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THE SARCOLEMMA OF MUSCLE CELLS

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Morphology and function of the striated muscles have ever since the beginning of research into their nature been invariably in the foreground of scientific interest. Although a great number of problems has been solved in this field, a host of new questions is incessantly arising [4]. While it seems safe to regard problems concerning the morphology and function of the contractile components of the muscle cells, *i. e.* the muscle fibrils, as more or less settled, there still exists many a detail which awaits elucidation in connection with the other principal component of muscle cells, *i. e.* the sarcolemma.

Meat research institutes are known to be highly interested in the intricate biochemical and biophysical properties of the striated musculature. Studies of this kind concern not only the outward appearance (white and red muscles), palatability (softness etc.) and biological value (protein composition) of the meat but also certain aspects of the technology of manufacturing (*e. g.* diffusion during pickling). We have discussed the quantitative and qualitative problems concerning the connective tissue of meat and meat products at the 1958 conference of the *European Meat Research Workers* [6], and studied the interconnections between the thickness of the striated muscle cells and the quality of meat [7].

While studying the two principal tissue-elements of meat, *i. e.* the muscle tissue and the connective tissue, we have endeavoured to analyse the two morphologically distinguishable parts of the muscle cell, the sarcoplasm (together with the fibrils) and the sarcolemma. The structure (thickness, etc.) of the sarcolemma presumably varies according to species and age and the part of the body in which it is situated. We have attempted to establish connections between the variable structure and the physical, chemical and organoleptic characteristics of the meat. Having dealt with the open problems concerning the structure of sarcolemma in a recent paper [8] we do not propose to expatiate upon them at present and content ourselves with noting that most of the authors regard the sarcolemma as composed of two layers, a structureless homogeneous (lipoprotein ?) membrane, *i. e.* the sarcolemma proper, and — around it — a fibrillar layer. Some authors hold that this second layer does

not really belong to the sarcolemma but forms part of the connective tissue around the muscle cells, *i. e.* the endomysium.

Method and results

Samples from the thigh muscle of adult oxen were obtained about 15 minutes after killing the animals. After having freed the specimens from all visible fatty elements and the coarse connective tissue, the 3 to 5 mm thick samples were homogenized with physiological saline in a Waring blender. Under both the phase contrast and the light microscope, the muscle fragments revealed a strongly refractive circular structure which, composed of fibres, appeared as a spiral rim around the cells. While this "winding", made up of connective tissue, was still more or less intact here and there (Fig. 1), it was loose at other points (Fig. 2) or, else, the ruptured circular fibres seemed to have slipped towards one another (Fig. 3). At some points, the ruptured fibril bundles were observed to have become free from the tight grasp of the connective tissue fibres (Fig. 4). At still other points, the originally dense and spirally arranged fibres appeared as the circlets of a disrupted spring (Fig. 5), that were still trying to hold together the masses of fibrils (well visible in the picture) contained in the cells which had evidently been deprived of their cell-membrane. The nature of all these changes depended on the extent of the injury caused at homogenization.

Careful search revealed in the homogenate comparatively intact fragments of tissue which bore a resemblance of somewhat impaired delicate springs (Fig. 6). These fine structures which undoubtedly formed part of the cells were — so to speak — empty, since the applied mechanical force had driven out all cellular contents. That the spring-like circular structures really belong to the muscle cells is irrefutably proved by Fig. 7, which shows a more or less unruptured bundle of cells where the fibrillar structure in question is well observable on the still cohering muscle cells.

Accepting the theory that the cell membrane in striated muscle tissues has two layers, viz. a fine, structureless lipoprotein membrane and, above it, a second layer composed of collagenous fibres, we suggest that the circular fibrous structure observed by us is identical with this second layer. This assumption means a step toward a reconciliation of the controversies existing between the different authors. For example, JONES and BARER [5], further CONTE and RIESER [2] reject the theory that the sarcolemma has a collagenous fibrous structure and regard it as a simple fine protein membrane, while WANG and other investigators recognize — apart from the homogeneous membrane — the existence of a fibrous envelope. RÓZSA et al. [9], while unable to confirm that the sarcolemma contained fine collagenous or reticular fibres, demonstrated

by electron-microscopy that it was covered by granules which they regarded either as connective-tissue fibres or as the points of attachment for myofibrils.

