnormal electromyographic pattern and an increased axonic collateral ramification.

Group 2. The 16 cases of this series were 7 alcoholic, 8 diabetic, and 1 both alcoholic and diabetic patients (Table 3). Investigations were performed in arreflexic limbs, 4 of them presenting symptoms of sensory neuropathy, but no reduction of motor power.

Histological examination of the biopsied muscles did not show conspicuous qualitative alterations of the contractile elements and of the connective tissue. The only changes were some ring fibers (cases 475 and 413, figure 12). In all the cases but one there was a wide range of muscle fiber sizes distributed at random in the pattern of single fiber atrophy but with a marked hypertrophy of many fibers (Figs. 4B and 13). There was, in some cases, a tendency to the grouping of small fibers (Fig. 14). These changes were reflected by an increase of the standard deviation of the mean surface. The predominance of the hypertrophic process in most of the cases resulted in an important increase of the mean surface value. The shifting of the histogram toward the high values (Fig. 10A) was also indicated by the larger proportion of surfaces exceeding the upper normal limit.

There was some increase in muscle nuclei and a large number of central nuclei in many cases (Fig. 14).

The intramuscular nerve fibers were abnormally ramified, and the terminal innervation ratio was normal in only 2 cases (517 and 550). Some axis cylinders were fine and beaded. The mean surface of the subneural apparatuses was normal, reduced, or increased, but the standard deviation was consistently increased in most of the cases, indicating an abnormally large spectrum of end-plate sizes, both in alcoholic and diabetic patients, exceeding the upper and lower normal limits in nearly all the cases (Fig. 10B).

The electromyographic detection disclosed an increased proportion of polyphasic or long-duration potentials, or both, in the majority of biopsied muscles (Fig. 3B). Only 2 had a normal electromyographic pattern (Fig. 3A). None of the cases had spontaneous activity. Nerve conduction velocity was moderately reduced in 8 cases and within the normal limits in 6 cases (Fig. 2A). Intensity-duration curve was normal in all examined muscles.

Group 3. These 8 patients, 5 alcoholic, 2 diabetic, and 1 both alcoholic and diabetic, were diagnosed as sensory motor polyneuropathy. In one patient (case 343), the biopsied muscle had a markedly reduced motor power.

(GROUP 2)

Histological data												
Muscle fibers							Innervation					
								SNA				
Mean surface μ2	σ	Range μ2	Percent outside normal range −	+	Peripheral nuclei (per fiber)	Central nuclei (%)	TIR:1	Mean surface μ2	σ	Range μ2	Percent outside normal range −	+
5,285	1,218	2,850-10,650	0	70	4,6	43	1,50	—	—	—	—	—
3,101	1,737	900- 7,800	0	14	4,0	11	—	268	153	40- 640	2	1
1,637	706	450- 3,750	10	0	2,3	3	1,36	269	128	40- 500	1	3
1,532	887	450- 3,300	12,5	0	2,1	5	1,40	194	92	60- 600	5	0
3,615	1,713	900- 8,100	0	27	5,7	7	1,80	392	199	40- 920	2	13
2,580	1,305	600- 7,500	4	8	5,0	7	1,44	326	142	80- 600	0	0
2,904	949	1,050- 6,150	0	5,5	3,2	11	1,70	420	180	140-1,100	0	9
3,798	1,707	450- 9,600	1,5	34	4,9	35	1,34	533	213	100-1,180	0	27
3,424	1,188	1,650- 7,650	0	15	3,6	10	1,57	560	188	180-1,220	0	30
3,373	1,407	600- 5,750	1	20	4,7	21	1,45	311	108	100- 680	0	1
2,390	871	600- 5,250	2,5	4	2,7	3	1,37	243	149	60- 720	3	2
1,887	590	300- 3,900	10	0	2,7	3	1,35	271	86	40- 520	1	0
1,751	790	300- 4,500	5	0	2,2	2	1,16	200	113	40- 560	4	0
3,300	740	900- 5,100	0	10	4,1	14	—	218	115	40- 880	3	1
1,704	913	600- 4,200	2	0	3,1	1	1,26	441	199	80-1,160	0	17
4,413	1,768	1,200- 8,400	0	22	4,0	7	1,60	242	121	80- 480	0	0

In the others, the weakness did not exceed grade 4.

