Skeletal muscle abnormalities associated with occupational exposure to mercury vapours

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Summary. There is scarce information on the possible effects of chronic exposure to mercury on skeletal muscle. Dental personnel are frequently exposed to inhalation of metallic mercury vapours. The skeletal muscle of five technicians and one dentist (females, age 36-55) was studied. All of them presented symptoms of chronic mercury poisoning. Needle biopsy was taken from the quadriceps femoris muscle and samples were prepared for light microscope histochemistry and for transmission electron microscopy. Selective atrophy of type IIB muscle fibres was found in patients, and in one of them there was fibre grouping. Most of the muscles showed increased fibre area per capillary. Atrophy was confirmed by the ultrastructural study, demonstrating increase of intermyofibrillar spaces, loss of myofibrils or complete disappearance in some fibres, and sarcolemmal foldings. Splitting of the fibres was also found. Some capillaries were altered, showing endothelial infoldings into the lumen, thickened basement membrane and partial or total occlusion. The alterations found in muscle may be secondary to nerve damage, to ischemia caused by capillary lesion and/or to a direct effect of mercury on muscle fibre proteins.

Key words: Mercury toxicity, Muscle fibre types, Muscle ultrastructure, Muscle histocytochemistry

Introduction

It is well known that dental personnel, both dentists and technicians, are frequently exposed to inhalation of metallic mercury vapours when handling and preparing amalgam filling and dental restorations (Sullivan and Krieger, 1992; U.S.D.H.H.S., 1993; Rumack et al., 1997). The repeated exposure in ill-ventilated rooms, can eventually produce chronic mercurialism. The symptoms of mercury-induced toxicity include loss of appetite, nausea, diarrhoea, ulceration of oral mucosa,

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gingivitis; neurological signs like tremors, irritability, memory deficits, decreased motor functions and muscle reflexes, and renal damage manifested as mild proteinuria and enzymuria, glomerular dysfunction and nephrotic syndrome (Sullivan and Krieger, 1992; U.S.D.H.H.S., 1993).

Metallic mercury is oxidised to the divalent inorganic cation in the red blood cells and lung, but may also occur in all other tissues. In the brain the divalent form becomes trapped due to the difficulty of crossing the barrier. The kidney is the organ with the highest mercury bioaccumulation (Zalups et al., 1993). Although mercury can be deposited in muscle, as shown in pig (Raszyk et al., 1996), dolphin (Augier et al., 1993) and fish (Gill et al., 1990), to our knowledge the effect produced in human skeletal muscle has not been described. The muscular symptoms of mercury poisoning have been thought to be secondary to nervous system damage.

In the present work we examined the skeletal muscle of a group of dental personnel that presented symptoms of chronic exposure to mercury and we found muscle lesions that are not necessarily secondary to neurological involvement, but which could be the consequence of vascular or direct muscle fibre damage.

Materials and methods

Six women, aged 36 to 55 years were studied. Five of them were dental technicians and the other one a dentist. They had been handling mercury for over 16 years and all of them presented some of the clinical symptoms of mercurialism, which were headache, hair loss, muscular pain, tiredness, dermatitis, nausea, vomiting, mood changes, loss of libido, memory deficits, motor disturbances and visual alterations. The dental technicians had all retired from work at least for a period of time, and three of them were treated with a chelator agent (Table 1).

The levels of mercury were determined in urine by the diphenyl tiocarbazon method (Taylor and Crable, 1977).

Muscle biopsy was taken from the vastus lateralis

Table 1. Characteristics of the patients.

PATIENT No.	AGE	URINARY LEVEL OF MERCURY (μg/L)	TREATMENT
1	36	65	α-mercaptopropionyl glycine stop work for a period of time
2	44	67	stop work for a period of time
3	43	35	α-mercaptopropionyl glycine stop work for a period of time
4	53	13	stop work for a period of time
5	55	140 (seven years before) 60	α-mercaptopropionyl glycine stop work for the last 5 years
6	41	25	homeopathy did not stop working

Table 2. Fibre type proportion (%).

PATIENTS	I	II A	II B	II C
1	34 *	37	29 *	0.5
2	39 *	45 *	16	
3	45	28	23	4 *
4	42	31	27 *	
5	62 *	21 *	17	
6	50	41	9	
N.V.	51±10	33±10	16±8	1.0

N.V.: Normal values. Mean±SD from Nygaard (1981). *: difference with normal values >1 Standard deviation

Table 3. Fibre area (μm^2)

PATIENT	I	II A	II B
1	5509**	5818**	4000*
2	4115	3278	1874*
3	3914	4715*	3182
4	4750*	5286**	2838
5	4528	3167	3187
6	3217	2080**	1655**
N.V.	3907 ± 651	3581± 732	3100± 618

N.V.: Normal values. Mean±SD from Nygaard (1981). *: difference with normal values >1 Standard deviation. **: difference with normal values >2 Standard deviations

part of quadriceps femoris muscle, with the Bergström needle (Bergström, 1962) The sample was divided in two parts. One was stretched between two pins, covered for 5 min with 3% glutaraldehyde in phosphate buffer, pH 7.4,̇ 320 mOsmol, cut into 2 mm diameter pieces and used for electron microscopy. After 30 min the muscle was post-fixed in 1% OsO_4 for one hour, pre-stained with uranyl acetate, dehydrated, infiltrated in propylene oxide and epoxic resine, and embedded in LX-112 resin (LADD Res. Inc., Burlington). Sections were cut with a diamond knife in a Porter-Blum MT2-B ultramicrotome and stained with uranyl acetate and lead citrate. Sections were observed in a Hitachi H-500 transmission electron microscope at an accelerating voltage of 100 kV.