Our experiments have definitely convinced us that — at least as regards cattle — there is no occasion for further controversies: there exists a separate fibrous structure which envelops the structureless sarcolemma proper. There remains the question whether Rózsa et al. [9] are right in believing that the

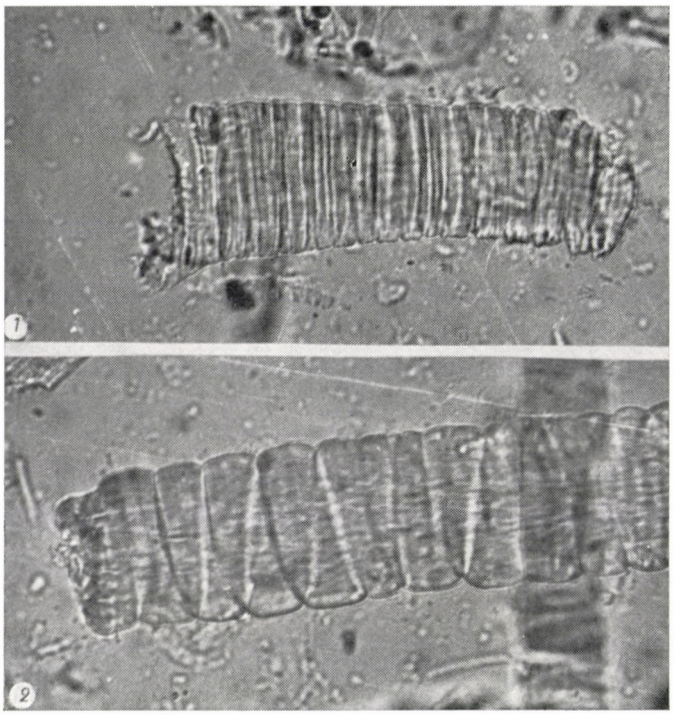

Fig. 1. The well preserved circular, dense collagenous-fibrous "winding" of the sarcolemma is clearly visible on the fragment of striated muscle cell. ×400

Fig. 2. The circular sarcolemmic fibres are loose and show an oblique course around the muscle cell. ×400

fibres (whose points of attachment they claimed to have found) belong to the endomysium and not to the muscle cells themselves, or whether those are right who regard the circular fibrous "windings" — which become easily detached from the cell membrane under the effect of mechanical force — as forming an integral part of the muscle cells. We are more inclined to accept the view of Rózsa et al.

It would also seem possible that the structure at issue is just a phenomenon of sarcoplasmic degeneration following the death of the cell, the like of which was observed by Gelei and Jendrassik [3] on the frog's thigh muscles;

on treatment with calcium, the sarcoplasm disintegrated into discs of a delicately granular structure and left the sarcolemma intact so that the photomicrograph showed a pattern of circular striation. We made microscopic preparations according to the technique of GELEI and JENDRASSIK but failed to observe the phenomenon described by them; the microscopic picture we saw was essentially