The qualitative histology revealed various patterns of atrophy: single fiber atrophy and hypertrophy in 6 cases (Fig. 13), small group atrophy (case 550), and large group atrophy (case 459, figure 15). The structure of the contractile elements was not consistently altered, except in the large groups of atrophied fibers (case 479), which had lost their striations and were filled by conglomerations of pycnotic nuclei. The great variety of sizes was indicated by a marked increase of standard deviation of the muscle fiber mean surface. The high value of this mean surface, found in most of the cases, is the result of the large

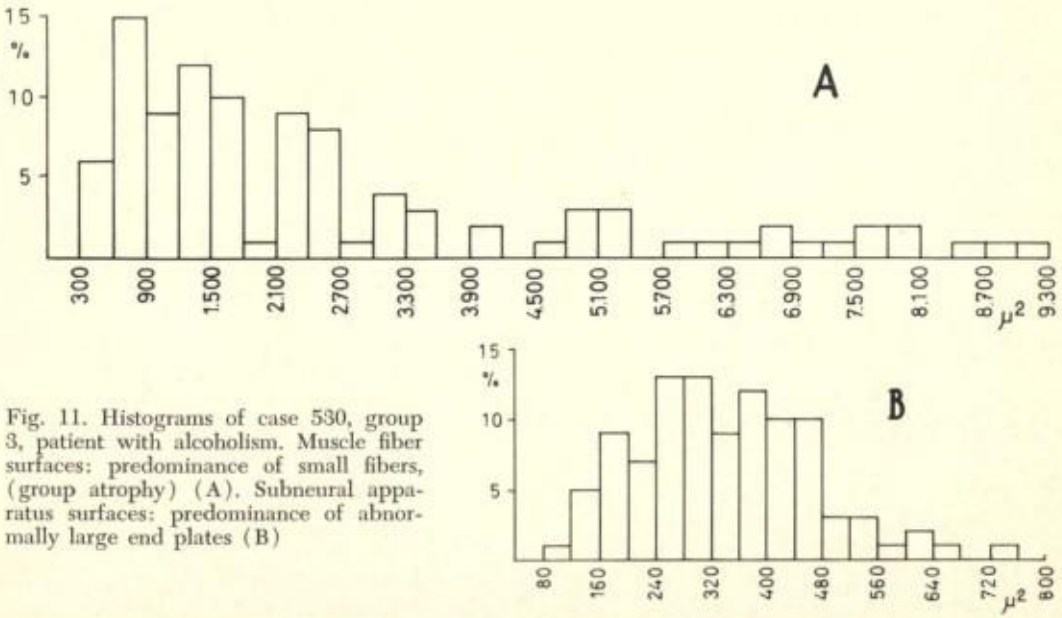


Fig. 11. Histograms of case 530, group 3, patient with alcoholism. Muscle fiber surfaces: predominance of small fibers, (group atrophy) (A). Subneural apparatus surfaces: predominance of abnormally large end plates (B)

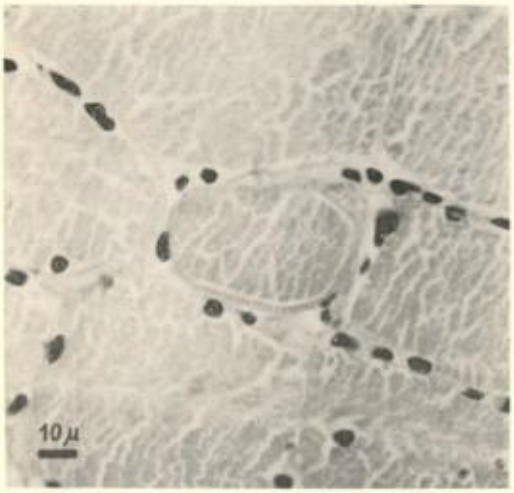


Fig. 12. Ring fibers. Case 475, group 2, P.A.H.

proportion of hypertrophic fibers. This figure does not apply to case 459, in which it was not possible to include the large groups of very small fibers in the planimetric measurements. In case 530 the relatively low mean value is related to the high proportion of small fibers (Fig. 11A). As in group 2, there was an important increase of central muscle nuclei in nearly all the cases.

The intramuscular nerve fibers showed numerous collateral branches ending in growth cones (Fig. 22) or forming small or large end plates. Many axis cylinders were fine and beaded. The quantitative expression of these changes was an increase of the terminal innervation ratio and a high value of the standard deviation of the subneural apparatuses mean surface in all cases (Fig. 11B).

The electromyographic pattern of the biopsied muscles was markedly altered in all examined cases, large proportion of polyphasic or long-duration potentials, or both. The nerve conduction velocity was reduced in 2 of the 4 patients in whom it had been measured (Fig. 2B). In one patient (case 459), there was no evoked potential in extensor digitorum brevis. The intensity-duration curve indicated a partial denervation in case 530 and 459 (biphasic curve) and a complete denervation in case 343. Another indication of denervation was the occurrence of fibrillation potentials in cases 486, 530, and 342.

