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Muscle Cell Studies.

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Dr.F. Lőrincz, Dr Ida Szeredy, Dr.J.Szinák

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For some years we have investigated the problems of finer morphology and chemistry of the striated muscle tissue /1-4/. Particular emphasis was given on the structural aspects of the cell membrane, the sarcolemma. To the elucidation of the problems yet unsolved we endeavoured to contribute all the more as the quantitative and qualitative relations of the connective tissue elements in the muscle tissue /meat/ are made generally responsible for the quality of meat. Sarcolemma is the connective tissue of the muscle cells, and its external, circular collagen layer plays at the same time a role in the connective tissue of the muscle tissue as well.

We do not wish to enter into details concerning the relation between meat quality and the properties of the connective tissue. Neither shall we tackle the morphological and chemical differences between muscle tissue in young and old animals. Our experiments, carried out together with Dr. Szeredy /1/, disclosed sufficient data in this respect.

Other results provided by us /3,4/ prove that sarcolemma with its homogeneous lipoprotein component might be considered on the one hand, as the membrane of the muscle cell /sarcolemma proper/ and on the other, with its external fibrous part /sarcolemma externa/ as part of the endomysium. We are, therefore of the opinion that the quality of the "meat tissue" is not determined by the rougher connective tissue elements /epimysium, perimysium, endomysium/ only, but sarcolemma also has a considerable contribution on it. We believe that well-defined differences exist between the finer structural properties and chemical compositions of the sarcolemma in young and adult animals and possibly in animals belonging to different breeds as well.

Our previous experiments dealt only with the structure of the muscle cell membrane in adult cattle. We didn't investigate closely however the sarcolemma-problems of young calves and other animals. Neither could we attempt the isolation of the sarcolemma membrane in pure form, though only the morphological and chemical analysis of the pure sarcolemma, isolated in sufficient quantity from muscles of animals of different ages and breeds, can clarify the exact effect of sarcolemma properties on meat quality. Our programme includes also the study of the developing musculature in different animals and special consideration was given to the development of the sarcolemma itself.

It is the purpose of this paper to review briefly the results attained within the past year.

I. Young bovine and porcine muscle cells were examined by means of the well-known method. The muscle tissue, cut out from the thigh muscle approximately 15 minutes after slaughter and freed from the coarse connective tissue elements, was cut into pieces of 3-5 mm and disintegrated in physiological NaCl solution in a Waring blender. Muscular cell fragments in the homogenate clearly show the sarcolemma structure found by us formerly in the muscular cells of the adult cattle /Fig. 1-3/.

Fig. 1. Muscular cell particles of a 10 week old calf. On both ends of the cell-fragment fine granular sarcoplasma-masses may be seen, which seem to "flow out" from the sarcolemma sheat. Some fine circular fibres on the sarcolemma surface are also visible/magnification about 1000/.

Fig. 2. Muscle cell particles of a 10 week old calf with clearly distinguishable circular fibres on the surface /magnification about 1000/.

Fig. 3. Muscle cell particles from adult pig. The circular fibrous structure of the sarcolemma is clearly visible /magnification about 1000/.

These three figures show on the one hand the sarcolemma structure, which we proved previously in adult cattle and supposed in young animals or animals other than cattle . The essential features are identical with those found in the muscle cells of adult cattle with perhaps the slight difference that muscle cells of calves and pigs show a finer circular fibre structure than those of the cattle.

II. Sarcolemma material was isolated according the methods of Kono and Colowick /5/ and McCollester /6/ respectively. Both authors start from the immediate post-slaughter leg muscles of decapitated rats and disintegrate in a Waring blender with alcaline buffer or $CaCl_2$ solutions at 0-+6°C. The sample is then freed from rough connective tissue elements by means of nylon or terylene filters of 1,5 mesh. Kono and Colowick treated the samples with a LiBr solution of 0,4 M final concentration, whereas McCollester used a NaCl solution, the pH of which had been adjusted to 7,4. After repeated washing the sarcolemma particles were extracted in the experiments of Kono and co - worker by means of a 1 M KCl solution /pH 8,2-8,4/; in those of McCollester with distilled water of pH 7,4. Finally, the former author separated empty sarcolemma-sheat parts at 8 000-25 000 g, whereas the latter used MSE Minor and MSE Major centrifuges respectively and separated the sheats at 2 000 g. Kono and co-worker carried out centrifugation using KBr solutions of different specific gravities.