The second portion of the biopsy was embedded with OCT compound (Tissue Tek II) and frozen in isopentane cooled with liquid nitrogen. Transverse 10 μm sections were cut in a cryostat at -20 °C and mounted on cover slips for staining for adenosine triphosphatase (ATPase), after alkaline (pH 10.3) and acid (pH 4.37, 4.6 and 4.8) preincubation (Brooke and Kaiser, 1970). Serial sections were also stained for reduced nicotinamide dinucleotide diaphorase (NADH-d, Novikoff et al., 1961), α-glycerophosphate dehydrogenase (α-GPD, Wattemberg and Leong, 1960) and haematoxylin-eosin.

Capillaries were visualised by the α-amylase-PAS reaction (Andersen, 1975). Photomicrographs at a final magnification of X200 were taken from these sections and the fibres were identified by comparison with the ATPase sections. An area of the photograph was delimited, measured with a planimeter, and fibres and capillaries were counted to calculate the mean area of the fibres, capillaries/mm^2, and capillaries/fibre ratio. All the fibres of one type were drawn together, and the area was measured by planimetry to calculate the mean area of each fibre type.

Results

In Table 1 the levels of mercury in urine of the subjects are presented.

All the subjects showed fibre type distribution that could be considered normal with the criterion that their proportion differed less than two standard deviations from the normal values reported by Nygaard (1981) for sedentary women (Table 2). Only patient 3 showed an increased proportion of IIC fibres. Patient 5 had a high proportion of type I fibres that showed grouping.

The main finding with light microscopy was the

Fig. 1. Serial sections of vastus lateralis part of quadriceps femoris muscle. Patient No. 1. **a.** ATPase reaction, preincubation pH 10.3. **b.** ATPase reaction, preincubation pH 4.6. **c.** ATPase reaction, preincubation pH 4.37 plus PAS-α-amylase reaction. **d.** haematoxylin-eosin stain. **e.** NADH diaphorase reaction. **f.** α-GPD reaction. Stars: type I fibre. Asterisks: type IIB fibre. Open circles: type IIA fibre. In b and c black triangles show IIB fibres with reduced cross sectional area. Note in d, between arrowheads, splitting of a fibre. Bar: 50 μm.
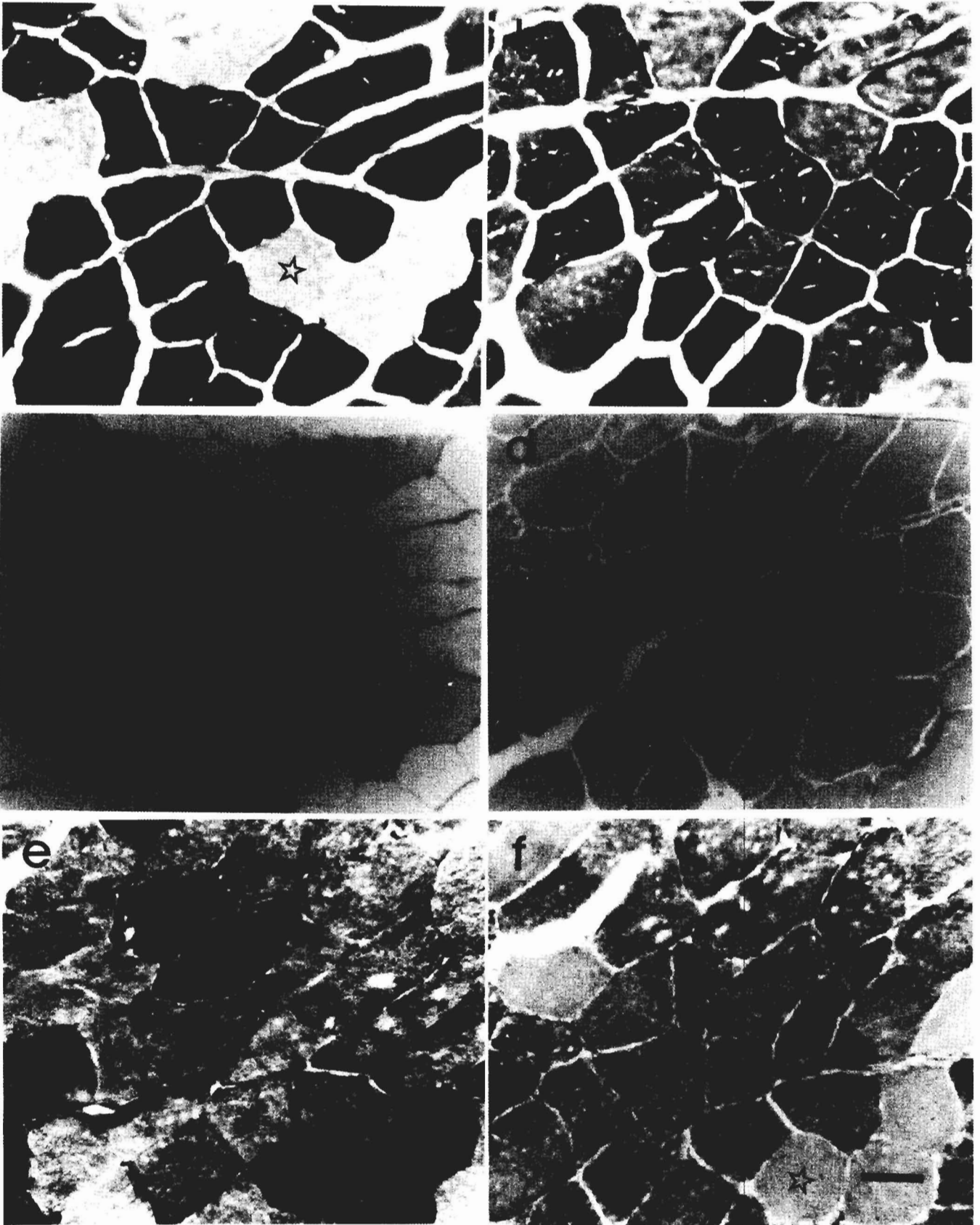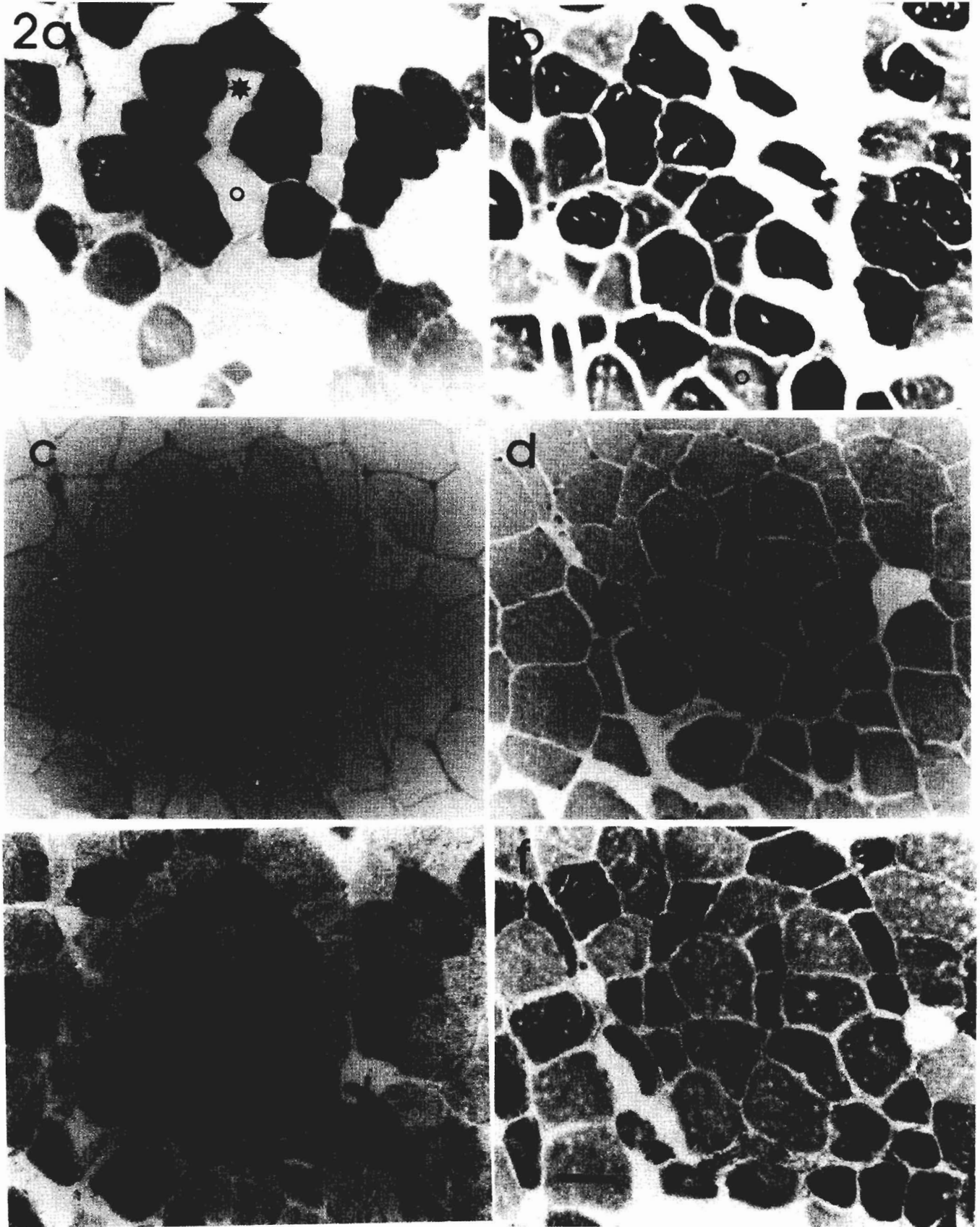
Fig. 2. Serial cross sections of vastus lateralis part of quadriceps femoris muscle. Patient No. 6. **a.** ATPas reaction, preincubation pH 4.37. **b.** ATPase reaction, preincubation pH 4.6. **c.** ATPase reaction, preincubation 4.37 plus PAS-α-amylase reaction. **d.** Haematoxylin-eosin stain. **e.** NADH-diaphorase reaction. **f.** α-GPD reaction. Fibre types as in Fig. 1. Note the reduced area of fibre type IIB (asterisk). Bar: 50 μm.

