when given for 5 days or less and increased biotransformation when administered for 4 weeks.

Many chemicals produce a biphasic change in biotransformation[5]. In these cases, an initial inhibition is followed by a stimulation 24–48 h after treatment. In the case of 2,6-DNT, an inhibition of biotransformation was observed 24–72 h after the last dose of 2,6-DNT. This was followed by a stimulation in metabolism when rats were fed 2,6-DNT for 4 weeks.

1 J.R. Fouts, Tox. appl. Pharmac. *17*, 804 (1970).
2 B. Testa and P. Jenner, in: Drug Metabolism Chemical and Biochemical Aspects. Marcel Dekker, New York 1976.
3 B.N. LaDu, H.G. Mandel and E.L. Way, in: Fundamentals of Drug Metabolism and Drug Disposition. The Williams and Wilkins Company, Baltimore 1971.
4 A. Goldstein, Biostatistics. Macmillan, New York 1964.
5 R. Kato, E. Chiesara and P. Vassanelli, Biochem. Pharmac. *13*, 69 (1964).

Thick filament size changes in contraction of human muscles[1]

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Summary. Measurements done on electron micrographs show that in myofibres with sarcomeres contracted to below 2.1 μm, proportional shortening of the A bands occurs. In muscles from patients with idiopathic scoliosis very short A bands are especially prominent.

In the course of a study of muscle biopsies taken from patients with scoliosis (lateral displacement and rotation of the spine) of varying etiology, several hundred human muscle specimens from various locations have been examined[3,4].

As part of the study myofilament dimensions were measured on electron micrographs greatly enlarged by projection. It was noted that while Z line and M band thickness and M band periodicity were fairly constant, the A bands and sarcomeres varied considerably. Significant differences were found in measurements of these 2 in muscle specimens from idiopathic scoliosis (adolescent disease of unknown etiology) as compared to muscles obtained from cases of secondary scoliosis (mainly paralytic) and from non-scoliotic patients (mainly spondylolysthesis) (table 1). Since this finding was unexpected and not easily explainable a more thorough examination of additional muscles in different states of contraction was undertaken.

Materials and methods. Specimen preparation for electron microscopy is known to cause many artifactual modifications. Numerous preliminary experiments, testing the effect of various modes of fixation on human muscle were therefore carried out.

In all the cases, biopsy specimens were obtained during corrective spinal surgery while the patients were under general anaesthesia using tubocurarine for respiratory control and nitroprusside to produce hypotension. Samples from the deep paraspinal, trapezius and the glutei muscles, were usually taken isometrically while those from deltoids and quadriceps by needle biopsy only. The muscle specimen (1.5×0.3 cm) was tied in situ to a sterile wooden 'matchstick', excised, and fixed while still splinted, in 2% glutaraldehyde at room temperature. The buffer was 0.1 M cacodylate (pH 7.4). In most of the samples no buffer rinsing or postosmication was done. The material was dehydrated rapidly and embedded in araldite. Postosmicated and unsplinted muscle samples were also examined.

Some muscle specimes were stretched to 130–150% of their in situ length by tying and cutting one end only at first and then pulling on it before applying the second suture and excising the specimen. Others were obtained in a stretched state by tying the muscle to the splint after flexing or extending the limb to vary the muscle length.

Longitudinal sections were cut at right angles to the fibre axis onto formvar-coated and uncoated 300 mesh grids. The sections were stained with uranyl and lead and photographed with a Philips 300 EM using both 35 mm film and 60×90 mm cut film.

3–5 blocks with good orientation were selected from each muscle sample and in each, 10–40 myofibres were measured (averaging at least 10 sarcomeres per myofibre). The enlargement was measured by repeated photography of a replica grating. Final measurements were performed in several ways. In one technique the photographic negatives were projected onto a screen in a large room at magnifications of half to one million times (table 1). This was done to assess intrafilamentous periodicities (especially the well defined ones of the M band) and to minimize the errors arising from the difficulty of defining the edges of the A band. Most negatives were examined with lower magnifications while in some a microdensitometer (Joyce-Loebl) was used to obtain photodensimetric graphs of the sarcomere structures and measurements were made on the tracing.