In our experiments we used mostly the method of Kono and co-worker. These methods, possibly in improved form, might help to clarify scientifically the changes occuring in sarcolemma-properties in the function of age or breed. They might also help to elucidate the relation between these properties and meat quality.

The three specimens here shown were taken from adult cattle. The sarcolemma structure of adult cattle seems to resist better the stress caused by the preparatory process.

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Fig. 4. Sarcolemma membrane of cattle muscle cell washed to almost complete purity. The sarcoplasma, already amorphous in form, seems to "flow out" from the orifices of the small tubes. The circular fibres of the membrane are recognizable well. The slide was prepared after treatment with $CaCl_2$ and washed with NaCl solution /magnification about 1000/.

Fig. 5. Sarcolemma particles, isolated from cattle, treated with $CaCl_2$ and washed with NaCl /magnification about 1300/.

Fig. 6. Sarcolemma tube particle from cattle muscle, after a LiBr treatment /magnification about 500/.

Of the above figures Fig. 5. and 6 are very similar to the photomicrogramme published by Kono and Colowick. On the other hand, Fig. 4 and 5 in their above quoted paper show the same circular fibrous structure which our samples displayed /see cit. lit. 3, 4/. Another feature in the work of the above mentioned authors wich deserves attention is the fact that they succeeded in separating the sarcolemmal material from non - sarcolemmic proteins: actin, myosin and other "impurities". The analysis of the amino acids in this, almost pure sarcolemma /according to their calculation the purity grade attains 99 %/ proved that it consisted mainly of collagen. This statement is also in agreement with the results which we gained by enzimatic differentiation /4/.

III. A third part of our studies, closely connected with the above described experiments, refers to the morphological

and chemical analysis of what we termed the "meat in development". Though morphological and chemical studies have already been carried out in this field, no meat researcher tackled the problem of muscular development yet. We believe that such details of muscle morphology and biochemistry might reveal many interesting data for meat research. Without wishing to prove the truth of this statement by an abundance of literary data, we want to point out two standard works only which could not alone stimulate meat research workers to further studies but offer an ample choice of previously published literary information as well /7, 8/.

As to our experiments, we selected three microphotogramme from the results of our investigations, started a short time ago. They convincingly prove that the questions of muscular development may help the solution of many a morphological problem.

Fig. 7. Longitudinal section from the leg muscle of a 2 month old cattle foetus. Muscle cells, still in the stage of myo-tubes, can be seen in almost full length. Muscle nuclei are in the centre of the cell. They remind us of pea seeds in an opened pea-pod /magnification about 500/.

Fig. 8. Cross section of leg muscle from a 2 month old cattle foetus. Clearly visible are the cross sections of myofibrils, ranging on the muscle cell periphery, the sharp outlines of the sarcolemma-membrane and the cross sections of the cell nuclei in the centre of the cells.

The latter are also marked by arrows /magnification about 500/.

Fig. 9. Part of the leg muscle of a 4 week old pig foetus. The longitudinal section shows muscular cell particles, which are embedded into the exceedingly loose connective tissue. These particles enclose two well developed muscular cells in their initial form with myofibrils on the periphery and cell nuclei in the middle. Sarcolemma membrane is yet undistinguishable /magnification about 500/.

Fig. 7-9. do not lend themselves to commentaries yet. They are demonstrated in order to illustrate investigations which we began a short time ago. The three photomicrographs prove the direction of our above outlined opinion, according to which results of the morphological studies on muscle tissue in development ought to be applied to the less known part of the muscle tissue: the sarcolemma.

Literature.

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Studien über Muskelzellen

X. Konferenz der Europäischen Fleischforscher /Roskilde 10-15.8.1964/
Dr. F. Lőrincz, Dr. I. Szeredy und Dr. J. Szinák.

Es wird über die Muskelzellenforschung berichtet, welche in drei Richtungen durchgeführt wurde.

I. In den bisherigen Arbeiten wurde die Zweischichtigkeit der Sarcolemma nur an erwachsenen Rindern bzw. ausserhalb der eigentlichen Sarcolemmahaut liegenden und teilweise zur Endomysium gehörenden, äusseren, zirkulär verlaufenden Kollagenfaserschicht, demonstriert. Jetzt wurde das Vorhandensein dieser Faserschicht /Abb. 1-3./ auch an den Muskelzellen junger Kälber und Schweine nachgewiesen.

