

## SKELETAL MUSCLE INVOLVEMENT IN SYSTEMIC SCLEROSIS\*

BY

JOHN M. THOMPSON,† RODNEY BLUESTONE, E. G. L. BYWATERS,  
J. DORLING, AND MARGARET JOHNSON

*From the Departments of Medicine and Histochemistry, Royal Post-Graduate Medical School of London,  
and the M.R.C. Rheumatism Research Unit, Taplow, Bucks*

The nature and extent of muscle involvement in systemic sclerosis has been the subject of considerable controversy from which it is difficult to form a coherent concept.

Clinical evaluation yields an estimate of significant abnormality, varying from none (Norton, Hurd, and Ziff, 1966) to over 60 per cent. (Medsgger and Rodnan, 1966). Serum enzyme studies appear to be of little value, since the abnormalities found failed to correlate with clinical or histological assessments (Rodnan and Medsger, 1966). Electrodiagnostic evaluation, however, seems to indicate a significant degree of abnormality. In two of the three patients examined by Medsger and Rodnan (1966), there was shortening of the mean potential duration and an increase in polyphasicity. Hausmanowa-Petrusewicz and Kozmińska (1961) reported significant shortening of the mean potential duration in nineteen of 25 muscles underneath involved skin and in ten of eleven muscles distant from skin involvement. In these patients, the mean potential duration was felt to be the most sensitive index of muscle involvement, since it was abnormal even when biopsy specimens of muscle were histologically normal.

Light microscopical abnormalities that have been described include interstitial fibrosis, muscle fibre degeneration, and focal infiltration of inflammatory cells (Medsger and Rodnan, 1966; Le Coulant and Texier, 1967; Michałowski and Kudejko, 1966). Medsger and Rodnan (1966) noted abnormalities in fifteen of 32 biopsy specimens, with an increased frequency where the muscle examined was beneath the involved skin. They noted fibrosis of the perimysium and epimysium and an increase in interstitial fat in eleven of these biopsies, with many specimens demonstrating scattered cellular infiltrates, primarily lymphocytes, both diffusely and in a perivascular distribution.

Norton and others (1966) have described, from electron microscopical examination of quadriceps needle biopsies in eight patients with systemic sclerosis, a characteristic lamination of the basement membrane in 65 per cent. of the capillaries. In none of these was there light microscopic evidence of muscle abnormality. Associated with this basement membrane lamination were noted basement membrane thickening, an increased mean diameter of blood vessels, endothelial swelling, increased numbers of pericytes around the capillaries, and decreased numbers of capillaries per cross-sectional area of skeletal muscle (Norton, 1968). Michałowski and Kudejko (1966) also noted thickening and lamination of the capillary basement membrane, capillary wall thickening, and endothelial proliferation. They described muscle fibre changes, including myofibril dissociation, granular degeneration and fragmentation, granular sarcolemmal change, nuclear indentation (*i.e.* damage) and mitochondrial changes.

We have studied skeletal muscle in fifteen patients with definite systemic sclerosis. The deltoid muscle was chosen because of its accessibility for study, because proximal muscles tend to be most frequently implicated in myopathic disease generally, and because of the allegedly common occurrence of muscle involvement at sites distant from areas of cutaneous involvement (Hausmanowa-Petrusewicz and Kozmińska, 1961). Electromyographic measurement of mean potential duration was completed in each, and twelve had muscle biopsies examined by light microscopy, histochemistry, and electron microscopy.

### Methods

#### (1) Clinical Assessment

Fifteen unselected patients (13 women, 2 men) aged from 28 to 72 years (mean 48·6) with systemic sclerosis were studied. In addition to evaluation of skeletal muscle, appropriate investigations to characterize

\*Given to a meeting of the Heberden Society on May 17, 1968.

†In receipt of a training grant from the Canadian Arthritis and Rheumatism Society.

cardiopulmonary, gastrointestinal, renal, articular, and vascular involvement were carried out. Only two patients had received corticosteroid therapy during the previous 3 months.

### (2) Electrodiagnosis

Using a standard Medelec (M.S.C.3) two-channel electromyograph unit, concentric needle electrode exploration of either right or left deltoid muscle was carried out. Approximately fifteen to twenty different motor unit potentials were photographed several times at minimal volition. The duration in milliseconds of each potential was then measured, and the mean potential duration (MPD) subsequently calculated for the muscle examined (Yates, 1963a).