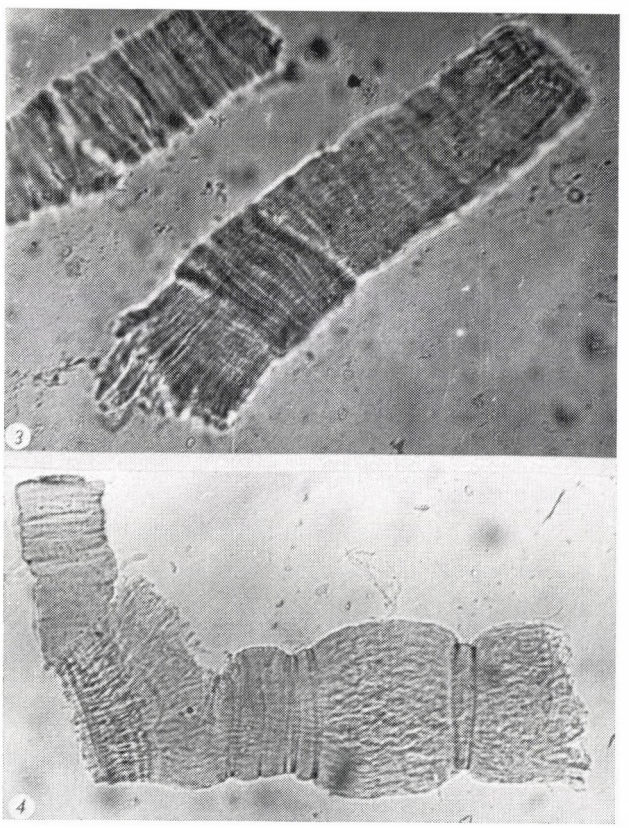

Fig. 3. The circular fibres of the sarcolemma, after having slipped towards one another, seem to be accumulated at a single point of the cell surface. The arrangement of the fibres is still regularly circular in the cell fragment visible at the periphery. ×300

Fig. 4. The circular collagenous fibres appear to be ruptured on the cell surface; masses of myofibrils, freed from the pressure of the collagenous fibres, can be seen to protrude beyond the original circumference of the muscle cell. ×300

different from theirs. The findings of the said authors should nevertheless warn us that many problems concerning the comparative biology and morphology of the striated muscles of different species are still unelucidated.

In earlier experiments [8] we have studied whether the fibres in question were collagenous or elastic. Hydrolysis by means of alkali and tartaric acid

seemed to prove that we are dealing with collagenous fibres. The elucidation of this question was continued in the present experiments. Knowing that the peptide linkages of collagen are less readily dissolved by trypsin than by pepsin, we performed digestion with both these proteolytic enzymes. That the said linkages are more resistant to trypsin was shown by BOYER et al. [1], and

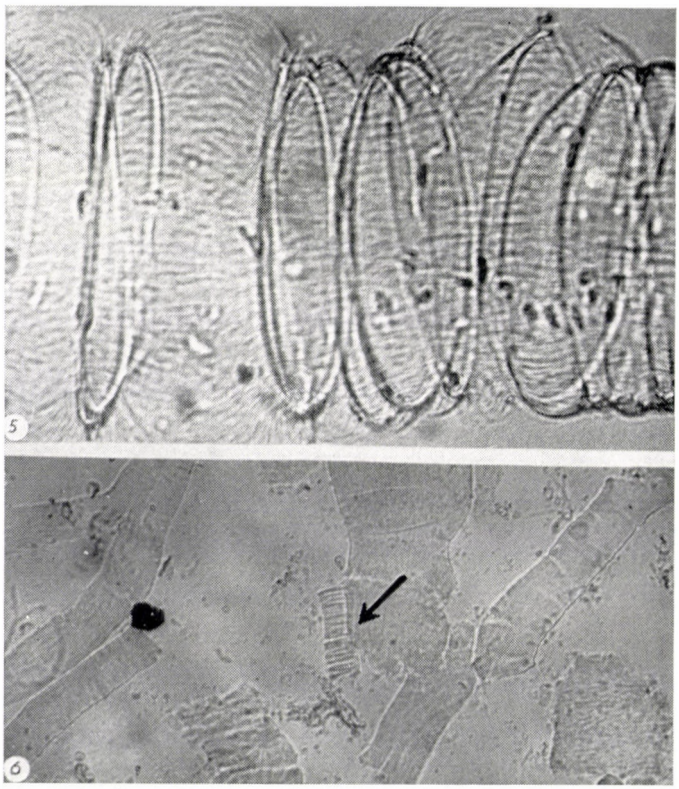

Fig. 5. Disrupted circular collagenous fibres can be seen at some points as coherent or just separating circlets, with masses of loose myofibrils within them. ×1000

Fig. 6. Among the muscle-cell fragments there is a portion of a still coherent collagenous fibre (indicated by arrow) which has slipped off the cell and resembles a piece of steel spring. ×160

it was just this property of the collagen which SCHÖNBERG and LOCHMANN [10] utilized for the determination of the connective-tissue contents of meats.

