^{3}H Leucine Incorporation Into Myofibrils of Normal and Dystrophic Mouse Skeletal Muscle

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SUMMARY: *The study of ^{3}H leucine incorporation into skeletal muscle of mouse muscular dystrophy (129 ReJ/dy Bar Harbour strain) shows the uptake of isotope into myofibrils. The techniques employed were light and EM autoradiography before and after glycerination (Szent-Gyorgyi 1947). The results indicate a marked drop in uptake of the ^{3}H-Leucine into myofibrils in the dystrophic animals, supporting the contention of Nihei et al (1971) that reduced myosin synthesis occurs in mouse muscular dystrophy.*

RÉSUMÉ: *Notre étude sur l'incorporation musculaire de la leucine tritiée chez le souris dystrophique (souche 129 ReJ/dy Bar Harbour) a révélé l'incorporation de l'isotope dans le myofibrille. Nous avons utilisé une technique autoradiographique sur des preparations de microscopie ordinaire et d'électronmicroscopie avant et aprés extraction proteinique du cytoplasme par la glycérine (Szent-Gyorgyi 1947). Nos résultats ont indiqué une diminution importante de l'incorporation de ^{3}H leucine dans les myofibrilles chez l'animal dystrophique résultats en accord avec le observations de Nihei et al (1971).*

Les auterus ont demontré une reduction de le synthèse myosinique chez le souris atteinte de dystrophie musculaire.

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It is now well recognized that in dystrophic mouse skeletal muscle there is an increased turnover of proteins (Dreyfus, 1960; Kruh et al, 1960; Coleman, 1959; Simon et al, 1962; Srivastava and Berlinquet, 1966). Attempts to determine the nature of this increased protein synthesis have demonstrated an increased rate of incorporation of amino acids by a reduced number of large polysomes (Nihei and Monckton, 1967). More recently it has been suggested that there is a reduction in the synthesis of myosin in contrast to cytoplasmic proteins (Nihei et al, 1971).

The object of this paper is to use applied histological methods to demonstrate the uptake of ^{3}H leucine into myofibrils after the removal of soluble cytoplasmic proteins by glycerination, and to see whether there is a reduced uptake in dystrophic mice.

MATERIALS AND METHODS

Six litter mate pairs of dystrophic and normal mice of the 129 ReJ/dy Bar Harbour strain were given intraperitoneal injections of ^{3}H L-leucine (Amersham-Searle). The dose was 20u Ci per gram of body weight. The mice were five to eight weeks old and all were male. After twelve hours the mice were sacrificed by decapitation, and the erector spinae and sartorius muscles were removed. Each specimen of muscle was divided for routine light microscopy and EM autoradiography, and for glycerination.

The technique of glycerination was described by Szent-Gyorgyi (1947). The muscle was placed in a 50% aqueous glycerol solution with 5mM EDTA at pH 7.0. This was kept at 4°C for 24 hours, after which the specimen was placed in fresh glycerol solution and kept at —20°C for three weeks. The muscle was then washed in a solution of 100mM KCl and 10mM di-basic sodium phosphate for one hour at 4°C. Both glycerinated and non-glycerinated muscle were fixed in 3% glutaraldehyde in phosphate buffer at pH 7.0, post fixed in OsO_4, dehydrated in ethanol and embedded in Epon 812. 0.5% PPO (2,5-Diphenyl oxazole, Amersham-Searle) was added to the propylene-oxide/Epon mixture of the embedding procedure (Fischer et al, 1971).

Thick (1μ) sections were cut and mounted on glass slides, then dipped in Ilford L4 nuclear emulsion and kept in light-tight containers. The slides were developed after a week to ten days, the sections were stained with a saturated aqueous solution of phenylenediamine and examined under a light microscope.

Thin sections on copper grids were stained with uranyl acetate and lead citrate, mounted onto glass slides, coated with a film of evaporated carbon, then dipped in Ilford L4 nuclear emulsion. After five to six weeks of exposure the sections were developed, examined under a Zeiss EM9 and photographed (Rogers, 1969).