COMMENT

The subclinical involvement of peripheral nerves, mentioned by Hurst in 1925,[18] seems to be a rather common feature among diabetic patients. Dolman[19] has observed histological lesions of peripheral nerves and neurogenic atrophy of muscles in patients free of obvious symptoms of neuropathy. Stimulation electromyographic studies have demonstrated a slowing of nerve conduction velocity in most of the diabetic patients, including those without overt neuropathy.[20,21] Thage and associates[22,23] have obtained abnormal electromyographic patterns in unaffected limbs of patients with polyneuropathy or mononeuropathy of various origins, including those who are diabetic and alcoholic.

In confirmation to these reports, the present

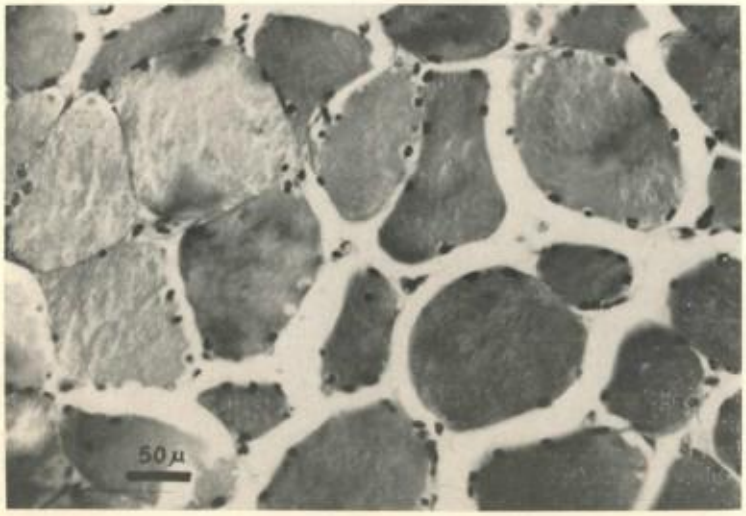


Fig. 13. Single fiber atrophy pattern with increased number of muscle nuclei. Case 343, H.E.

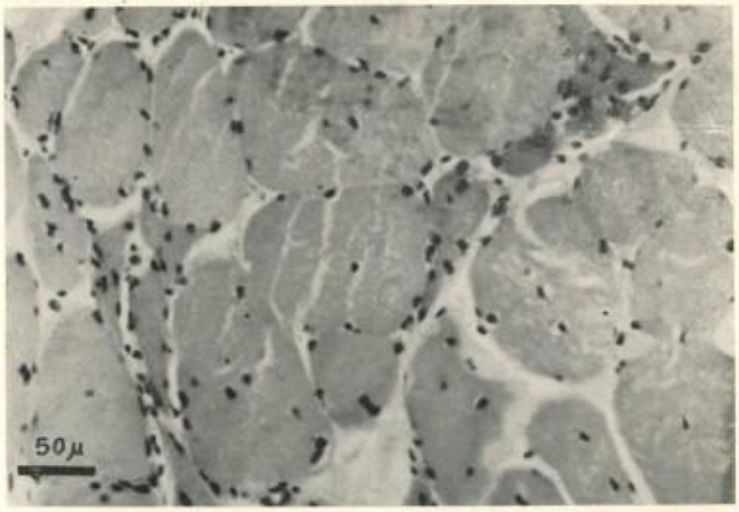


Fig. 14. Small group atrophy pattern with numerous central nuclei. Case 429, group 2, H.E.

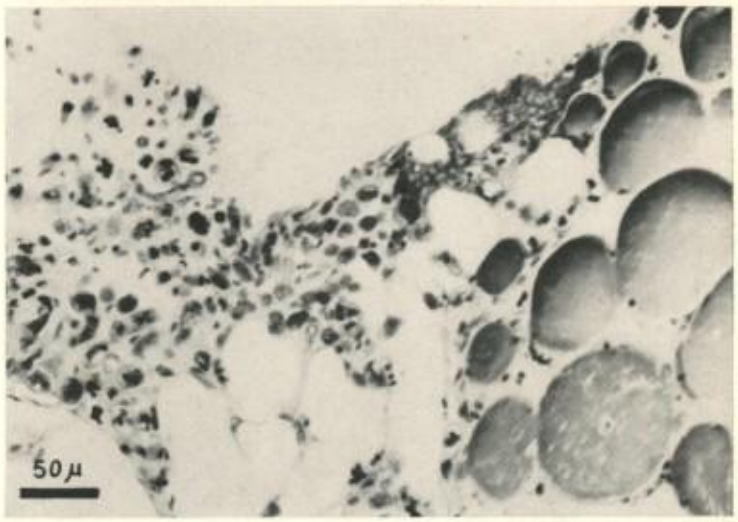


Fig. 15. Large group atrophy pattern. Case 459, group 3, P.A.H.