In addition to the fifteen patients with systemic sclerosis, electromyographic (EMG) examination of nineteen normal subjects (18 women, 1 man) aged from 23 to 78 years (mean 48) was carried out. In the measurement of motor unit potentials and subsequent calculation of MPD, the recordings of patients and controls were arranged in a random manner with identifying labels removed.

### (3) Biopsy and Histological Preparation

Drill biopsies were carried out in all patients except Cases 13, 14, and 15. Specimens were obtained from the deltoid muscle opposite to that examined electromyographically, either at the time of EMG or within 24 hours. This technique has previously been described by Yates (1963a). Specimens were aspirated through the

drill cannula into a syringe containing heparinized sodium citrate; they were then frozen in Arcton 12 (Dichlorodifluoromethane) cooled to minus 150° C. in liquid nitrogen. Cryostat sections were then cut at 6 $\mu$  and fixed in formol-calcium at 4° C. for 1 hour. For histological studies, sections were stained by haematoxylin-Van Gieson and haematoxylin and eosin methods.

Acid phosphatase and alkaline phosphatase were demonstrated using the azo dye techniques described by Burstone (1958 a, b). Unfixed frozen sections were used for the estimation of mitochondrial oxidative enzyme activity (Scarpelli, Hess, and Pearse, 1958).

A portion of each biopsy was fixed in Palade's buffered osmium tetroxide fixative, embedded in Epon, and stained in uranyl acetate and lead citrate for electron microscopic examination.

## Results

### (A) Clinical (Table I)

Muscle weakness and atrophy were clinically apparent in only three patients (Cases 7, 12, 13), one of whom (Case 12) was on concurrent low dose corticosteroid (5 mg. prednisone daily). Three (Cases 3, 4, 12) demonstrated sclerodermatous involvement of the skin overlying the tested deltoid muscle.

### (B) Mean Potential Duration Studies

The normal range of mean potential duration as ascertained in the nineteen control subjects is shown

TABLE I  
SYSTEMIC SCLEROSIS: 15 PATIENTS STUDIED

Case No.	Age (yrs)	Sex	Duration of Disease (yrs)	Clinical Severity	Clinical Myopathy	L.E.-Cells	Antinuclear Factor	Electromyograph "abnormality"
1	39	F	15	+	0	0	+	
2	72	F	1	++	0	0	+	+
3	42	F	12	+	0	0	0	
4	28	F	1	+++	0	+	+	
5	66	F	7	+++	0	0	+	
6	61	M	20	++	0	0	0	
7	49	F	10	+++	+	+	+	+
8	58	M	17	+	0	0	+	
9	34	F	5	++	0	0	+	
10	62	F	15	+	0	0	+	
11	33	F	16	+++	0	0	+	
12*	51	F	5	+++	+	0	0	+
13	30	F	6	+	+	0	0	
14	66	F	5	++	0	0	+	
15†	39	F	3	+	0	+	+	+

\*Recent prednisone therapy—5 mg. daily  
†Recent prednisone therapy—10 mg. daily

in Fig. 1. As in other similar studies there is a gradual increase in MPD with age, although the correlation coefficient here is 0.273, which is not at a level of statistical significance.

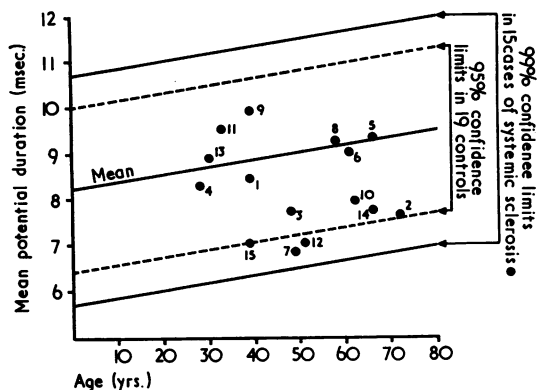


Fig. 1.—Range of mean potential duration in systemic sclerosis and in normal subjects.

Values obtained in patients with systemic sclerosis are also shown in Fig. 1. Four patients demonstrated MPDs at or just below the 95 per cent. confidence limit (Cases 2, 7, 12, and 15).