By using 0.5 g of dehydrated pepsin dissolved in 100 ml of 2 per cent hydrochloric acid, it was possible to dissolve the fibrous elements of the muscle fragments at 37° C within 15 to 20 minutes. The sarcoplasm retained the outlines of the muscle cell as a granular and structureless substance (Fig. 9). An exact reiteration of this experiment with 0.3 g of dry trypsin dissolved

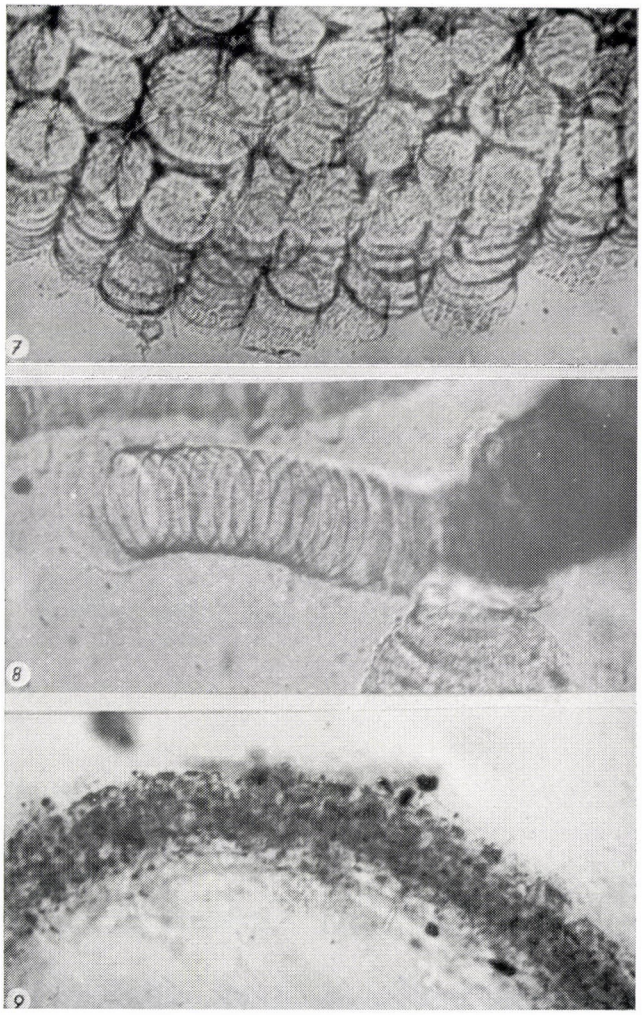

Fig. 7. Muscle cell bundle at oblique angle, with strongly refractive circular collagenous fibres on the surface of the cells. The original pattern of the fibres seems to have been deranged at certain points during homogenization. ×200

Fig. 8. Fragment of muscle cell after 15-minute trypsin treatment at 37° C. The "winding" of collagenous fibres is undigested. ×300

Fig. 9. Fragment of muscle cell after 15-minute pepsin treatment at 37° C. The structure of circular collagenous fibres is not visible; the outlines of the muscle cell are shown by the digested amorphous granular substance. ×300

in 100 ml of sodium carbonate failed to digest the fibres (Fig. 8). The longer the process of digestion, the less became the difference between pepsin- and trypsin-treated preparations, so that, after a few hours, dissolution of tissue elements of the muscle fragments was complete.

Although the staining of our preparations presented manifold technical difficulties under the given conditions, we have applied several dyes (Van Gieson's, Mallory's triple dye, resorcin-fuchsin) in order to ascertain the collagenous or elastic nature of the observed fibres, but none of the employed methods ensured reliable differentiation.

Our next investigations will be concerned with the further elucidation of this problem, as also with the behaviour of the observed fibrous structure at different times after the death of the animals; we wish, moreover, to study the structure of muscle cells in different species and different age groups.