selective atrophy of type IIB fibres shown by the decrease in area of those fibres. Atrophy was marked in patients 2 and 6 (Fig. 2), and moderate in patients 1 (Fig. 1), 3 and 5; patient 4 did not show any sign of atrophy. Most fibres were of normal size (Fig. 1), however, some IIB fibres were smaller (Fig. 1b,c), and fibre splitting is also shown (Fig. 1d). In table 3 the results of measurements of cross sectional area are shown. Patient 6 showed a frank reduction in the area of types IIA and IIB fibres. Although the other patients had normal or even high mean areas, the examination of the sections showed the presence of some small IIB fibres in patients 1, 3 and 5, which were not reflected in the mean values.

The results of capillary evaluation are shown in Table 4. No changes were found in capillary density, or in capillary per fibre index. However, when mean area of a fibre type was divided by the mean number of capillaries surrounding that fibre type (mean fibre area per capillary), a deficit of capillaries was demonstrated in all patients except number 5.

The study of the ultrastructure revealed signs of atrophy, from moderate to severe. For comparison has been included a section of normal muscle (Fig. 3). Intermyofibrillar spaces were widened and sarcomere width was decreased (Fig. 4). The sarcolemma showed foldings (Figs. 5, 6). In some fibres muscle basement membrane was thickened (Fig. 6). Fibres with marked atrophy lost their myofibrils, their nuclei were hyperchromatic, and some of these fibres were fragmented (splitting) (Figs. 7, 8). Glycogen granules were abundant, not only in the muscle fibres but also in the capillaries (Figs. 4-8). Lipofuscin granules (residual bodies) were also frequent (Fig. 6). Abnormal capillaries were found, showing infoldings into the lumen (Fig. 5), partial or total occlusion (Fig. 8) and in some instances thickened basement membrane (Fig. 8).
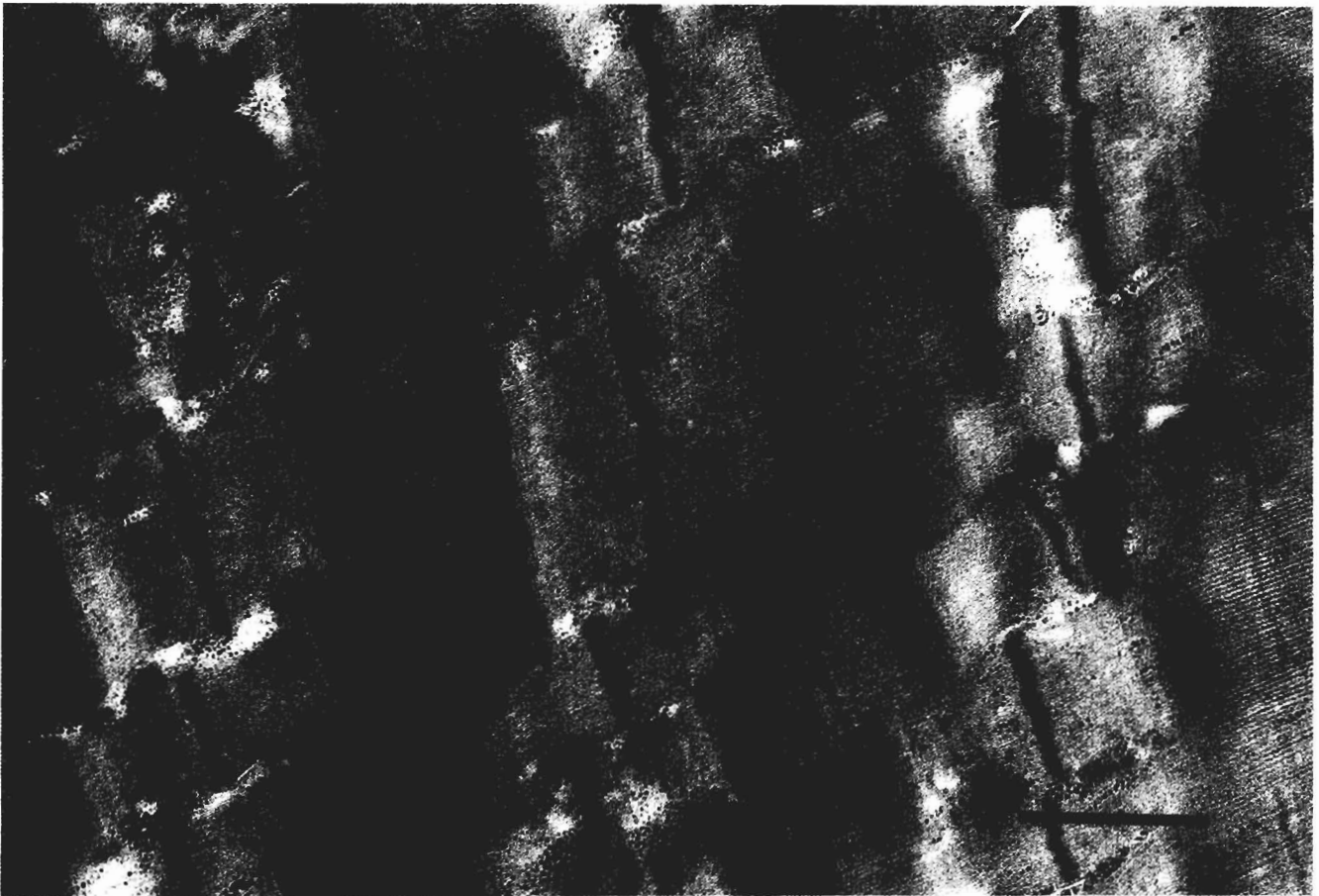

Fig. 3. Electronmicrograph of a longitudinal section of vastus lateralis part of a normal quadriceps femoris muscle. Note that there is very little space between adjacent myofibrils (arrows).

Discussion

The mean total mercury level in urine of the general population is 4 μg/L (U.S.D.H.H.S., 1993). Patient 4 had the lowest level (13 μg/L) and though she presented clinical symptoms of mercury toxicity, no lesions were found in muscle. Patients 1, 3 and 5 were treated with α-mercaptopropionyl glycine, and showed moderate atrophy of type IIB fibres, although on electron microscope examination some muscle fibres and capillaries were found with severe damage (Fig. 8). In patient 5 a higher proportion of type I fibres was present and the fibres were grouped; this may have been the consequence of a previous selective destruction of type II fibres or to nerve damage with fibre grouping produced by reinervation. The severest atrophy of type

Table 4. Capillaries per mm², capillaries per fibre, capillaries adjacent to each fibre type, and mean fibre area per capillary.