(C) Histology (Table II)

Muscle changes observed were not generally extensive. The most common abnormality was an increase in the number of centrally placed nuclei (3 to 4 per 100 fibres cut transversely being regarded as normal), the abnormality being minimal in all except Case 11. The incidence of very small atrophic fibres was marked in one biopsy and was noted to a lesser degree in six others. A slight increase in the amount of interstitial fibrous tissue accompanied these changes in a majority of cases.

Infiltration of phagocytic cells sufficient to constitute a myositis was observed in only two biopsies (Cases 7 and 12). There was little evidence of fibre necrosis and no regenerative elements were seen.

(D) Histochemistry (Table II)

Six of the biopsies showed increased activity of acid phosphatase. This was due partly to an increase in the number of macrophages present between the muscle fibres but the fibres themselves also showed raised enzyme levels. Perinuclear (lysosomal) acid phosphatase was frequently increased and in the more severely affected fibres, the enzyme was found between the myofibrils.

Oxidative enzymes (*e.g.* N.A.D. diaphorase) were generally normal. However, abnormally large aggregations of mitochondria around muscle nuclei and underneath the sarcolemma were observed occasionally.

Levels of alkaline phosphatase in capillaries and arterioles were within normal limits except in two cases. In one patient (Case 4) the enzyme was considerably raised, though the number of capillaries appeared normal. The other abnormality was in a patient (Case 12) with large foci of inflammatory cells—in these areas the actual number of capillaries was increased.

(E) Electron Microscopy

The ultrastructural changes in the muscle fibres themselves were minimal in the majority of cases. In those patients in which atrophied fibres were found at the light microscope level, however, there was some evidence of loss of myofibrils within small diameter fibres and such fibres occasionally contained areas where the breakdown of myofibrils and

TABLE II  
MAIN HISTOLOGICAL AND HISTOCHEMICAL FINDINGS IN MUSCLE FROM  
TWELVE PATIENTS WITH SYSTEMIC SCLEROSIS

Case Number	1	2	3	4	5	6	7	8	9	10	11	12
Central nuclei	+	+	+	+	—	+	+	+	+	+	++	+
Atrophy	—	+	+	+	+	++	+	—	—	—	+	—
Fibrous tissue	+	+	+	+	—	—	—	—	—	+	—	+
Phagocytic cells	—	—	—	—	—	—	+	—	—	—	—	++
Hypertrophy	—	—	—	+	—	—	—	—	—	—	—	—
Necrosis	—	—	—	—	—	—	—	—	—	—	—	—
Regeneration	—	—	—	—	—	—	—	—	—	—	—	—
Fat	—	—	—	—	—	—	—	—	—	—	—	—
Acid phosphatase	N	+	+	N	N	+	+	N	N	+	N	++
Mitochondrial NAD diaphorase	N	N	N	+	N	N	N	+	—	+	+	N
Capillary alkaline phosphatase	N	N	N	++	N	N	N	N	N	N	N	+

— Absent  
+ Minimal  
++ Moderate  
N Normal

sarcoplasmic components was severe. A moderate amount of lipofuscin (wear and tear pigment) was found, especially in those biopsies in which the acid phosphatase level was raised. The nature of the pigment granules was confirmed histochemically and its association with the enzyme acid phosphatase suggests that the granules may represent a form of cytolysosome produced as a result of autolytic activity.

Because of the reported thickening of the basement membrane material of muscle capillaries in systemic sclerosis (Norton, 1968), a linear scanning technique (Findlay and Brown, 1967) was applied to electromicrographs of muscle capillaries from eight of our patients in an attempt to assess quantitatively any change in the observed amount of basement membrane material. The amount of basement membrane substance was calculated in relation to the cross-sectional area of the capillaries. It was impossible to detect any quantitative increase in the basement membrane component in these cases as compared with a small number of measurements in normal subjects. Furthermore, the mean capillary diameter did not appear to differ significantly between control and patient specimens. However, some degree of lamination of the basement membrane was demonstrable in a high proportion of muscle capillaries (Fig. 2, opposite and overleaf).