Summary

Ox muscle 15 minutes after killing the animal was homogenized in physiological saline. A circular fibrous structure, loosely connected with the sarcolemma, was observed on the lacerated cells. It was found to be of collagenous origin and seemed to envelop the structureless sarcolemma proper as a second layer. This envelope presumably forms part of the connective tissue surrounding the muscle cells, and its connection with the sarcolemma is rather loose. Like the sarcolemma itself, this second layer follows the contraction or relaxation of the muscle cells, a phenomenon which cannot be clearly perceived in the usual histological preparations. It was the fact that we homogenized the muscles immediately after death which has made it possible to discover the peculiar structure at issue which disappears or becomes less conspicuous when rigor mortis sets in, and especially in the course of the subsequent colloid-chemical processes. It is our intention to study the fibrous structure at longer intervals after death, further in different species and in animals of different ages.

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UNTERSUCHUNGEN AN MUSKELZELLEN: SARKOLEMMA

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In bezug auf die zwei, morphologisch und funktionell gut differenzierbaren Teilen des quergestreiften Muskels, den Myofibrillen und dem Sarkolemma, bestehen ungeklärte Fragen besonders hinsichtlich des letzteren.

In frischen, noch lebenswarmen Muskelzellen vom erwachsenen Rind wurde eine bisher noch nicht beschriebene regelmäßige zirkuläre Fasernstruktur beobachtet die, obwohl

sie eng zu den Zellen gehört, anscheinend dennoch nur schwach mit ihnen verbunden ist. Falls die zweiteilige Struktur des Sarkolemma den Tatsachen entspricht, so stellt die neu beobachtete Fasernstruktur ein auf der äußeren Oberfläche der lipoproteinen Zellmembran vorhandenes und auch von anderen Autoren erwähntes Stützgewebeelement dar. Die Tatsache, daß diese bindegewebige zirkuläre Fasernstruktur auf mechanische Einwirkung mit der Zellmembran nur lose verbunden zu sein scheint, bestätigt den endomysialen Ursprung dieser Fasern.

Es wurde unternommen, die Natur dieser Fasern mit Hilfe von Differenzialfärbung sowie durch enzymatischen und hydrolytischen Abbau zu klären. Mit alkalischer Hydrolyse und insbesondere mit Pepsin- und Trypsinverdauung ließ sich nachweisen, daß es sich um Fasern kollagenen Ursprungs handelt, da sie bei völlig gleicher Versuchsanordnung und gleicher Einwirkungszeit in saurer warmer Pepsinlösung intensiver abgebaut wurden, als in Trypsinlösung. Dies entspricht den Feststellungen, laut welchen Pepsin die Peptidbindungen des Kollagens schneller löst bzw. daß diese Bindungen Trypsin gegenüber resistenter sind.

ИССЛЕДОВАНИЕ МЫШЕЧНЫХ КЛЕТОК: САРКОЛЕММА

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Было установлено, что из двух частей поперечнополосатой мышцы хорошо отделяемых, как с морфологической, так и с функциональной точек зрения, миофибриллы и сарколеммы, в отношении последней имеется больше невыясненных вопросов.

В ходе исследований мышечных клеток авторы на свежих, еще теплых мышечных клетках взрослого крупного рогатого скота обнаружили до сих пор неизвестную равномерную циркулярную волокнистую структуру. Эта структура хотя и принадлежит к клеткам, но она повидимому только слабо связана с ними. Поскольку можно согласиться с разделенной на две части структурой сарколеммы, то описанная авторами волокнистая структура представляет собой упомянутый уже другими авторами элемент опорной ткани, располагающийся на наружной поверхности собственной клеточной оболочки липопротеинового характера (**sarcolemma proper**). Ввиду того, что эта соединительнотканная циркулярная волокнистая обмотка по исследованиям авторов при механическом воздействии по-видимому только слабо связана с клеточной оболочкой, то они придерживаются того мнения, что эти волокна предположительно происходят из эндомизия.

Авторы старались выяснить природу волокон при помощи дифференциального окрашивания, ферментативного и гидролитического расщепления. Щелочный гидролиз, и, в частности, опыты по пепсиновому и трипсиновому перевариванию показали, что речь идет о волокнах коллагенного происхождения, так как они расщеплялись в кислом, теплом растворе пепсина — при идентичных экспериментальных условиях — одинаковой продолжительности воздействия — более выраженно чем в растворе трипсина. Это соответствует установлениям, согласно которым пепсин быстрее разлагает пептидную связь коллагена, или что эта связь устойчивее в отношении к трипсину.

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