study gives evidence of electromyographic abnormalities in a large number of the diabetic and alcoholic patients examined. The nerve conduction velocity is significantly reduced in 9 patients, moderately reduced in 7 others, and within the normal limits in 9. It must be pointed out that the proportion of reduced conduction velocity is about the same in clinically affected and nonaffected neuromuscular regions (6 to 7 in the first group, 8 to 14 in the second group, and 2 to 4 in the third group). The electromyographic pattern during voluntary contraction is abnormal in 15 and within the normal range in 8. Here, the proportion of abnormal traces is definitely higher in muscles having a clinical indication of neural involvement: 1 in the 6 records performed in the first group (normal muscles), 8 in the 11 records of the second group (arreflexia), and in all recorded cases of the third group (reduced motor power). Fibrillation potentials were observed only in 3 patients of the third group, 3 of whom also had abnormal intensity-duration curves. In all other cases the intensity-duration curve was not significantly altered in the biopsied muscle.

Histological observation of the muscle tissue discloses that the most common change is a pattern of single fiber atrophy, in which the small fibers are scattered among normal or hypertrophied fibers. Such a distribution is not considered as characteristic of the neurogenic

TABLE 4

Case	Sex	Age	Diagnosis	Clinical data: Tendon reflexes S.L.	Inf. L. P.	Inf. L. An.	Strength	Paresthesia	Sense	Studied region: Muscle	Studied region: Nerve	I/T	Physiological data: Conduction velocity (meters per second)	Normal potential (%)	Polypotential (%)	Long-duration potential (%)	Spontaneous potential
397	M	46	A.	0	0	0	4	+	V.P.	P.L.	M.	N.	—	—	—	—	—
457	M	54	A.+D.W.	+	0	0	4-5	0	N.	T.A.	L.P.	N.	—	63	23	14	0
508	M	41	A.W.	0	0	0	4-5	+	V.P.Atax.	T.A.	L.P.	N.	39,0	—	—	—	—
402	M	50	A.T.B.	0	0	0	4-5	++	V.P.	P.L.	M.	N.	51,6	78	22	0	0
486	M	64	A.	0	0	0	4-5	+	P.Atax.	T.A.	L.P.	N.	27,8	65	0	35	+
530	M	33	A.	0	0	0	4	++	P.Sup.	P.L.	M.	B.	40,0	85	15	0	+
459	F	67	D.	+	0	0	4-5	0	N.	T.A.	L.P.	B.	0	76	24	0	0
343	F	71	D.	0	0	0	3	++	N.	T.A.	L.P.	D.	—	64	36	0	+

*In large fibers groups D. = Denervation curve B. = Biphasic curve P. = Position sense

atrophy and has been interpreted as an indication of a primary muscular involvement in diabetic amyotrophy.[24,25] This assumption will be discussed in the light of the neural changes observed in our patients. We may already remark that the pattern of single fiber atrophy cannot be considered as the hallmark of a myogenic process in the absence of degenerative changes in the muscle fibers. In amyotrophies of neural origin, showing the group atrophy pattern, it is customary to observe a great variety of sizes in the large fiber groups. This is the case of our biopsy 459 (Fig. 15). The absolute increase of muscle nuclei with multiplication of central nuclei is usually more pronounced in primary muscle diseases[15,26]