### Discussion

Because measurement of the MPD has been shown to be an accurate index of myopathic change (Pinelli and Buchthal, 1953), we had hoped by its use to identify patients with systemic sclerosis in whom myopathy was present. However, in only four of the fifteen patients studied were MPDs obtained that might be considered abnormally short—although these values were still within the 99 per cent. limits of confidence. Two of these patients (Cases 12 and 15) had recently received adrenocorticosteroid therapy; in at least one (Case 15, on 10 mg. prednisone daily), this therapy could well have been responsible for shortening of the MPD (Yates, 1963b). The use of adrenocorticosteroids, not uncommon in systemic sclerosis, may have led to the high incidence of electromyographic abnormality previously described (Hausmanowa-Petrusewicz and Kudejko, 1961; Medsger and Rodnan, 1966), although information pertinent to this is not available in those published reports.

Clinical weakness, present in three of the fifteen patients, and "abnormal" MPD values coincided in only two patients. Furthermore, the electromyographic findings showed no correlation with the

extent of the systemic disease, its duration, or the presence or absence of either L.E.-cells or anti-nuclear antibody.

Histological abnormalities were present in nearly all biopsy specimens, but were limited primarily to an increased number of centrally placed nuclei and, to a lesser extent, muscle fibre atrophy and a slight increase in interstitial fibrous tissue. In none was severe muscle fibre necrosis or regeneration a feature. There appeared to be no difference in histological findings between patients with "normal" MPDs and those with "abnormal" electromyographic studies.

When atrophied muscle fibres were identified by light microscopy, electron microscopic study showed myofibrillar and sarcoplasmic degeneration. In contrast to previous reports, preliminary study in specimens from eight patients failed to reveal an increase in mean capillary diameter (Norton, 1968) or a quantitative increase in basement membrane substance (Norton, 1968; Michałowski and Kudejko, 1966), at least in comparison with a small number of control specimens. Confirmation of this must await further control specimen measurements since there has been, to date, no published study of these parameters in a normal population. We did find, however, some degree of lamination of the capillary of the basement membrane in a high proportion of vessels studied. The significance of this latter finding is open to speculation, but similar capillary laminar changes have been described in diabetes mellitus (Banson and Lacy, 1964) and polymyositis (Shafiq, Milhoiat, and Gorycki, 1967).

### Summary

Fifteen unselected patients (13 female, 2 male) with systemic sclerosis were studied. Shoulder girdle weakness and wasting, interpreted as clinical myopathy, was present in three. Eleven had normal electromyographic findings; four showed borderline or slight reduction of the mean potential duration. There appeared to be no correlation between the duration and severity of the disease, the presence of clinical myopathy, or electromyographic findings. It is suggested that previously reported electromyographic abnormalities may have been due to concurrent corticosteroid administration. Light microscopic abnormalities were present in a majority of muscle biopsies, but were minimal and non-specific. Where muscle fibre atrophy was noted, electron microscopic study showed frequent myofibril loss and occasional degenerative changes in the sarcoplasm. The previously described increase in capillary basement membrane substance and mean capillary diameter could not be confirmed, but we