II. Es wurde nach den Methoden von Kono und Colowick bzw. M^{c}Collester die Isolierung der reinen Sarcolemmahaut angestrebt /Abb. 4 bis 10./. Nach der Meinung der Autoren andert sich die morphologische und chemische Qualitat der Sarcolemma je nach Tierarten und innerhalb dieser mit dem Lebensalter. Es wird vorausgesetzt, dass zwischen der Qualitat der Sarcolemma und des Fleisches ein Zusammenhang besteht.

III. Um die feineren geweblichen und biochemischen Eigenschaften des Muskelgewebes zu erkennen, ist die Untersuchung der Muskel während der Entwicklung unentbährlich. Im Zusammenhang der diesbezüglich begonnenen Forschungen, wurden einige Feststellungen gemacht und einige charakteristische Präparate vorgelegt /Abb. 7 bis 9/.

Abbildung 1 : Der Bruchteil der Muskelzelle eines 10 jährigen Kalbes. An beiden Enden des Zellenteiles ist die blasse fibrillär - körnige Sarcoplasmamasse zu sehen, die aus der Sarcoplasmahülle quasi herausströmt; an der Oberfläche der Sarcolemma sind auch einige feine zirkulare Faser zu beobachten /etwa 1000-fache Vergrösserung/.

Abbildung 2 : Ein Teil der Muskelzelle eines 10 jährigen Kalbes; an der Oberfläche sind die zirkularen Fasern scharf zu sehen /etwa 1000-fache Vergrösserung/.

Abbildung 3 : Ein Teil der Muskelzelle eines erwachsenen Schweines. Die zirkuläre Faserstruktur der Sarcolemma sticht gut ins Auge /etwa 1000-fache Vergrösserung/.

Abbildung 4 : Beihane rein gewaschene Haut der Sarcolemma der Muskelzellenteile des Rindes. Bei der Mündung der kleinen Röhrchen fliesst quasi die schon amorphe Sarcoplasma heraus. Die zirkuläre Fasrigkeit der Sarcolemma ist gut zu erkennen. Das Präparat stammt aus einem Material das mit $CaCl_2$ behandelt und nachher mit NaCl gewaschen wurde /etwa 1000-fache Vergrösserung/.

Abbildung 5 : Ein Teil der isolierten Sarcolemma, welche aus Rindermuskel entstammt, mit $CaCl_2$ behandelt und mit NaCl gewaschen wurde /etwa 1300-fache Vergrösserung/.

Abbildung 6 : Ein Teil des Sarcolemma-Röhrchens das aus Rindermuskel entstammt, nach der Behandlung mit LiBr. /etwa 500-fache Vergrösserung/.

Abbildung 7 : Längschnitt aus dem Foetus-Schenkenmuskel eines etwa 2-jährigen Rindes. Die Muskelzellen die im Stadium der Muskelröhre /Myotubus/ sind, können in ihrem Verlauf fast vollkommen beobachtet werden. Die Muskelkerne sind in der Mitte der Zelle angeordnet; sie erinnern an die in der Hülle der grünen Erbsen entlang eingeordneten Erbsenkerne /etwa 500-fache Vergrösserung/.

Abbildung 8 :Querschnitt aus dem Foetus-Schenkelmuskel eines etwa 2-jähringen Rindes. Es sind so die Querschnitte der Myofibrillen, welche sich auf der Randzone der Muskelzellen befinden, wie die gut abgezeichnete Sarcolemmahaut und die Querschnitte der Zellenkerne in der Mitte der Zellen gut zu sehen./Bezeichnung mit Pfeilen; etwa 500-fache Vergrösserung/.

Abbildung 9 : Ein Teil aus der Foetus-Schenkelmuskulatur eines etwa 4 wöchigen Schweines.Auf den Längschnitten sind in äusserst losem Bestand wellenartig ablaufende Muskelzellenteile, zwischen diesen zwei gut entwickelte Anlagen mit Myofibrillen, die sich auf den Periferien lagern, mit den Zellenkernen, die sich in der Mitte anreihen, zu erkennen. Die Haut der Sarcolemma ist noch nicht zu entdecken /500-fache Vergrösserung/.