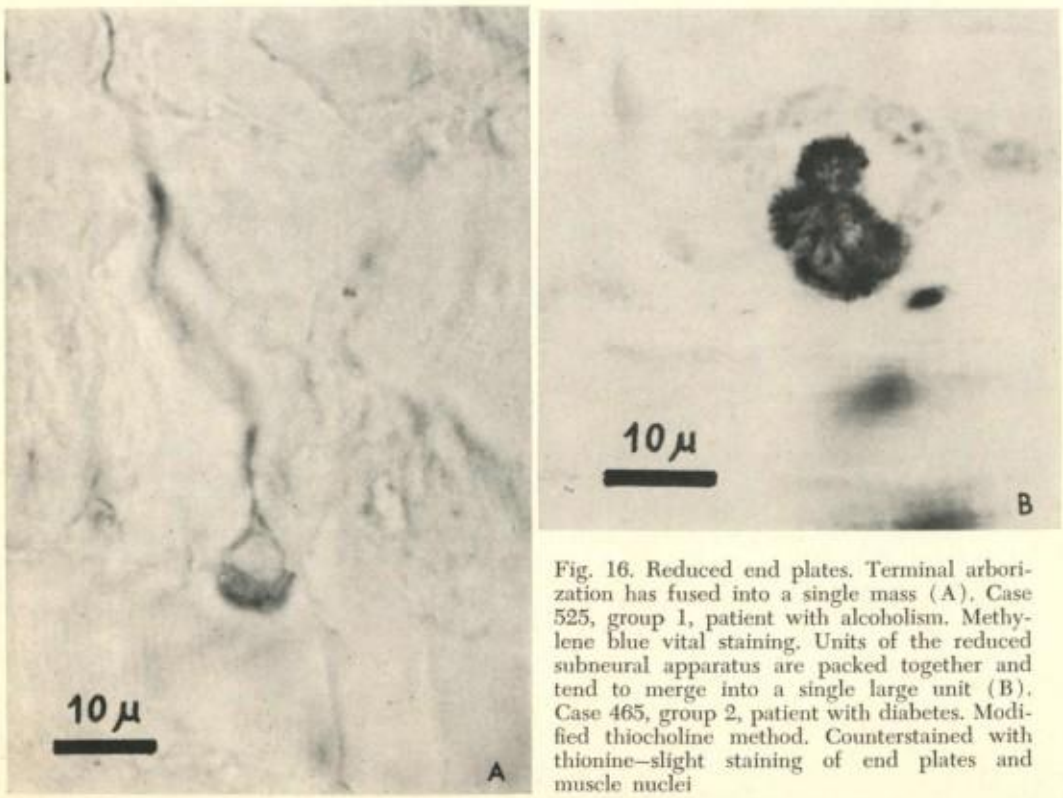


Fig. 16. Reduced end plates. Terminal arborization has fused into a single mass (A). Case 525, group 1, patient with alcoholism. Methylene blue vital staining. Units of the reduced subneural apparatus are packed together and tend to merge into a single large unit (B). Case 465, group 2, patient with diabetes. Modified thiocholine method. Counterstained with thionine—slight staining of end plates and muscle nuclei

(GROUP 3)

Histological data												
Muscle fibers							Innervation					
								SNA				
Mean surface μ2	σ	Range μ2	Percent outside normal range −	+	Peripheral nuclei (per fiber)	Central nuclei (%)	TIR:I	Mean surface μ2	σ	Range μ2	Percent outside normal range −	+
3,931	1,751	600- 8,700	1	31	6,1	9	2,70	352	168	60- 800	1	9
4,258	985	2,250- 8,400	0	15	3,2	9	1,31	364	173	60- 900	3	8
3,978	1,874	900- 8,400	0	36	4,9	23	1,45	386	209	40-1,000	2	11
2,712	1,545	750- 5,850	1	10	3,8	8	1,32	338	180	120- 800	0	3
4,278	1,990	600- 8,100	2	45	3,2	22	1,42	309	213	40- 800	3	7
2,508	2,463	300- 9,300	21	19	3,3	3	1,45	320	143	80- 720	0	2
4,850*	2,437	600-10,500	3	60	4,9	19	1,65	327	204	80- 860	0	7
3,373	1,407	300- 9,900	7	21	5	15	1,60	312	149	60- 820	1	3

Sup. = Superficial sensibility Sp.Pot. = Spontaneous potential T.B. = Pulmonary tuberculosis V. = Vibration sense

but is in no way specific of them. In this respect, the small foci of lymphocytes observed in our case 506 and the ringed fibers present in cases 413 and 475 cannot be interpreted as a proof of primary muscle involvement, both changes being quite unspecific processes, occasionally found in otherwise normal muscles.[1,26,27]

In fact, the pathological changes of the muscle tissue, if not very marked, can hardly be interpreted in the absence of information on the morphological changes of the motor innervation.

When we come to this part of our observations, we see that the very first changes in the innervation pattern, clearly seen in group 1, happen at the level of the myoneural junction. Some of the terminal arborizations shrink and their telodendrial expansions become closely packed together. Eventually they fuse into a single mass. The subneural apparatus follows this evolution and takes the form of a single large unit, with a well-preserved laminated border (Fig. 17) resulting from the conglomeration and merging of its elements. These changes in diabetic neuropathy were first described by Woolf and Malins.[28] In the present series they have been also observed in alcoholic patients, although somewhat more frequently in the diabetic of the first group. Other end plates have an opposite evolution and, through a process of neurocladism of the telodendrion, become abnormally large and expanded, with corresponding enlarged subneural apparatuses. In some motor arborizations, an alternate pattern of reduction is observed: One of the terminal expansions enlarges, whereas the others become small and eventually disappear (Fig. 20).