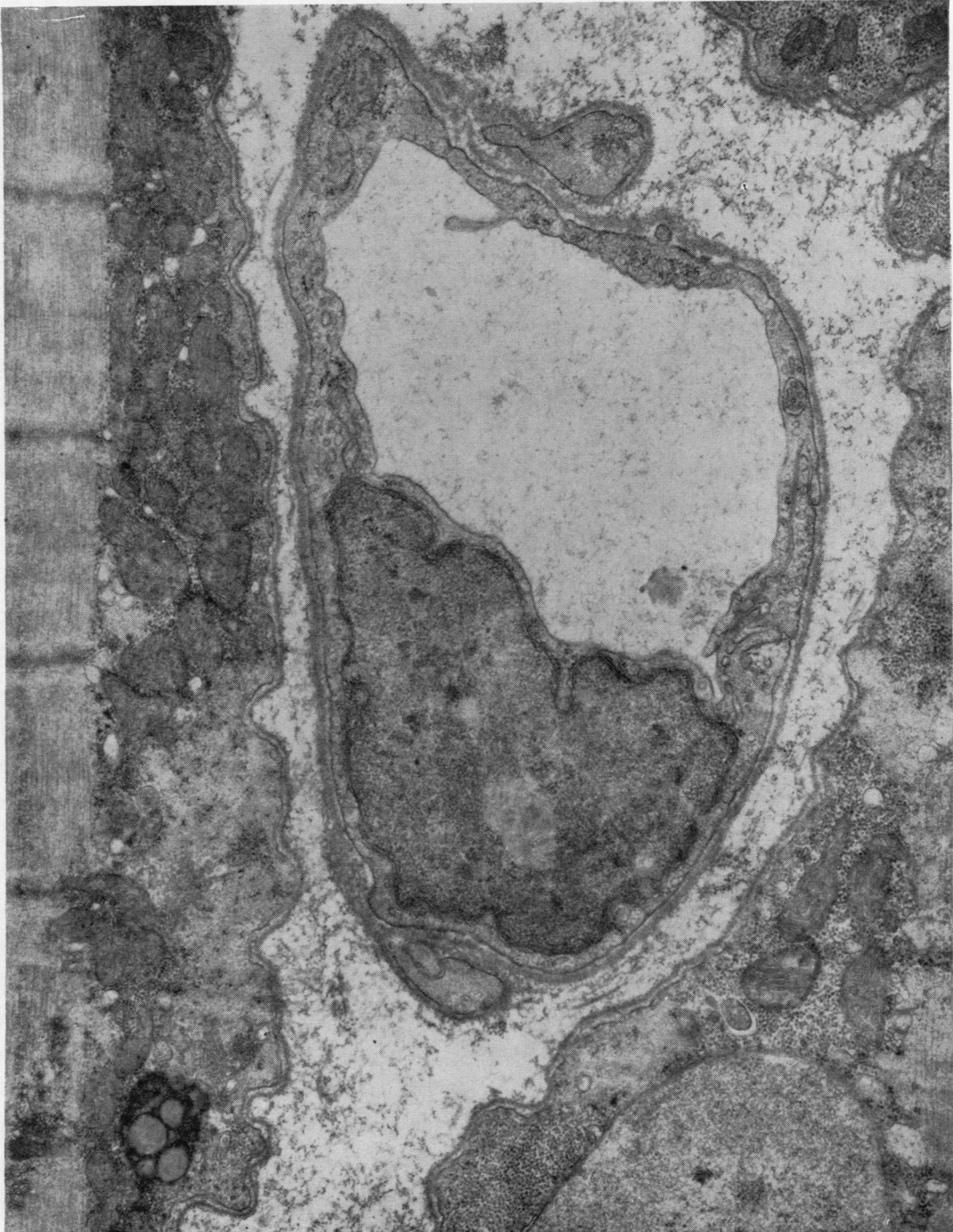


Fig. 2(a).—Electron micrograph ( $\times 24,400$ ) of muscle capillary in normal control subject.