RESULTS

A. *Light Microscope Autoradiography:*

Grain counts were carried out over an area of 250 microns^2 for both non-glycerinated and glycerinated material in the normal and dystrophic mice. The histogram (Fig. 1) shows the results of grain counts obtained from the whole muscle. It should be noted that in the non-

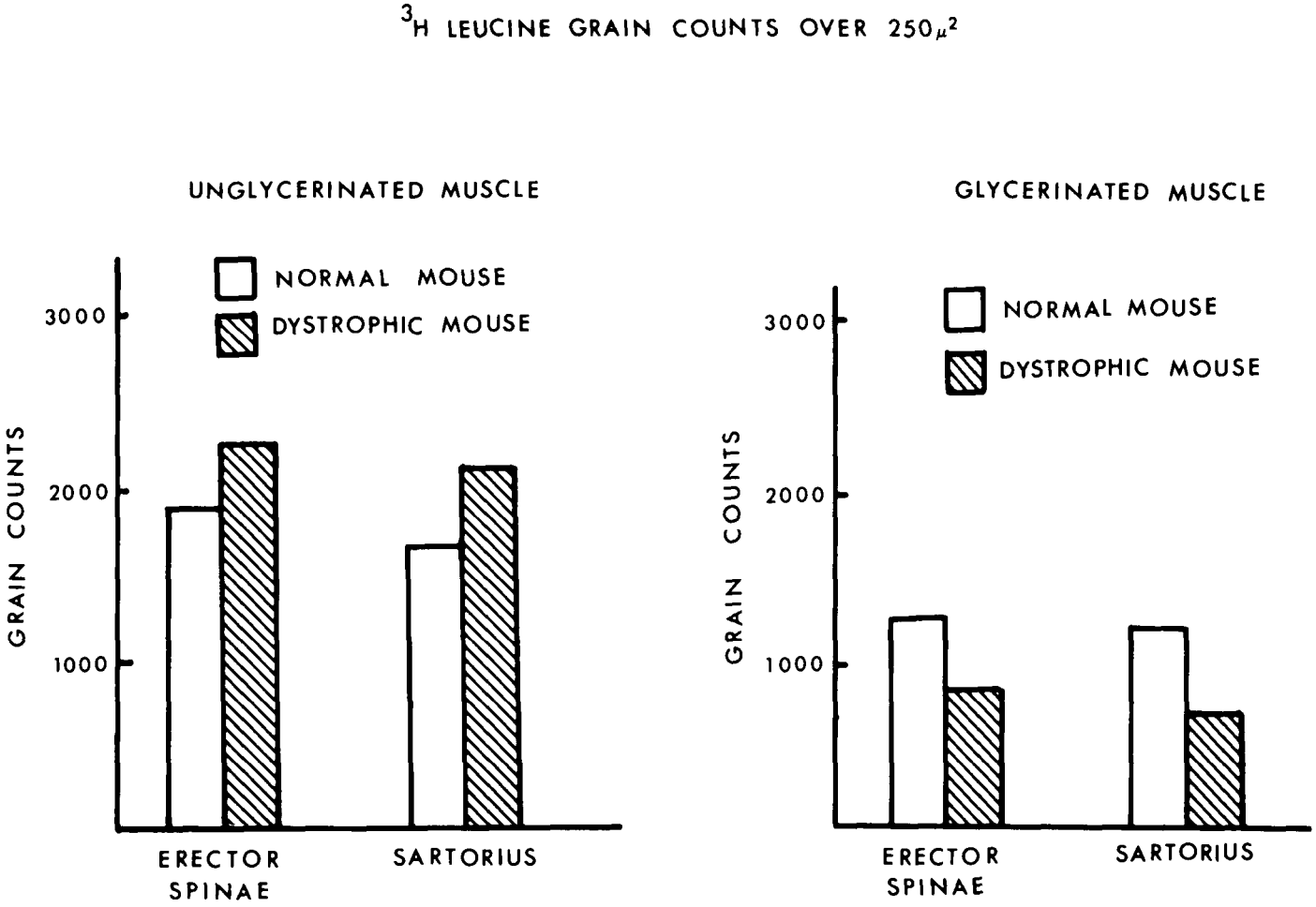

Figure 1. Histogram showing the mean grain counts of ^{3}H leucine incorporation over 250 microns^2 from normal and dystrophic mouse muscle before and after glycerination. Application of the Student's t-test showed these means to be significantly different ($p < 0.05$).

glycerinated muscle there is a definite increased uptake of ^{3}H leucine, both in the erector spinae and the sartorius muscle, which is consistent with previous findings (Monckton, 1971). After glycerination it can be seen that there is a marked reduction in the residual activity both in the normal and dystrophic mice, but that the degree of reduction is greater in the dystrophic than in normal muscles.