These processes were observed in the absence of other conspicuous changes in nerve fibers in several biopsies of the first group of clinically normal cases, in which there were no significant changes in muscle fiber sizes. It may be deduced that: (1) the modification in nerve ending structure is not secondary to muscle fiber alterations, (2) these morphologically abnormal end plates have retained their trophic action on the contractile units, and (3) they are able to ensure a satisfactory

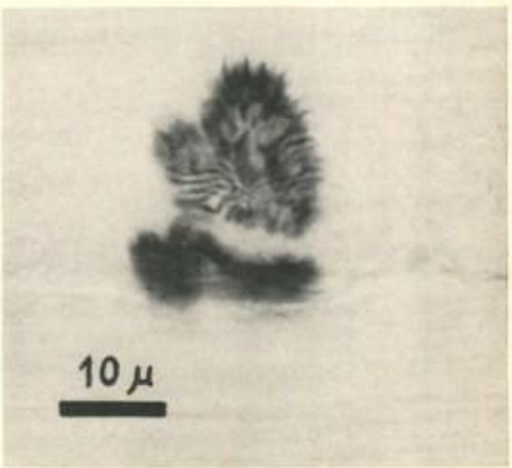


Fig. 17. Reduced subneural apparatus. Case 459, group 3, subject with diabetes. Note preservation of well-laminated border. Modified thiocholine method

Fig. 18. Expanded and irregular terminal arborizations. Case 475, group 3. Methylene blue

neuromuscular transmission which results in the preservation of a good motor power and even a normal electromyographic pattern. Actually it is not excluded that such end plates should be unable to sustain a repetitive activity at high frequency which might result in a myasthenic-like irregular decrease of 50 evoked potentials per second.[29] In this respect it is tempting to correlate the myasthenic reaction described in some cases of small cell carcinoma of the lung[38] with the finding of reduced end plates in carcinomatous neuropathy.[4,30] So far, the only definite physiological indication of a neural dysfunction, at the stage of diabetic and alcoholic neuropathy in which the histological alterations are limited to the myoneural junction, is a slowing of nerve conduction velocity.

It may be assumed that the next step in the evolution of reduced end plates should be their degeneration with subsequent fragmentation of axis cylinders. This process of disintegration beginning distally has been occasionally observed in acute denervation[2,3] but never in chronic neuropathies, which is not surprising, according to the probable quickness of this process and to the fact that the axonic debris hardly stain with methylene blue. More stable is the collateral ramification of nerve fibers which has been considered as a reaction to degeneration in compensation to the loss of nerve fibers.[3,4,31,32] This collateral sprouting is regularly observed in biopsies of group 2, corresponding to neuromuscular territories in which the only clinical neuropathic sign was a loss of deep reflexes. These biopsies contain also beaded fibers ending in growth cones that are other indications of a regenerating process (Fig. 22).

Therefore, we may consider the single fiber atrophy pattern observed in biopsies of group 2 as secondary to the loss of scattered terminal nerve fibers. Even if these fibers are grouped in a subunit pattern, the concomitant process of collateral ramification with subsequent random reinnervation of muscle fibers should account for the type of atrophy observed. We may conclude that the single fiber atrophy pattern, though generally attributed to myogenic atrophy, is perfectly consistent with a neurogenic process occurring distally. Such is the large increase of muscle nuclei observed in most of the cases.

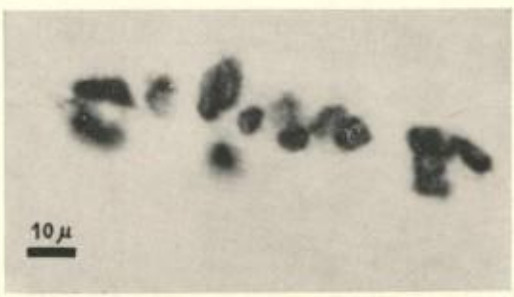


Fig. 19. Expanded subneural apparatus. Case 475, group 3. Modified thiocholine method

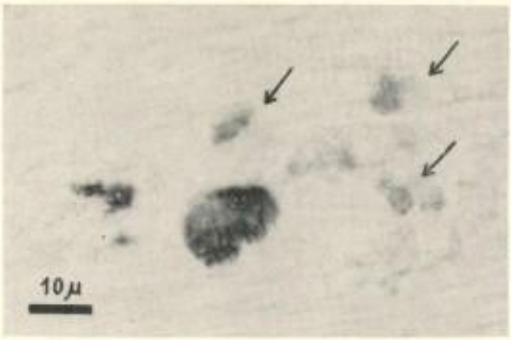


Fig. 20. Irregular subneural apparatus. Case 515, group 1. One unit abnormally large, others small; some very pale and probably degenerating (arrows)

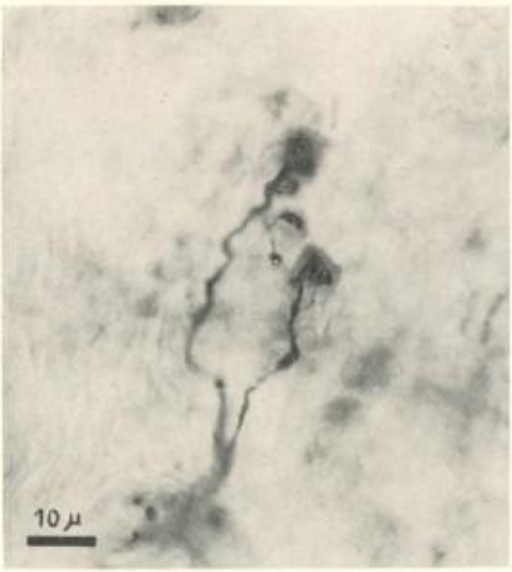


Fig. 21. Small and irregular terminal expansions. Case 505, group 2. Methylene blue