Results. As can be seen from tables 1 and 2 normal human muscles, splinted at slack rest length and kept splinted for glutaraldehyde fixation (whether osmified or not) has sarcomeres around 2.2 μm and A bands ranging from 1.35 to 1.60 μm. Cutting out a piece of muscle, fixing and further treating it freely, resulted in sarcomere shortening to

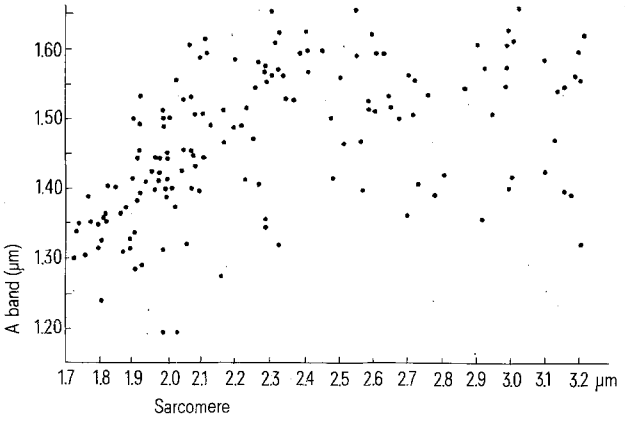

Fig. 1. Scattergram of myofibres in 3 normal muscles fixed in various ways. In sarcomeres longer than 2.1 μm the A bands remain fairly constant. At short sarcomere lengths the A bands become correspondingly shorter. Sarcomeres below 1.75 μm were very rare.

around 2 μm, while needle biopsies produced excessively contracted sarcomeres and very short A bands.

In the isometrically taken samples the results were within the limits reported by others[5–8] and therefore the degree of shrinkage attributable to specimen preparation is unlikely to have exceeded 15%. The shrinkage was not eliminated by keeping the muscles splinted throughout dehydration and embedding. It is noteworthy that the appearance of sections was not uniform. In each sample sarcomeres were seen to be contracted, relaxed and even extended, in the same or neighbouring myofibres. The A bands too were of variable length being notably shorter in very contracted sarcomeres. Occasionally, short A bands were also seen in an apparently relaxed state. When muscles held stretched during fixation were compared with slack, unrestricted or osmium contracted ones, the A bands correlated more obviously with the sarcomere length (figures 1, 2a and b).

Figure 1 is a cumulative scattergram of A bands plotted against sarcomeres from normal control gluteus, trapezius and paraspinal muscles. It shows theat in sarcomeres below

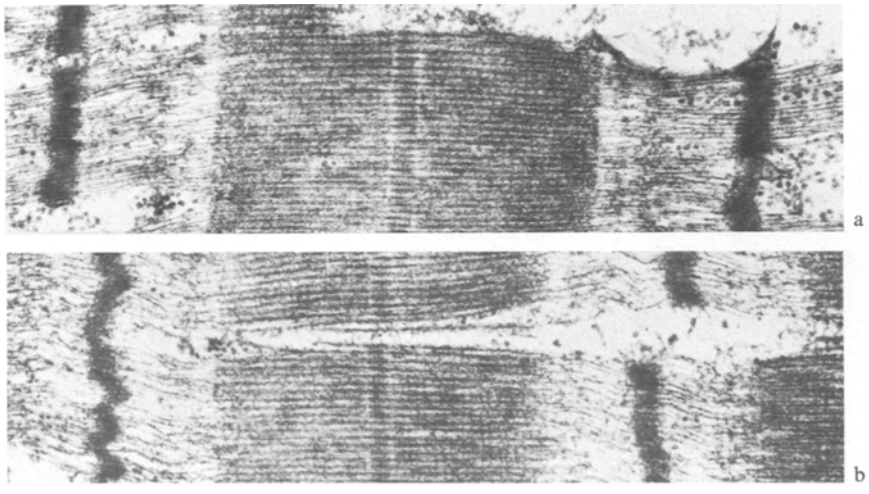

Fig. 2. *a* and *b* Electron micrograph of 2 samples from the same normal muscle fixed in glutaraldehyde (no postosmication). The upper myofibre is stretched; its sarcomere is 3.05 μm, the A band is 1.63 μm. The lower one has a sarcomere of 2.40 μm and an A band of 1.43 μm. × 30,000.

Table 1. Measurements of myofilaments at very high magnifications in paraspinal and gluteal muscles of patients and controls (mean ± SD)

	Idiopathic scoliosis (58 muscles from 20 patients	Secondary scoliosis and normal controls (36 muscles from 12 patients)	Statistical significance (Student's t-test)
M band period (Å)	199 ± 20	202 ± 19	NS
M bands (Å)	1153 ± 120	1186 ± 110	NS
Z lines (Å)	955 ± 170	1019 ± 160	NS
Sarcomeres (μm)	1.83 ± 0.20	2.14 ± 0.21	$p < 0.001$
A bands (μm)	1.30 ± 0.14	1.46 ± 0.09	$p < 0.001$