B. *Electron Autoradiography:*

Figures 2 and 3 are representative pictures obtained from normal non-glycerinated and glycerinated mouse muscle. Figure 2 from the normal non-glycerinated muscle shows grains adjacent to the Z band which is one of the most frequent locations for grains to be found in skeletal muscle. In Figure 3 the glycerinated preparation of muscle shows three grains present, only one of which is within the A-I junctional region, the other two being within the A band area. It can be seen that the glycerinated muscle cell appears empty save for organelles and intact myofibrils, which suggests a fairly complete removal of soluble cytoplasmic contents. Figures 4 and 5 are from the dystrophic animal. Figure 4 illustrates a non-glycerinated, and Figure 5 a glycerinated preparation. The latter again shows the effective removal of cytoplasmic contents with comparatively slight disruption of myofibrils. The positions of 450 grains were noted in both normal and dystrophic material before and after glycerination and their location was noted on a sarcomere diagram (Fig. 6). This shows, in each case, a fairly substantial reduction of grains in the I band after glycerination, suggesting this localization for synthesis of soluble proteins which are increased in the dystrophic animals.

CONCLUSIONS

The data obtained from light autoradiography shows initially a distinct increase in the uptake of ^{3}H leucine into dystrophic mouse muscle. After glycerination (Szent-Gyorgyi, 1947), which removes the majority of cytoplasmic proteins and soluble contents, there is a drop in the observed uptake which presumably represents the ^{3}H leucine which is incorporated into the myofibrils. In the dystrophic animals

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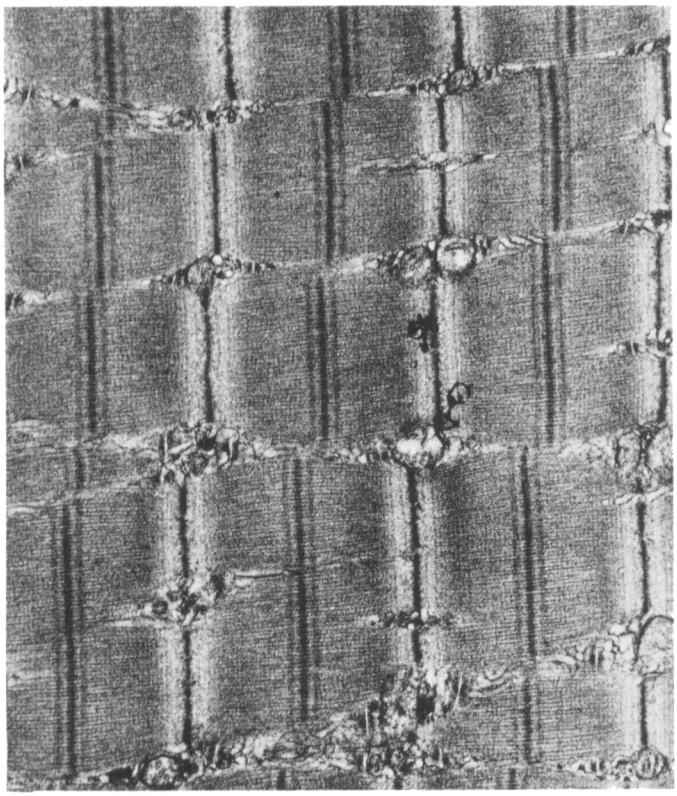