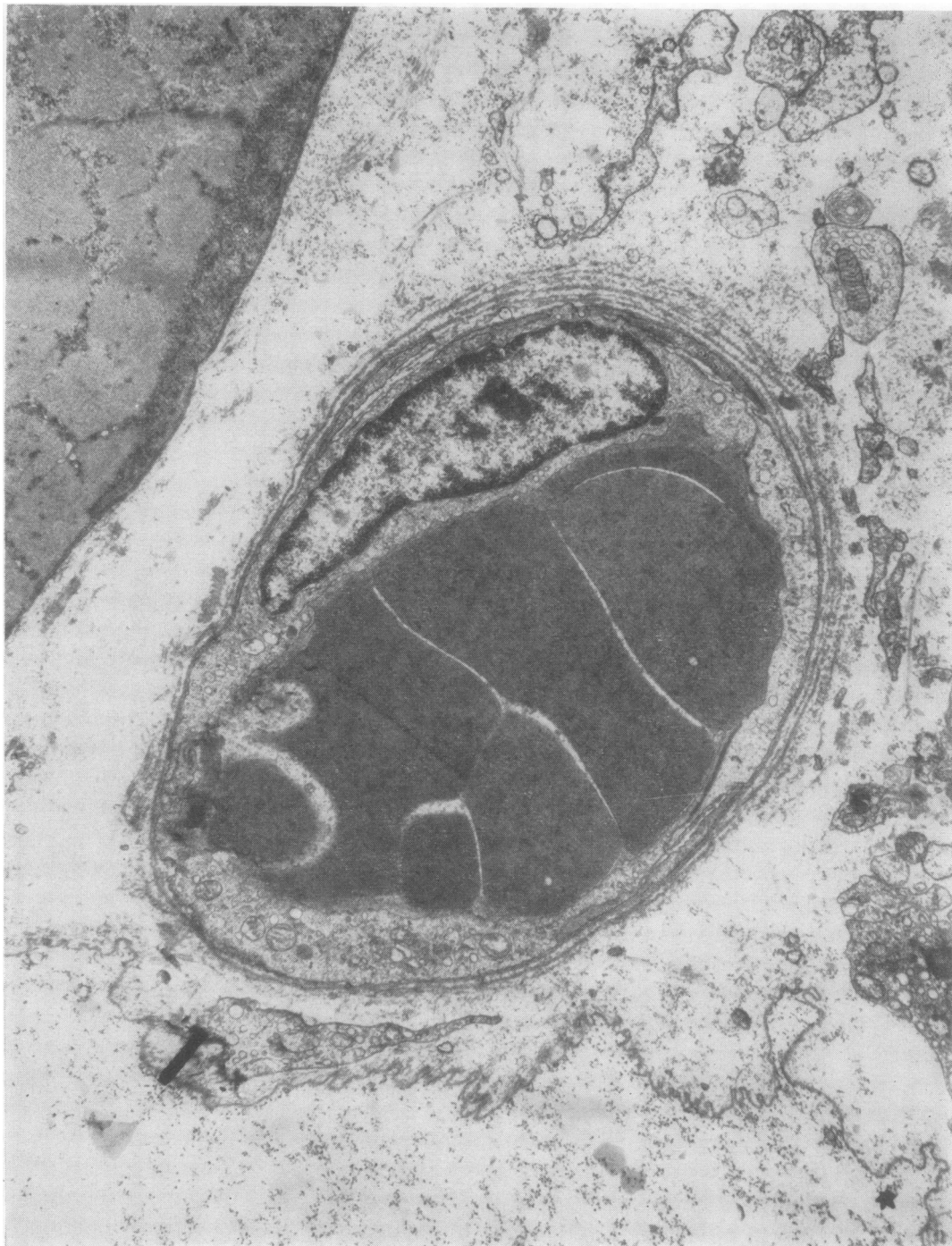


Fig. 2(b).—Electron micrograph ( $\times 9,750$ ) of muscle capillary in a patient with systemic sclerosis.

found a high incidence of capillary basement membrane lamination as noted in other electron microscopic studies.

Judged on the basis of this study, the inclusion of skeletal muscle involvement in the diagnostic

criteria for systemic sclerosis does not appear to be warranted.

Our thanks are due to Dr. Elaine Allen for her help with the electromyographic studies.

#### REFERENCES

- Banson, B. B., and Lacy, P. E. (1964). *Amer. J. Path.*, **45**, 41 (Diabetic microangiopathy in human toes).
- Burstone, M. S. (1958a). *J. nat. Cancer Inst.*, **21**, 523 (Histochemical demonstration of acid phosphatases with naphthol AS-phosphates).
- (1958b). *Ibid.*, **20**, 601 (Histochemical comparison of naphthol AS-phosphates for the demonstration of phosphatases).
- Findlay, J. A., and Brown, D. H. (1967). *J. Sci. Technol.*, **13**, 44 (Apparatus for selecting and measuring cell inclusions from electron micrographs).
- Hausmanowa-Petrusewicz, I., and Kozmińska, A. (1961). *Arch. Neurol.*, **4**, 281 (Electromyographic findings in scleroderma).
- Le Coulant, P., and Texier, L. (1957). *Brit. J. Derm.*, **69**, 299 (Histological lesions of muscle in dermatomyositis).
- Medsker, T. A., and Rodnan, G. P. (1966). *Arthr. and Rheum.*, **9**, 524 (The nature of skeletal muscle involvement in progressive systemic sclerosis [scleroderma]). Abstract of Rodnan and Medsker (1966).
- Michałowski, R., and Kudejko, J. (1966). *Brit. J. Derm.*, **78**, 24 (Electron microscopic observation in skeletal muscle in diffuse scleroderma).
- Norton, W. L. (1968), personal communication.
- , Hurd, E., Ziff, M. (1966). *Arthr. and Rheum.*, **9**, 870 (A capillary abnormality in scleroderma). (abstract).
- Pinelli, P., and Buchthal, F. (1953). *Neurology (Minneap.)*, **3**, 347 (Muscle action potentials in myopathies with special regard to progressive muscular dystrophy).
- Rodnan, G. P., and Medsker, T. A. (1966). *Bull. rheum. Dis.*, **17**, 419 (Musculo-skeletal involvement in progressive systemic sclerosis [Scleroderma]).
- Scarpelli, D. G., Hess, R., and Pearse, A. G. E. (1958). *J. biophys. biochem. Cytol.*, **4**, 747 (The cytochemical localization of oxidative enzymes. I. Diphosphopyridine nucleotide diaphorase and triphosphopyridine nucleotide diaphorase).
- Shafiq, S. A., Milhorat, A. T., and Gorycki, M. A. (1967). *J. Path. Bact.*, **94**, 139 (An electron-microscope study of muscle degeneration and vascular changes in polymyositis).
- Yates, D. A. H. (1963a). *J. Neurol. Neurosurg. Psychiat.*, **26**, 458 (The estimation of mean potential duration in endocrine myopathy).
- (1963b). *Ann. rheum. Dis.*, **22**, 342 (Muscular changes in rheumatoid arthritis).