PATIENTS	CAP/mm²	CAP/FIBRE	CAP. ADJACENT TO FIBRE TYPE			MEAN FIBRE AREA PER CAPILLARY		
			I	II A	II B	I	II A	II B
1	284	1.38	3.80	4.30	3.10	1450**	1353*	1290**
2	278	0.91	3.30	2.60	1.60*	1247**	1261*	1171**
3	259	1.07	3.03	2.04*	1.33*	1291**	2311**	2392**
4	258	1.40	3.04	2.62	2.54	1563**	2018**	1117**
5	331	1.39	4.10	3.70	3.40	1104	856	937*
6	347	1.07	2.49	1.80 *	1.31*	1292**	1156	1263**
N.V.	301±58	1.2±0.3	4.0±1.1	3.7±1.1	2.9±0.9	976±120	968±200	785±100

N.V.: normal values; Mean±SD from Nygaard (1981). *: difference with normal values >1 Standard deviation. **: difference with normal values >2 Standard deviations.
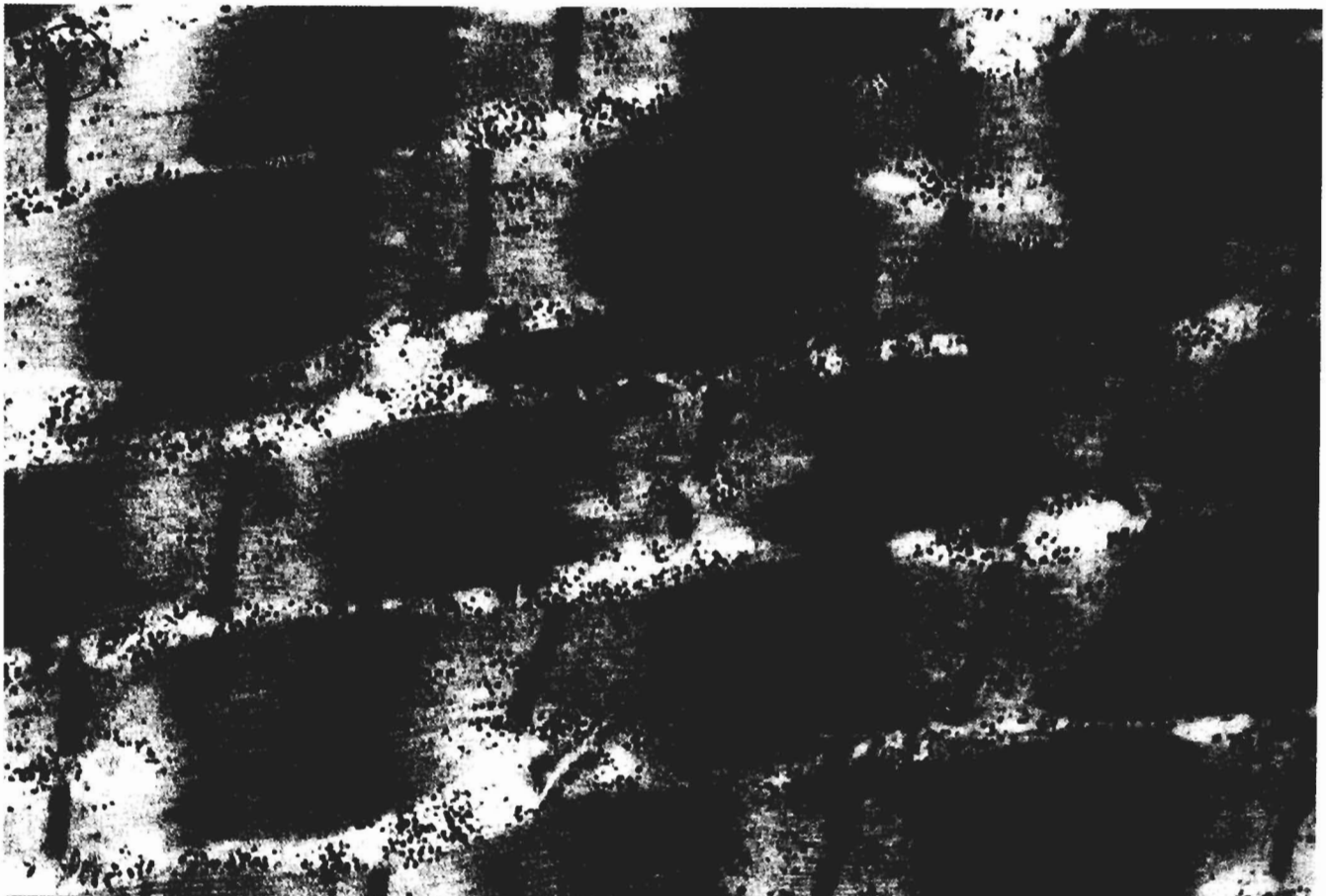

Fig. 4. Electronmicrograph of a longitudinal section of vastus lateralis part of quadriceps femoris muscle. Patient No. 1. Moderate atrophy of some muscle fibres (arrow). Note increased space between myofibrils. Bar: 1 μm.

IIB fibres was found in patients 2 and 6, which had not received treatment with chelator agents, and in the case of patient 6, the beginning of the symptoms (2 years before) was closer to the time when the biopsy was taken.

The mechanism of mercury action is believed to be its high affinity to sulfhydril groups of protein. These groups are an integral part of the structure of many proteins. Therefore, the precise effect of mercury is not easy to point out. It is possible that mercury affects structural proteins, transport processes and that it inactivates various enzymes (U.S.D.H.H.S., 1993). Wroblewski et al. (1995) have found that mercuric chloride produced changes in membrane permeability of cultured myoblasts, increasing intracellular sodium and chlorine concentrations together with morphological changes like folding and perforations of the membrane and shrinkage or flattening of the myoblasts. In rat papillary muscles mercury chloride produces changes in contractile force, time to peak tension and potentiation of post rest contractions, which were attributed to dose-dependent toxic effects on heart muscle via actions on the sarcolemma, the sarcoplasmic reticulum, and contractile proteins (Oliveira et al., 1994). Modulation of enzyme activities produced by mercury chloride, was found by Gill et al. (1990) in muscle of the Rosy barb.

The atrophy of large or small groups of fibres, characteristic of muscle denervation (Dubowitz, 1985) was not found in this group of patients. Only one patient showed fibre type grouping that could suggest nerve involvement. On the other hand, capillaries were damaged, showing infoldings protruding into the lumen, basement membrane thickening and partial or total closure. Atrophic changes of muscle fibres together with capillary damage is found in a wide range of muscle pathology, i.e. autoimmune diseases (Finol et al., 1990) or in the paraneoplastic phenomenon (Finol et al., 1997). These findings point out to muscle alterations secondary to ischemia. In the case of the present results, muscle fibre damage produced by a direct effect of mercury on muscle fibre proteins cannot be ruled out.