Table 2. Effect of specimen preparation on normal gluteal and spinal muscles (tied in situ at 'slack' rest length or removed freely)

	Sarcomere (μm)	A band (μm)
Splinted throughout all procedures (including dehydration and embedding) (42)	2.22 ± 0.23	1.47 ± 0.09
Splinted for glutaraldehyde fixation, freed for dehydration and embedding (41)	2.44 ± 0.44	1.48 ± 0.12
Splinted for glutaraldehyde fixation, freed and then osmified, etc (18)	2.43 ± 0.54	1.46 ± 0.11
Unsplinted glutaraldehyde fixation etc (no osmication) (46)	2.03 ± 0.23	1.44 ± 0.11
Needle biopsies from deltoids, trapezius and quadriceps (± osmication) (33)	1.89 ± 0.34	1.36 ± 0.27

Mean ± SD. In brackets are number of myofibres measured. (In each myofibre 10 sarcomeres were averaged, they were taken from 3–4 different blocks in each muscle.)

Table 3. Sarcomere and A band measurements (μm) in various muscles of patients with scoliosis

		Idiopathic Sarcomere	A band	Paralytic Sarcomere	A band
Paraspinal	Concave	(26) 1.85 ± 0.33	1.34 ± 0.17	(11) 2.22 ± 0.31	1.47 ± 0.15[b]
	Convex	(25) 2.11 ± 0.37[a]	1.36 ± 0.26	(12) 2.32 ± 0.16	1.46 ± 0.09
Gluteus	Concave	(20) 1.80 ± 0.28	1.36 ± 0.19	(6) 1.91 ± 0.16	1.43 ± 0.10
	Convex	(4) 1.72 ± 0.16	1.34 ± 0.19	(2) 2.02 ± 0.12	1.45 ± 0.11

The figures are means ± SD for each muscle type. In brackets are number of patients. [a]The sarcomeres of paraspinal muscles on the convex side are longer than those of the concavity ($p < 0.05$). [b]The A bands and sarcomeres of muscles in idiopathic scoliosis are shorter than in paralytic scoliosis ($p < 0.01$).

2.2 µm the A band length changed directly with the sarcomere (coefficient of correlation R = 0.77), while at higher sarcomere lengths it remained fairly constant (R = 0.25). It should be noted that in these muscles from non-scoliotic patients there were few sarcomeres below 1.8 µm.

Although the conditions for taking and processing muscle samples were kept similar in all cases, in idiopathic scoliosis the measurements differed from those of paralytic scoliosis or non-scoliotic controls in that excessively contracted myofibres with sarcomeres below 1.8 µm were common, especially in paraspinal muscles from the concave side of the deformity and the glutei (table 3, figures 3 and 4, a).

In addition, very short A bands were frequently seen in relatively relaxed as well as markedly shortened sarcomeres. They sometimes had frayed edges and a 'spool-like' shape (figure 4, b).

Discussion. The excessive and asymmetric state of contraction (? tonicity) of muscles in idiopathic scoliosis, and the thick filament alterations may be artefactual. Nevertheless a disease-related difference in muscle reactions to external agents suggests an inherant myogenic abnormality which would contribute towards the genesis of the deformity by an uneven muscle action. Other expressions of myogenic pathology have been recorded in histometric and biochemical studies[3,4].

The finding of A band variability, especially in apparently normal muscles, has however more general and fundamental implications. Although the sliding filament theory of muscle contraction, which postulates a constant dimension for the A bands, is generally accepted[9-11], changes in thick filament length and thickness in relation to contraction are the rule in some species[12-14]. Reports exist of such changes occurring in mammals as well, in particular conditions[15-19]. The findings presented here suggest that A band variability occurs in human muscle also and indicate the need to investigate further the possibility of filament shortening as well as sliding in muscle action.

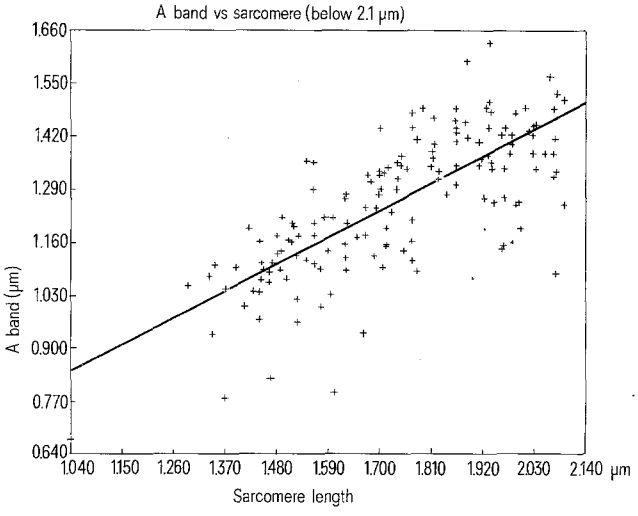

Fig. 3. Scattergram of various muscles from patients with idiopathic scoliosis with sarcomeres below 2.1 µm showing the correlation between A bands and sarcomeres.