#### L'atteinte des muscles du squelette dans la sclérose systémique

##### RÉSUMÉ

Quinze malades non-sélectionnés (13 femmes et 2 hommes) atteints de sclérose systémique ont été étudiés. Une faiblesse de la ceinture scapulaire et une atrophie, interprétées comme une myopathie clinique, étaient présentes chez 3 malades. Onze avaient une électromyographie

#### Complicación del músculo esquelético en la esclerosis sistémica

##### SUMARIO

Se estudiaron los casos de quince pacientes no seleccionados (13 mujeres, 2 hombres) con esclerosis sistémica. En tres de ellos se halló debilidad y atrofia de la cintura escapular, interpretada como miopatía clínica. Once tenían registros electromiográficos normales;



normale; quatre montraient très peu de réduction ou bien une légère réduction de la moyenne de la durée du potentiel. Il semblait qu'aucune corrélation n'existait entre la durée et la sévérité de la maladie, la présence de la myopathie clinique, ou les observations électromyographiques. Il est suggéré que les anomalies électromyographiques rapportées antérieurement pouvaient avoir été dues à l'administration simultanée des corticostéroïdes. Des anomalies vues au microscope optique étaient présentes dans la majorité des biopsies musculaires, mais étaient minimes et non-spécifiques. Là où l'atrophie des fibres musculaires avait été notée, l'étude au microscope électronique montrait des pertes fréquentes de myofibrilles et quelquefois, une dégénérescence du sarcoplasme. L'augmentation, décrite antérieurement, des capillaires de la couche sous-épithéliale et de la moyenne du diamètre capillaire n'avaient pu être confirmées, mais nous avons trouvé une forte incidence de laminage de la couche sous-épithéliale capillaire comme il a été noté dans d'autres études faites au microscope électronique.

D'après la base de cette étude, l'inclusion de l'atteinte des muscles du squelette dans les critères diagnostiques de la sclérose systémique ne semble pas être justifiée.

cuatro mostraron reducciones ligeras o dudosas de la duración potencial media. Parecía no haber correlación entre la duración y la severidad de la enfermedad, la presencia de miopatía clínica o los registros electromiográficos. Se sugiere que las anomalías electromiográficas notadas previamente quizá se deban a la administración corticosteroide concurrente. Anormalidades microscópicas leves se hallaban presente en una mayoría de biopsias musculares, pero eran mínimas y no específicas. Allí donde se notó atrofia de las fibras musculares, un estudio con microscopio electrónico reveló pérdida frecuente de miofibrilla y cambios degenerativos esporádicos en el sarcoplasma. No pudo confirmarse el aumento de la substancia membranosa en la base capilar y del diámetro capilar medio, pero hallamos una alta incidencia de laminación membranosa en la base capilar, según se ha notado en otros estudios con microscopio electrónico.

Juzgado sobre la base de este estudio, la inclusión de la complicación del músculo esquelético en el diagnóstico de la esclerosis sistémica no parece tener justificación.