Selective atrophy of IIB fibres is seen in many muscular diseases, especially in those accompanied by muscle disuse (Dubowitz, 1985). However, in the cases
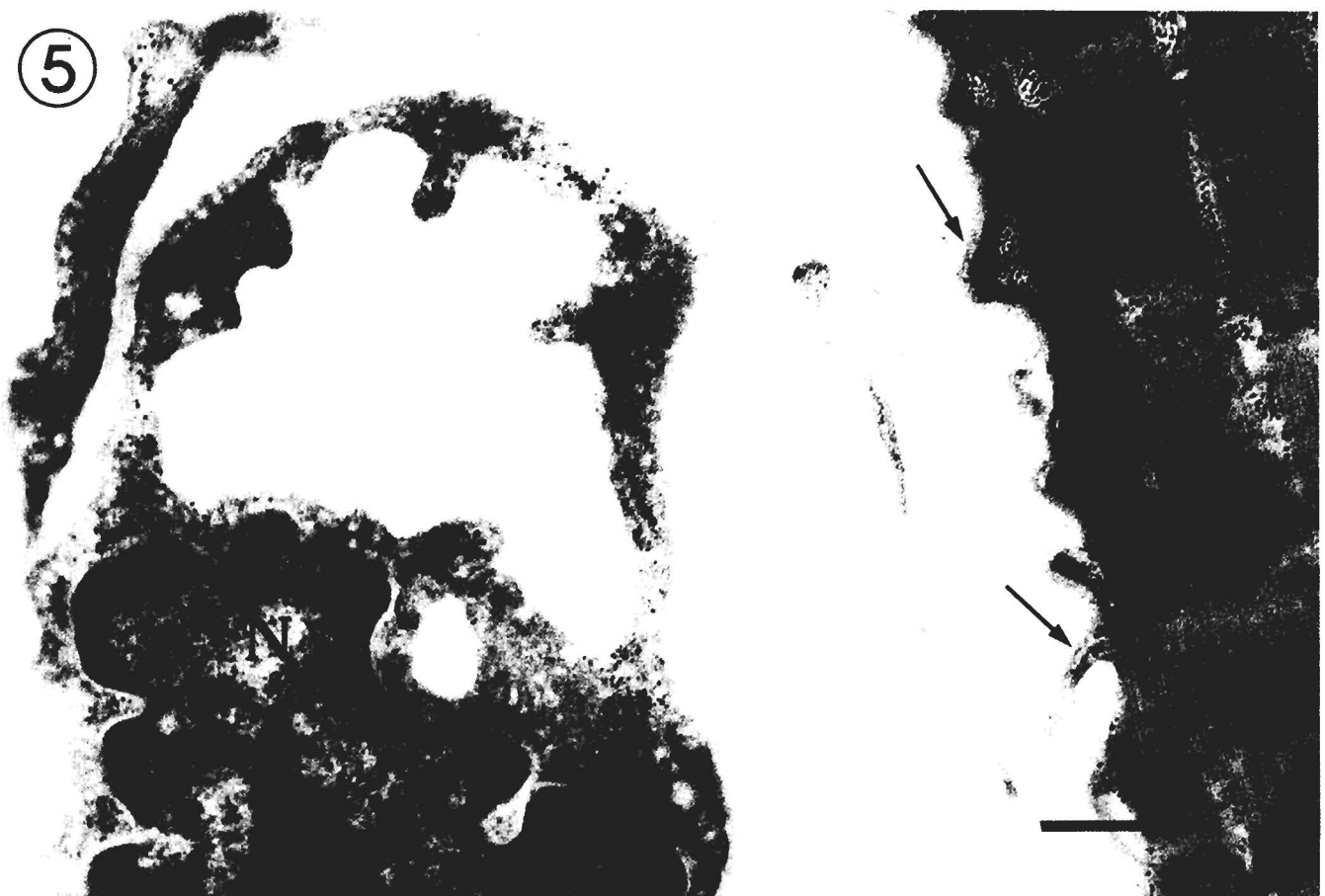