The measurement of the muscle fiber sizes reveals the importance of hypertrophy which, in many cases, definitely overcomes the atrophy. Both hypertrophy and collateral ramification account for the preservation of a normal muscle strength. However, the heterogeneity of muscle fiber sizes and the distortion of innervation pattern are reflected in a high proportion of polyphasic potentials in biopsied muscles. This is in good agreement with Buchthal's statement[33] that such potentials are the expression of a greater temporal dispersion of the subunit potentials in relation to a slowing of conduction and transmission in regenerating nerve fibers and endings. In this respect, it is worth pointing out that the only muscle having an increased number of polyphasic potentials in group 1 had also an increased terminal innervation ratio.

If the general correlation between the occurrence of collateral sprouting and polyphasic potentials is quite satisfactory, this correlation is not significant in individual cases, in spite of the fact that both histological and electromyographic investigations were carefully performed in the same fasciculi. Even in such conditions it should have been unlikely that the recording electrode had picked up selectively the electrical activity of the biopsied elements in moderate voluntary contraction. Therefore, the lack of parallelism between the proportion of polyphasic potentials and the degree of collateral ramification in each case does not invalidate the general correlation. Besides, the great variety of muscle fiber sizes is another factor of temporal dispersion of motor unit potentials which must be accounted for in the interpretation of electromyographic patterns.

The third group illustrates the decompensation of the balance between regressive and regenerative processes. The loss of contractile elements becomes apparent as a slight reduction of motor power. This step has no clear-cut electromyographic or histological expression in 5 cases which show the same features as group 2. However, we may assume that the pattern of group atrophy which appears in cases 459 and 530 is the histological expression of the increased number of denervated muscle fibers, which appear as a high proportion of small fibers (Fig. 11) and, on the other hand, may result in an abnormal intensity-duration curve of the biphasic type and in spontaneous denervation potentials.

The innervation pattern is not obviously different in groups 2 and 3 and the terminal innervation ratio is increased within the same range. The motor end plates are of the reduced, expanded, irregular, or normal type. Methylene blue preparations seem to contain more fine beaded fibers and less normal axis cylinders in some biopsies of group 3, but these features cannot be quantitatively estimated, and it is not excluded that these differences should sometimes be the result of artificial variation of staining intensity.

The reduced and expanded end plates in

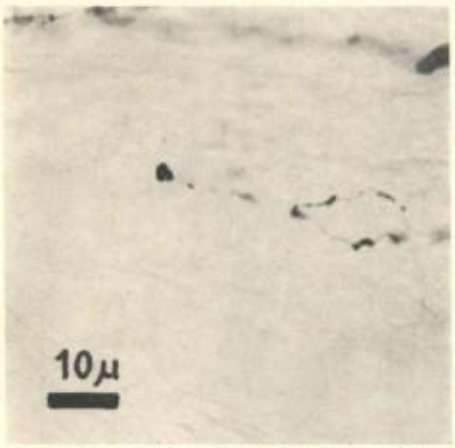


Fig. 22. Ramified and beaded fiber endings in growth cone. Case 530, group 3. Methylene blue

groups 2 and 3 raise the problem of the specificity of these morphological changes. We have previously pointed out[2,34,36] that the small regressive end plates, seen in chronic neuropathies, closely resemble the first stage of differentiation of the myoneural junction, either in normal development or in regeneration, when the axoplasmic growth cone reaches the sarcoplasm and before it starts its ramification. As for the enlarged end plates, they could represent an adaptation to the hypertrophy of the muscle fibers, according to the general rule that the size of the motor end plates is proportional to the size of the muscle fibers.[3,36] Therefore, in groups 2 and 3, these ambiguous pictures cannot be safely interpreted as regressive or regenerative phenomena. Nevertheless, in group 1 at least, the small end plates may undoubtedly be considered as the first stage of regression in the absence of any indication of axonal degenerative or regenerative changes and of conspicuous changes in muscle fiber sizes.

CONCLUSION

The study of our material has demonstrated the importance of the subclinical involvement of neuromuscular apparatus, not only in diabetic but also in alcoholic patients. The histological and electromyographic features and their evolution are not significantly different in either condition. Only at the first stage is the proportion of reduced end plates somewhat higher in diabetic than in alcoholic subjects.

