

## REFRIGERATION, FREEZING AND THAWING

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CHANGES IN THE FINE STRUCTURE OF SINGLE MUSCLE FIBRES  
AT DIFFERENT TEMPERATURES

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The effect of heat on muscle tissue, as it is applied in cooking, results in some changes in the morphology of the muscle cells or fibres which can only be observed by electron microscopy. Whereas other workers have made similar observations on blocks of heated tissue, this paper describes the changes observed in single fibres. The appearance of myofibrils and their filamentous components over a range of temperatures from 10° to 70° will be described.

DES CHANGEMENTS DE LA STRUCTURE FINE DES FIBRES MUSCULAIRES  
INDIVIDUELLES A DIVERSES TEMPERATURES

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L'effet de la chaleur sur le tissu musculaire, dans le contexte de la cuisson, provoque certains changements de la morphologie des cellules ou des fibres musculaires qui ne peuvent être observés qu'au moyen d'un microscope électronique. Tandis que d'autres chercheurs ont fait des observations semblables concernant des blocs de tissu chauffé, ce document décrit les changements observés dans des fibres individuelles. L'apparition de myofibrilles et de leurs composés filamenteux dans une gamme de températures de 10° à 70° sera décrite.

ÄNDERUNGEN DER FEINSTRUKTUR VON EINZELNEN MUSKELFASERN  
BEI VERSCHIEDENEN TEMPERATUREN

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Die Wirkung von Hitze auf Muskelgewebe, wie zum Beispiel beim Kochen, führt zu einigen Änderungen der Morphologie der Muskelzellen oder Fasern, die nur mit einem Elektronenmikroskop beobachtet werden können. Andere Forscher machten ähnliche Beobachtungen bei Blocks von angeheiztem Gewebe, jedoch soll dieser Bericht die Änderungen beschreiben, die bei Einzelfasern festgestellt wurden. Das Verhalten von Muskelfibrillen und ihrer Faserbestandteile bei einem Temperaturbereich von 10° - 70° wird beschrieben.

ИЗМЕНЕНИЯ ТОНКОЙ СТРУКТУРЫ ОДИНОЧНЫХ  
МЫШЕЧНЫХ ВОЛОКОН ПРИ РАЗНЫХ ТЕМПЕРАТУРАХ

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Воздействие теплоты, прилагаемой в процессе варки, на мышечные ткани выражается в некоторых таких изменениях морфологии мышечных клеток или волокон, которые можно обозреть только при помощи электронной микроскопии. В то время как другие исследователи подвергли этому виду исследований отдельные блоки нагретой ткани, в настоящей статье описываются изменения отмеченные в случае одиночных волокон. Описано также появление миофибрилл и их волокнистых компонентов в диапазоне температур от 10 до 70 °C.

## REFRIGERATION, FREEZING AND THAWING

Changes in the fine structure of single muscle fibres at different temperatures.

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Changes in the structure of single muscle fibres after heating, as observed by direct or light microscopy, consist of an increase in the optical density of the fibre and a change in the periodicity of the striations which is characteristic of skeletal muscle (Voyle & Restall, 1974). Such changes occur over a temperature range which is similar to that which is associated with change in water-binding properties of muscle protein, as reported by Hamm & Deatherage (1960).

The morphological changes which occur at the myofibrillar level are best resolved by the use of electron microscopy. Some work has been reported in which observations were made using strips or slices of muscle tissue from porcine muscles (Giles, 1969; Schmidt & Parrish, 1971). In using single fibres we have hoped to avoid any confounding influence of excessive amounts of connective tissue and to assess the effect of heat on myofibrillar structure.

Material & Method

The *M. Psoas* of the laboratory rat was used as a model system. Animals were slaughtered by a sharp blow on the head and held for 24 hours at 10°C. The dead animal was positioned so that its back was arched, thus exerting a degree of stretch in the *M. Psoas*. This ensured good visibility of both thin actin and thick myosin filaments in the sectioned material. Single fibres were carefully teased from the muscle and, after heating to the desired temperature in Ringer solution, were fixed in 2.5% glutaraldehyde buffered with 0.1M Na cacodylate at pH 7.2. Post fixation was carried out in similarly buffered 1% Osmium tetroxide.

Fixed fibre fragments, 2-3 mm in length, were dehydrated, and embedded in paraffin using the method of Bencosme & Tsutsumi (1970). Toluene was used as a clearing agent instead of propylene oxide. Thin sections, stained with uranyl acetate followed by lead citrate, were examined in an AEI EM6B electron microscope.

Results

The maximum temperature to which fibres were heated was 70°. At this temperature, which was attained in about 3 minutes, very marked changes had occurred in both the A-band and the I-band. The A-band had lost completely its characteristic filamentous appearance which was replaced by an amorphous electron-dense band. This band was substantially narrower than the A-band in unheated samples. The M-line, normally occupying a central position in the I-band, was obscured by the electron-dense material described above. The thin actin filaments of the I-band were completely disrupted so that the denatured protein was aggregated into a linear array at either end of the A-band and a short distance from it. Measurements suggested that the distance between these aggregates were similar to the width of the A-band prior to heating. More data are required to establish this point statistically. There was a

complete absence of Z-band material but vesicles of sarcoplasmic reticulum were still recognisable.

Samples heated to lower temperatures showed some of the changes described above but to a lesser degree. At 50° the fine periodicity of the actin filaments could no longer be observed. At 60° the Z-band had disappeared from many of the samples examined and clumping of actin filaments in the I-band had occurred. In the A-band the filamentous appearance was obscured but the M-line was still distinct. At 65°, however, the M-line was no longer visible and the width of the A-band was reduced to a little more than half its original value.

There were no detectable changes in morphology at temperatures below 50°.

Discussion

The observations made on heated single fibres are similar in some respects to those of Giles (1969) and Schmidt & Parrish (1971) on muscle tissue heated as a strip or slice. Some differences are worth noting, however. The changes in A-band structure described by Giles occurred more slowly than in our experiments where a temperature of 70° was attained in about 3 minutes. Neither Giles, nor Schmidt & Parrish, who did not state the time taken for their samples to attain the desired temperature, reported the disappearance of the Z-band. The latter group of workers used muscles which had been subject to 7 days aging, which in itself was sufficient to induce changes in the fine structure of the Z-band. These changes may have rendered the Z-band resistant to the effects of heating.

Clearly, a single fibre will reach equilibrium with its environment in terms of temperature much more rapidly than a bundle of fibres. It is feasible, therefore, that the rate of heating will determine the extent of morphological change within the fibre, just as will the temperature attained. In addition there may well be a relationship between the length of time a fibre is exposed to a particular temperature and the extent of the structural damage sustained; the higher the temperature the shorter the time required for damage to occur.

This concept can be projected into the realm of cooking practice to explain the variation in the degree of 'doneness' through a piece of meat cooked *en masse*, and the relative effects of 'slow' and 'fast' cooking. It is clear that there will be changes in the mechanical strength of the cooked tissue, compared with raw tissue, which will be reflected in the texture of the meat. The cumulative effect of increased fragmentation at the myofibrillar level will offset, at least in part, the similarly cumulative effect of hardened, denatured fibrillar proteins in determining texture. It is essential to add to this the contribution of collagen which will have been solubilised under heat treatment, the degree of solubilisation depending on the extent to which intra- and inter-molecular cross-linking has occurred in the native collagen.

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