Fig. 5. Electronmicrograph of a longitudinal section of vastus lateralis part of quadriceps femoris muscle. Patient No. 2. M: muscle fibre. Note the folding of the sarcolemma (arrows). E: Endothelial cell of the capillary. Note the infoldings into the lumen (asterisks). N: Endothelial cell nucleus; P: pericyte. Glycogen: open circles. Bar: 1 μm.
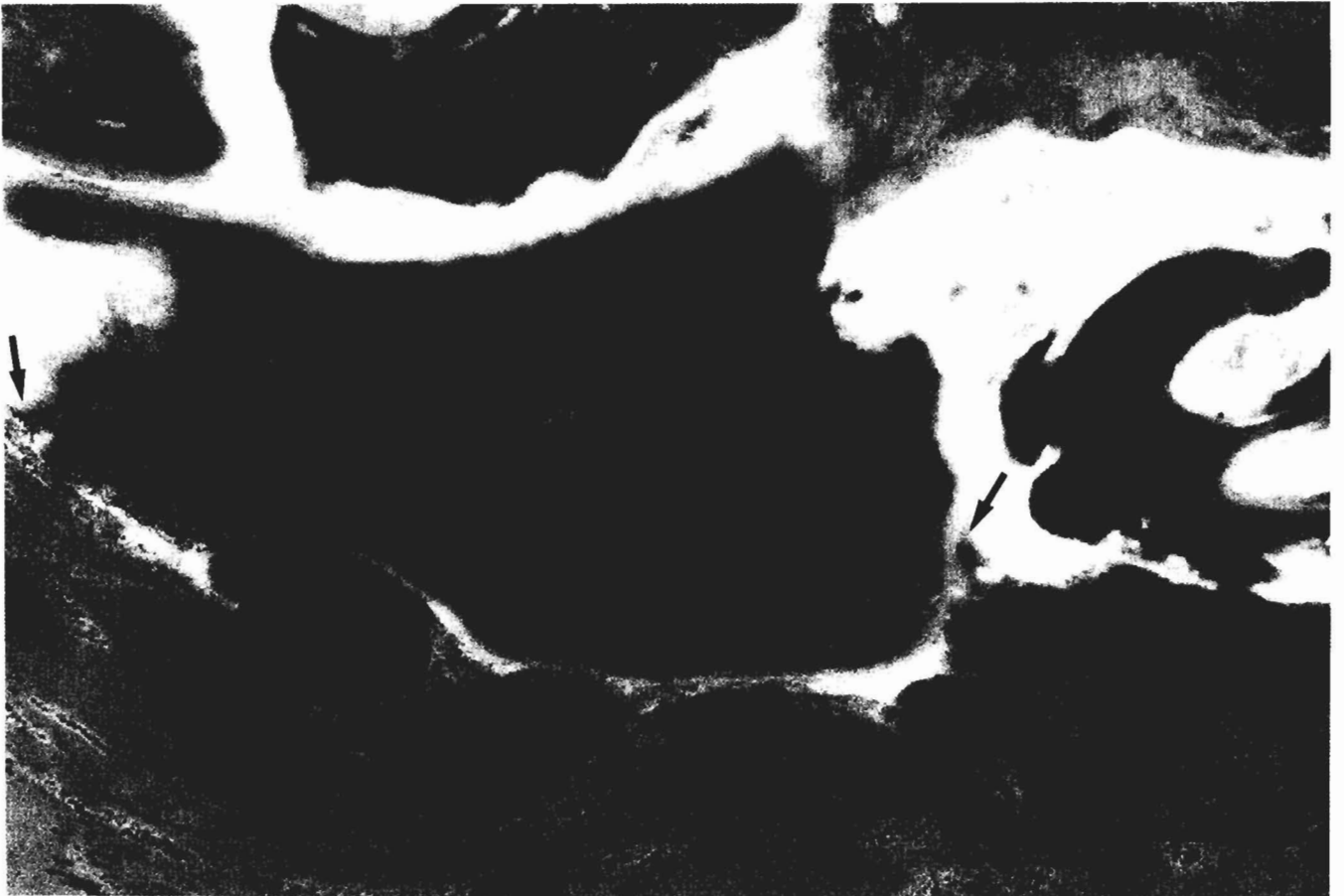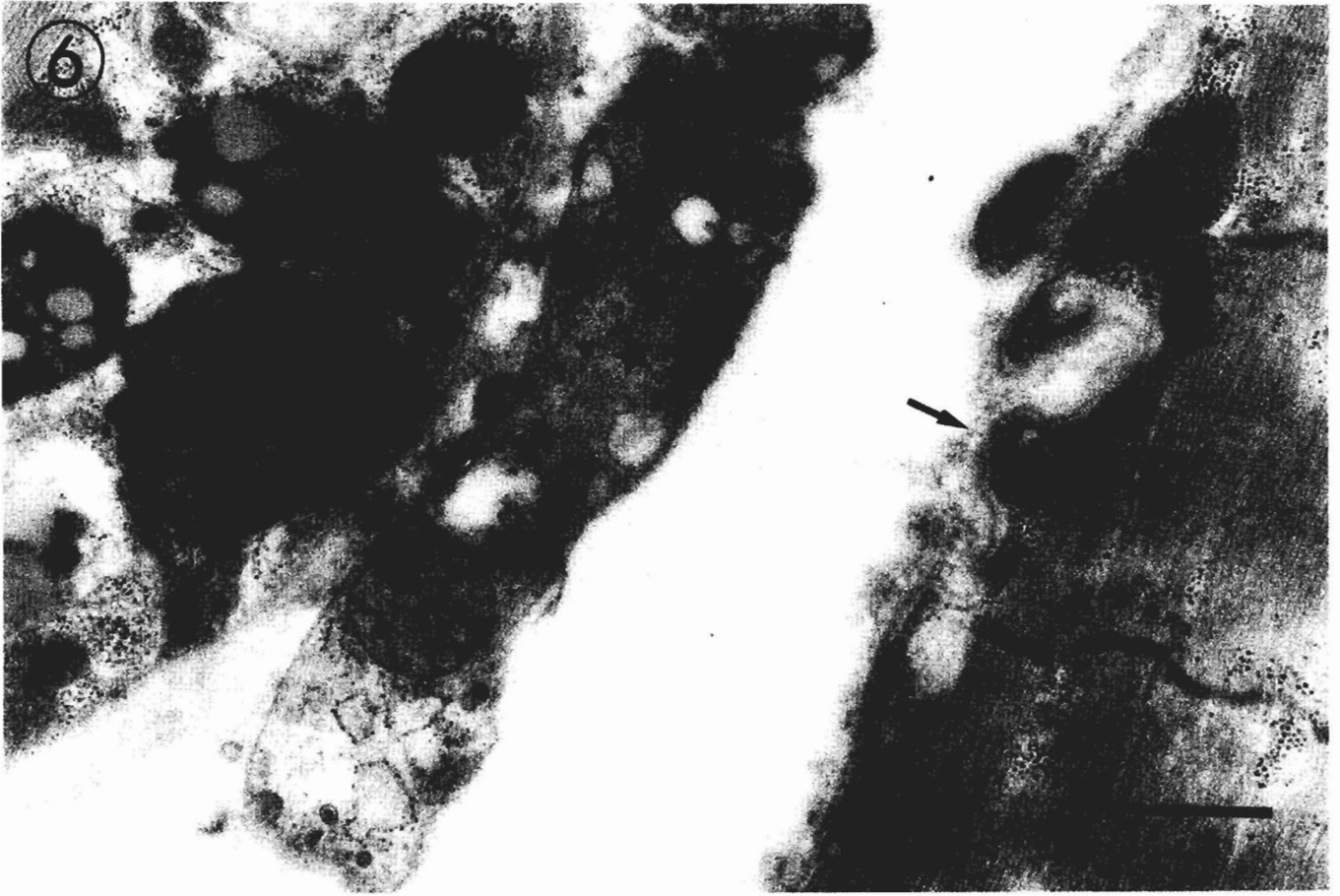
Fig. 6. Electronmicrograph of a longitudinal section of vastus lateralis part of quadriceps femoris muscle. Patient No. 1. M: muscle fibre. Note the thickening and folding of the sarcoplasma (arrow). Macrophage: star. L: Lipofuscin granules (residual bodies). Bar: 1 μm.