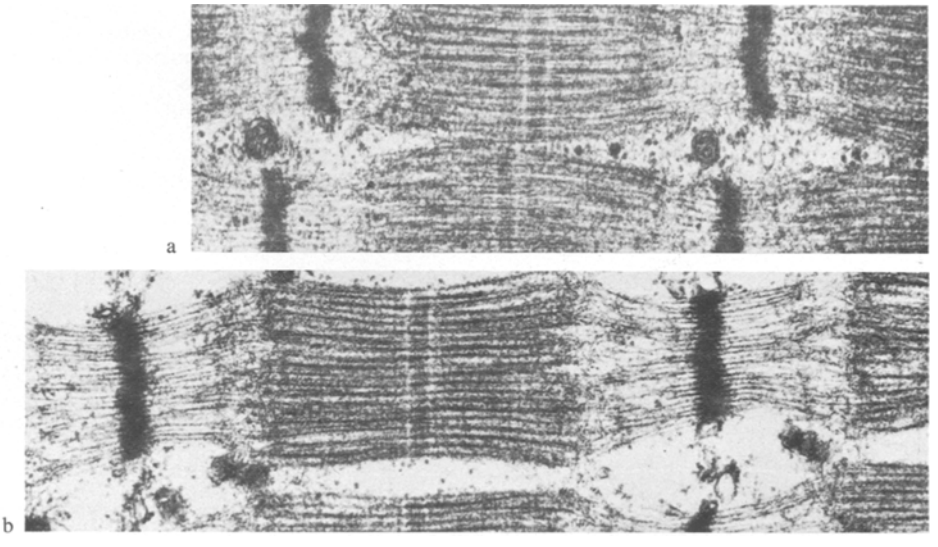

Fig. 4. *a* and *b* Electron micrograph of 2 samples from the same muscle in idiopathic scoliosis showing very short A bands. In a) the sarcomere is 1.79 µm, the A band 1.23 µm. In b) the short A band (1.28 µm) is in a sarcomere of 2.36 µm and has an abnormal appearance. The enlargement is the same as in figure 2.

1 This work was supported by the Muscular Dystrophy Association of America.
2 We wish to thank H. Orgal and Y. Havivi, for their technical assistance, O. Carmi and B. Ghidoni for their help with the measurements and N. Vagenberg for the computer analyses.
3 R. Yarom and G.C. Robin, Spine, *4,* 13 (1979).
4 A. Muhlrad, G.C. Robin and R. Yarom, Proc. 8th Eur. Muscle Club Meeting, Heidelberg 1979.
5 F. Carlsen, G.G. Knappeis and F. Buchthal, J. Biophys. Biochem. Cytol. *11,* 95 (1961).
6 S.G. Page and H.E. Huxley, J. Cell Biol. *19,* 396 (1963).
7 F.S. Sjöstrand and E. Andersson-Cedergnen, J. Ultrastruct. Res. *1,* 74 (1957).
8 R.H. Locker and N.G. Leet, J. Ultrastruct. Res. *55,* 157 (1976).
9 A.F. Huxley and R. Niedergerke, Nature *173,* 971 (1954).
10 H.E. Huxley and J. Hanson, Nature *173,* 973 (1954).
11 H.E. Huxley, Proc. Roy. Soc., Series B *187,* 131 (1970).
12 M.M. Dewey, B. Walcott, D.E. Colflesh, H. Terry and R.J.C. Levine, J. Cell Biol. *75,* 366 (1977).
13 G. de Villafranca, J. Ultrastruct. Res. *5,* 109 (1961).
14 G. de Villafranca, J. Ultrastruct. Res. *9,* 156 (1963).
15 N.A. Gabelova and K.S. Aleynikova, Biophysica *21,* 89 (1976).
16 V.P. Gilev, J. Cell Biol. *12,* 135 (1962).
17 F.S. Galey, J. Ultrastruct. Res. *11,* 389 (1964).
18 E.B. Kofman, Biophysica *21,* 491 (1976).
19 N.V. Samosudova, A. Lyndkovskay and G.M. Frank, Biophysica *20,* 445 (1975).