Figure 2. Normal mouse muscle with grains over Z band. X 22,200

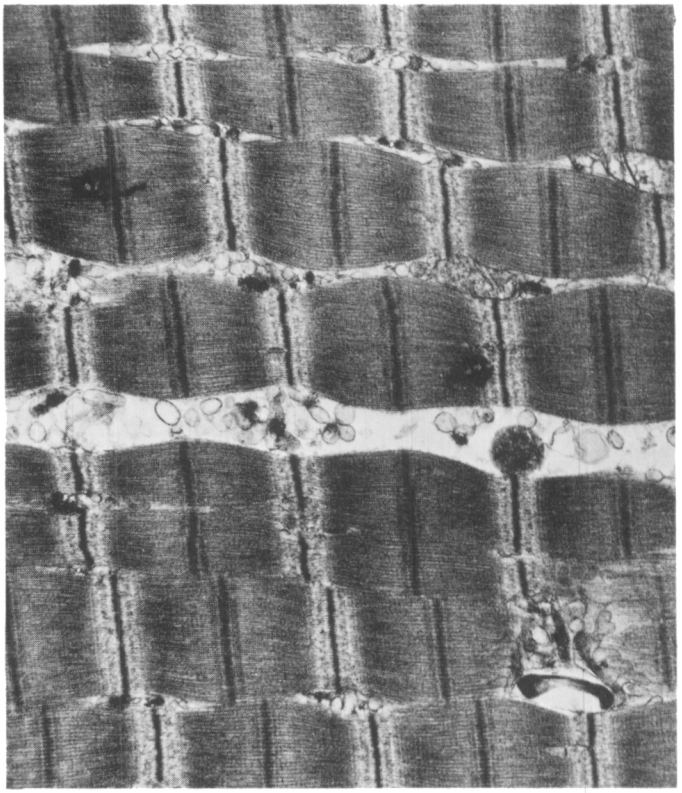

Figure 3. Normal mouse muscle after glycerination showing grains of ^{3}H leucine located over A band. Note emptiness of cell save for organelles and myofibrils. X 21,000

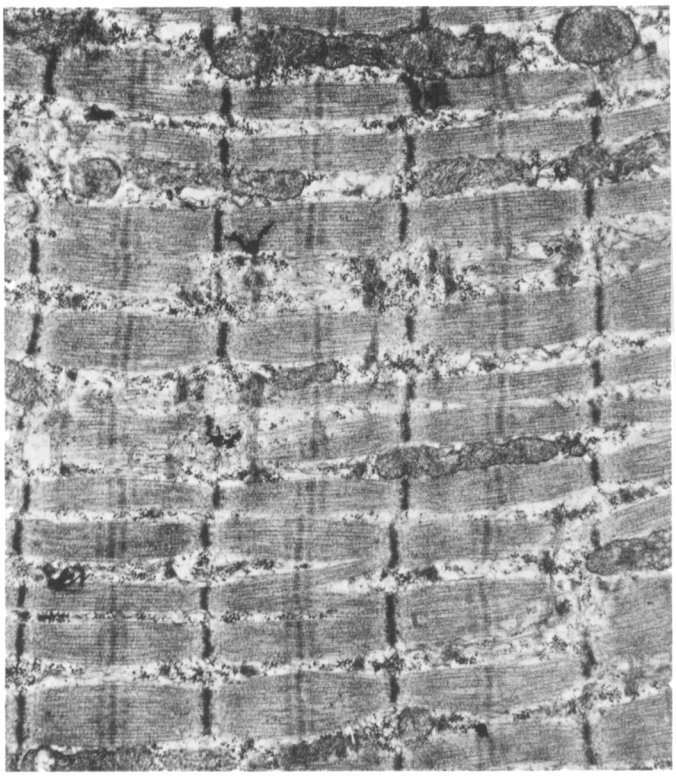

Figure 4. Dystrophic mouse muscle. ^{3}H leucine incorporation. X 17,000

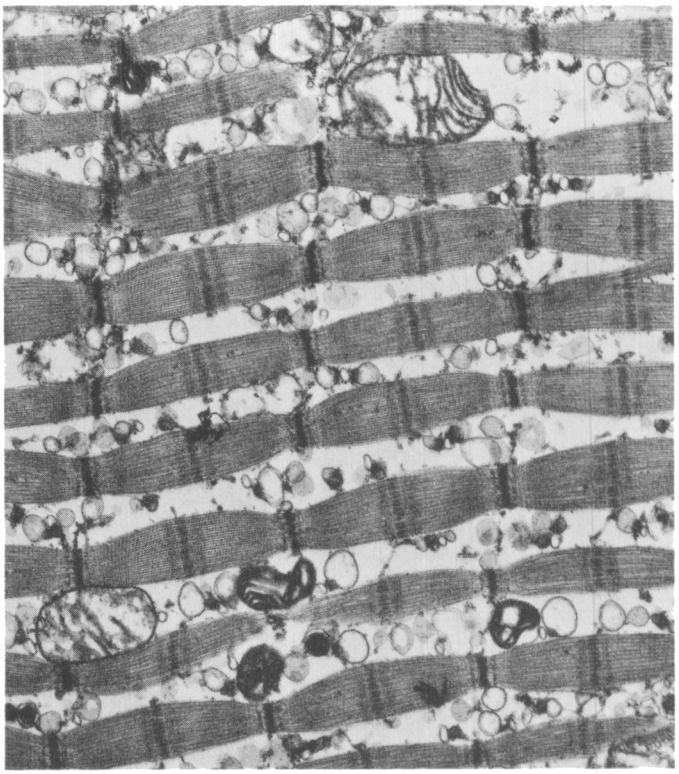

Figure 5. Dystrophic mouse muscle. ^{3}H leucine incorporation after removal of cell sap by glycerination. X 21,300