Fig. 7. Electronmicrograph of a longitudinal section of vastus lateralis part of quadriceps femoris muscle. Patient No. 6. M: muscle fibre with loss of structure. Splitting of the fibre is shown between arrows. N: hyperchromatic nucleus. Bar: 1 μm.
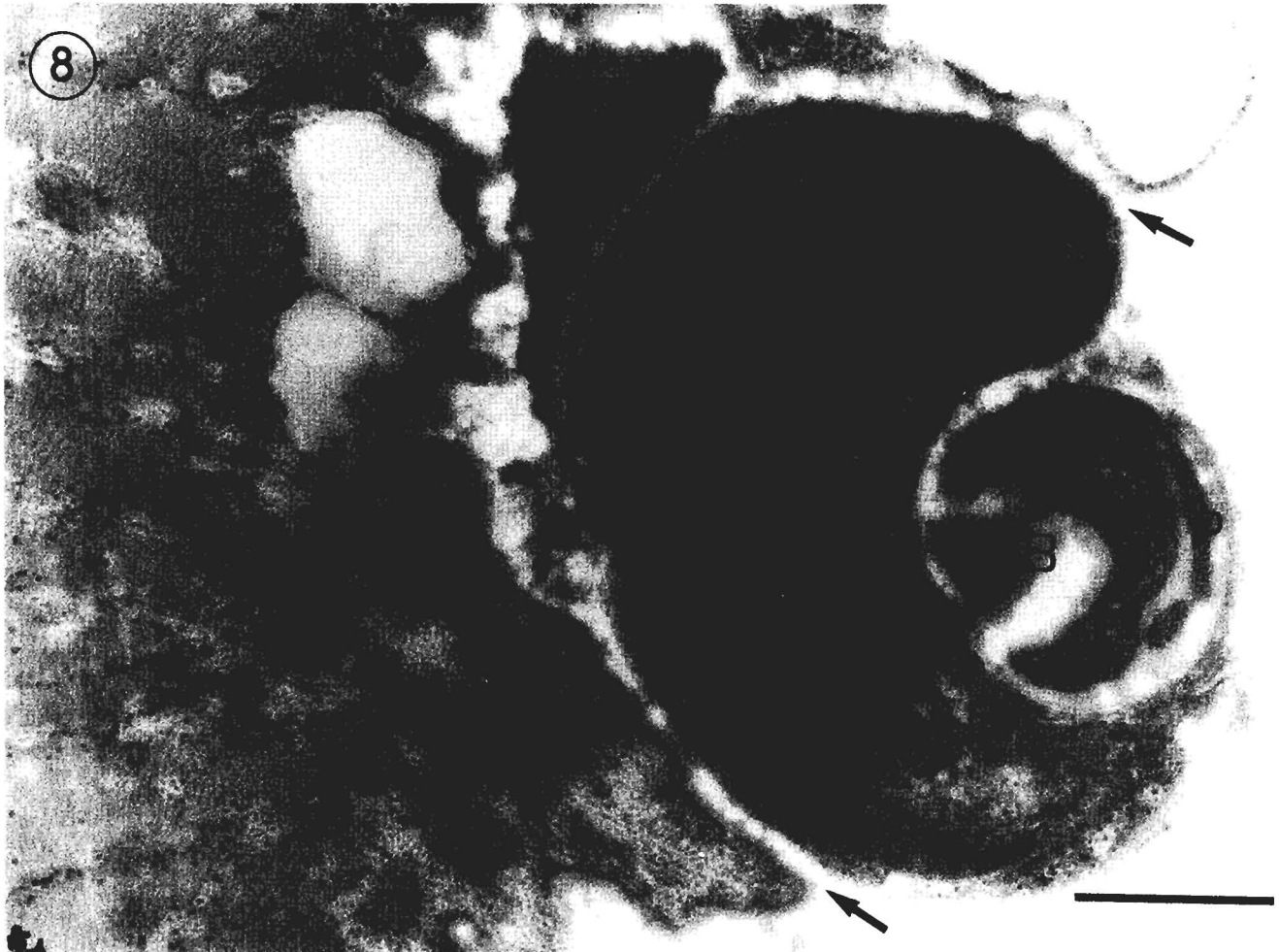

Fig. 8. Electronmicrograph of a longitudinal section of vastus lateralis part of quadriceps femoris muscle. Patient No. 3. M: muscle fibre with loss of structure. Note the extracelular space (arrows) separating a fragment of fibre containing the nucleus (splitting of muscle fibre). C: occluded capillary; N: hyperchromatic nucleus, B: thickened basement membrane of the capillary; P: pericyte. Bar: 1 μm.

presented, there was no particular decrease in the use of leg muscles.

In conclusion, chronic exposure to mercury vapours in dental personnel was associated with muscle damage in the 5 patients with the highest levels of urinary mercury. Selective atrophy of IIB muscle fibres was found, some fibres showed loss of structure, and capillary damage was present. The lesions seemed to be less widespread in the patients that were treated with a chelator agent.

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