Исследования мышечных клеток.

X. Европейский конгресс работников научно-исследовательских институтов, состоящий в г. Рошкилде от 10-го до 15-го августа 1964-го года.

д-р Ференц Лёринц, д-р Ида Середи,
д-р Янош Синак.

Резюме.

Авторы излагают исследования мышечных клеток проведенные ими в трех направление.

I.-Проведенными авторами до сих пор работами (см. лит. п. 3 и 4) они показали исключительно на мышечных волокнах взрослого крупного рогатого скота двухслойное состояние сарколеммы или циркулярный коллагеновый слой волокна находящийся вне настоящей оболочки сарколеммы и принадлежащчий в частности эндомизию. Существование слоя волокна они теперь доказывают на мышечных клетках молодых телят и свиней (см. рис. I-3).

II.-Использовая методы Коно и Коловика или Мак Коллестера авторы старались изолировать чистую сарколемму (см. рис. 4-6). По нашему мнению морфологическое и химическое качество сарколеммы изменяются по видам скота и внутри этого по возростам. Предпологается зависмость между качествами сарколеммы и мяса.

III.-Для ознакомления более тонких тканевых и биохемических свойств мышечных тканей авторы считают необходимым исследовать развивающую мышцу. Они излагают связано с этом некоторые данные и показывают несколько характерных препаратов (см. рис. 7-9).

Рис. I.- Показано часть мышечной клетки теленка возростом 10 неделей. На двух концах части клетки видно, вроде вытекающей из гильзы сарколеммы, бледно фибрилярно--зернистая масса саркоплазмы, на поверхности сарколеммы можно

наблюдать несколько циркулярных волокон (увеличивание прибл. I000 кратное).

Рис. 2.- Показано часть мышечной клетки теленка возростом I0 неделей, на поверхности которой наблудается яркие циркулярные волокна, (увеличивание прибл.I000 кратное).

Рис. 3.- Показано часть мышечной клетки взрослой свиньи -- циркулярная структура волокна сарколеммы хорошо видно, (увеличивание прибл. I000 кратное).

Рис. 4.- Показано почти до читой вымытая оболочка сарколеммы частей мышечных клеток крупного рогатого скота. У горловины мельких трубочках, вроде вытекает находящая уже в аморфном состоянии саркоплазма. Циркулярная волокнистая структура сарколеммы хорошо различаемая. Препарат обработан хлористым кальцием ($CaCl_2$) и после этого вымыто хлористым натрием (NaCl). (Увеличивание прибл. I000 кратное.

Рис. 5.- Показано часть изолированной сарколеммы происходящая из крупного рогатого скота после обработки хлористым кальцием ($CaCl_2$) и мойки хлористым натрием (NaCl). (Увеличивание прибл. I300 кратное).

Рис. 6.- Показано часть трубочки сарколеммы происходящая из мышцы крупного рогатого скота после обработки бромистим литием (LiBr). (Увеличивание прибл. 500 кратное).

Рис. 7.- Видно продолный разрез происходящий из мышца бедра двухмесячнего зародыша крупного рогатого скота. Мышечные клетки находяшчиеся в стадии мышечной трубочки(миотобус) видны почти во всей их величине. Ядро мышц располагаются в центре клеток, похожие на зеленый горох в открытом стручке. (Увеличивание прибл. 500 кратное).

Рис. 8.- Показано продолный разрез взятый из мышцы бедра двухмесячного зародыша крупного рогатого скота. -- Хорошо видны продолные разрезы миофибрилей располагающихся на периферии мышечных клеток. Ярко выраженная оболочка сарколеммы и в центре клеток разрезы ядра клеток. (Намечено стрелами; - увеличивание прибл. 500 кратное).

Рис. 9.- Видно часть из мышца бедра 28 дневнего зародыша свиньи. На продолном разрезе видны часты волнистых мышеч-

ных клеток находящиеся в очень рыхлом составе соединительных тканей, среди этих есть два хорошо развитая начатка мышечных клеток периферриально располагающимися миофибрилями; ядро клеток расположен центральным образом. Оболочка сарколеммы еще не обнаружется. (Увеличивание прибл. 500 кратное).