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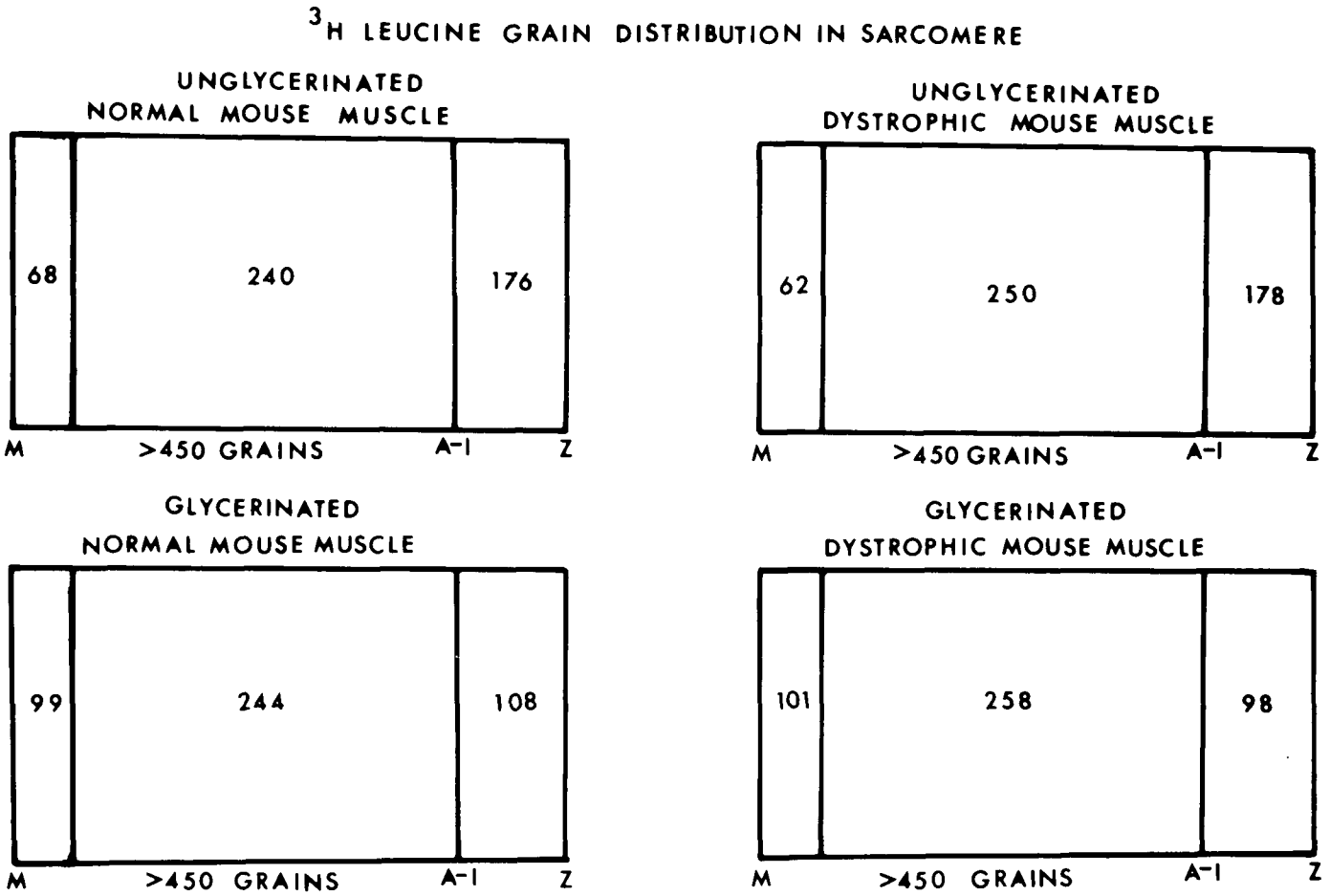

Figure 6. Plot of grains in sarcomere before and after glycerination. Note shift of grains from I band to A band.

this uptake is approximately one-half of the normal. These observations are in accord with the biochemical findings of Nihei et al (1971), suggesting a significant reduction in the synthesis of myosin in mouse muscular dystrophy. It should, however, be emphasized that we have no direct evidence as to which protein or proteins are incorporating ^{3}H leucine in the myofibril. The residual activity observed in the I band by EM autoradiography may conceivably be associated with ^{3}H leucine incorporation into actin. The question must remain open at the present time as to whether these findings represent a primary defect of synthesis or a secondary phenomenon due to a more basic disturbance of function in the muscle cell.

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