The morphological changes in end-plate structure, preceding the loss of nerve fibers and contractile units, are the first indication of a generalized involvement of peripheral nerves, the physiological expression of which being a slowing of motor nerve conduction velocity. The axonic degeneration may eventually lead to a loss of muscle fibers and a reduction of motor power, in spite of the reactive collateral sprouting of nerve fibers and of the hypertrophy of muscle fibers.

The demonstration of a primary involvement of motor axons excludes the possibility that the pattern of single fiber atrophy generally observed could be the expression of a primary muscular injury.

The generalized nature of the subclinical involvement of peripheral nerves in diabetes is in good agreement with the assumption that diabetic polyneuropathy is due to a metabolic defect in relation to insulin deficiency[37] in the same way as alcoholic polyneuropathy is related to an inefficient metabolism of carbohydrate. It may be assumed that various nutritional or toxic factors could produce similar changes in peripheral nerves.

It is hoped that the detailed study of neuromuscular apparatus in other conditions leading to chronic polyneuropathy, particularly the malignant tumors, will contribute to the understanding of some ill-defined features, such as the myasthenic-like reaction in some cases of small-cell bronchogenic carcinoma.[38]

Fig. 23. Abnormal collateral ramification: 4 small end plates coming from same axon (1, 2, 3, 4) and beaded fibers (arrow). Case 413, group 1

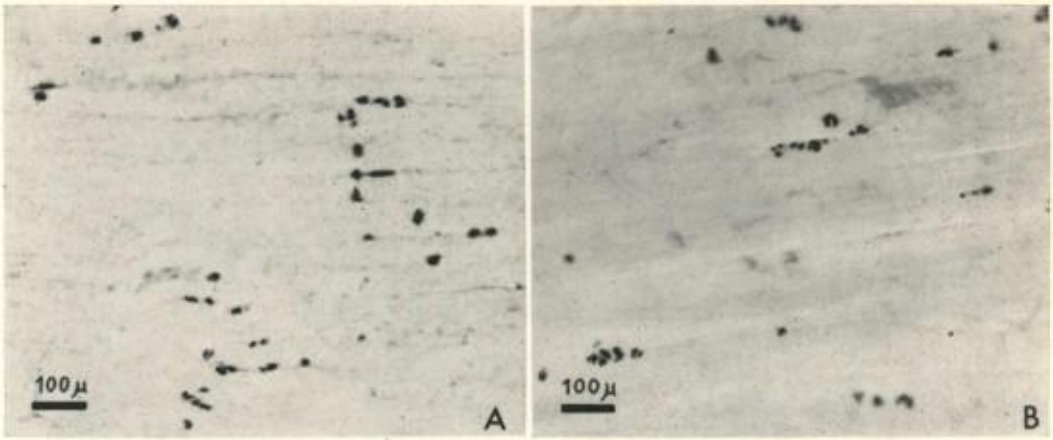


Fig. 24. Low-power magnification of preparations stained with modified thiocholine method for cholinesterase. Predominance of small end plates. Case 465, group 2, patient with diabetes (A). Abnormally large end plates. Case 397, group 3, patient with alcoholism (B)

SUMMARY

1. Selected neuromuscular territories were studied in 30 patients, 13 diabetic, 15 alcoholic, and 2 both diabetic and alcoholic, by several electrophysiological and histological means, including intensity-duration curves, stimulation and detection electromyography, quantitative histology of muscle tissue, and intramuscular innervation. Investigations were carried out in clinically normal muscles (group 1), in muscles belonging to a region having lost its deep reflexes (group 2), and in moderately weak muscles (group 3).

2. The first morphological indication of a neural involvement, constantly observed in group 1, is limited to the level of myoneural junction as a reduction, an irregularity, or an abnormal expansion of motor end plates. These changes, which have no clinical expression, are neither associated with histological changes in muscle tissue nor with abnormal electromyographic patterns. Their only physiological counterpart is a slowing of nerve conduction velocity.

3. The next step of latent neuropathy appears regularly in group 2. It is characterized by an increased collateral ramification of motor nerve fibers and a marked variety of muscle fiber sizes distributed at random, the hypertrophic process being usually prominent. These changes probably correspond to the distal degeneration of scattered nerve fibers. Their electromyographic expression is an increased number of polyphasic or long-duration potentials, or both. Neither denervation potentials nor abnormal intensity-duration curves occur in this group. The collateral ramification and muscle fiber hypertrophy account for the preservation of motor power.

4. The reduction of strength of the muscles studied in group 3 is not indicated by a clear-cut change in the innervation pattern or in conduction velocity, as compared with group 2. However, the loss of innervated fibers may appear histologically as a pattern of group atrophy and physiologically as spontaneous denervation potentials and as abnormal intensity-duration curves.

5. The type and evolution of histological and physiological changes are similar in both diabetic and alcoholic patients, and it may be concluded that the same generalized subclinical involvement of peripheral nerves may result from various metabolic or nutritional defects.

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