V

THE STRUCTURE AND CHEMISTRY OF MUSCLE

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There are different types of muscle, such as the voluntary cross-striated body muscle, the cross-striated automatic heart muscle, the smooth muscle performing the slow automatic motion of internal organs, the insect muscle, clam muscle, etc. What is commonly called "meat" and is one of the most important human foods is the voluntary ("red") cross-striated body muscle which moves the animal body. I will limit my remarks to this sort of muscle only. The nutritional qualities of this muscle are intimately connected with

1) its structure,
2) its function, and
3) the physiological state of its activity or degradation.

The light-microscope reveals that the muscle consists of fibers, 0.05 - 0.1 mm wide and rather long. These fibers are surrounded by a thin sheath, the sarcolemma, and contain in their interior the contractile matter. The contractile matter fills the fiber homogeneously and is not composed of fibrils, as was thought earlier. The contractile matter is homogeneous cross-wise but along the fiber axis, lengthwise, it shows periodic variations which make the fiber resemble a roll of coins. Isotropic and anisotropic segments alternate and in the middle of each of these we find a very thin cross-membrane, called Z in the former, and M in the latter.

The contractile matter is a complex protein, made up of two proteins, actin and myosin, both of which are present as very thin filaments of molecular dimensions. The actomyosin is a rather soft, elastic gel which, especially at rest, is easily damaged and disorganized mechanically. Accordingly, the protection it needs depends on its location and its exposure. Muscles lying superficially, especially on the extremities, need much protection. This is not provided by the sarcolemma itself, which is a rather thin membrane. Its protection is provided by the connective material, which is present in two different forms. The one is represented by the connective tissue lying between muscles, or inside the muscle between its fibers and fiber bundles. This connective material can form continuous layers, called fasciae. Between the muscle fibers we also find a rich net of collagen. The electron microscope has lately revealed that there is also another sort of fiber closely attached to the sarcolemma, where they form a thick net. These fibers resemble collageneous fibers but differ from them in many respects. The tenderness of meat is dependent, in the first place, on the quantity of these rather tough connective elements, which have to be tough since they are there to protect the muscle from mechanical injury. The contractile matter itself is soft, regardless of the location of the muscle. With advancing age the connective material seems to gain in quantity, making

the meat tougher. The main factor deciding its quantity, however, is the location of the muscle and natural protection it needs. The most protected muscles are those lying in the abdominal cavity, immediately in front of the vertebral column or along side it. These muscles are well protected in front by the organs of the belly and on the other side by the vertabral column, and are thus poor in connective matter. This explains the high culinary value of the musculus psoas, the mignon steak. Next in culinary value come the deep muscles of the back and then muscles of the deeper layers of the thigh.

The second factor which decides the nutritional qualities of muscle is its function. What is of importance here is whether the muscle serves in vivo to produce short but violent motion or rhythmic motion of long duration. The former sort of motion demands a different physiological mechanism from the latter. It is not the actomyosin itself which is essentially different, but the machinery which provides actomyosin with energy.

As far as we know today, the immediate source of energy in the contracting muscle is the high-energy-phosphate of ATP (Adenosine-tri-phosphates). The last (terminal) one of the three phosphates of the ATP molecule can be attached to the molecule only by using up 11,000 cals of energy. If this last phosphate is split off, these 11,000 cals are liberated again. This is what happens in muscle contraction and it is this energy which is used to produce motion. The energy needed to produce ATP is derived from foodstuffs and can be invested into the ATP molecule by two entirely different processes, one being oxidation, the other fermentation. In oxidation the foodstuff molecule, for example sugar, is burned to water and CO_2; in fermentation it is merely decomposed to give lactic acid. Lactic acid contains somewhat less energy than the sugar from which it was derived and this energy-difference can be used to build ATP. The difference in energy between lactic acid and sugar is not very great and therefore fermentation is a rather wasteful process. In order to render it more economical, oxidation must be coupled to fermentation, which oxidizes a small part of the lactic acid and, with the energy thus gained, converts the rest of the lactic acid into sugar again.

The main difference, from our present point of view, between fermentation and oxidation is that oxidation demands a rather bulky mechanism. It also seems that the solid state is essential for it. It is not a very efficient but rather slow process which depends on the oxygen supply. Fermentation can be effected by a number (a dozen or so) of soluble enzymes, thus it demands no solid structure or bulky molecular machinery. This is much the simpler process. It is true that eventually it also depends on oxidation but if time is given, a relatively poor oxidative apparatus will be sufficient to clear away the lactic acid produced. To all this we have to add that fermentation can work very fast. In itself, without oxidation, it could not work for very long because lactic acid would be accumulated, but for a short and rapid production of ATP it is more suited than oxidation.

Let us discuss now the two sorts of motion mentioned before and take rather extreme examples. A muscle typically made for short and violent motion is the breast muscle of the turkey or chicken. Actually these are wing-muscles. The wings of these domestic fowls are very poor, while the body is rather heavy. Very violent motion is thus needed to produce flight. However, these birds do not cover distances and use their wings merely to cover a few yards, to fly to a low branch of a tree or to the top of a low ladder. We can expect to find in these muscles a relatively high concentration of ATP which can supply the energy for a few violent strokes directly. Actually, the highest ATP concentration I have ever found was in the turkey

breast muscle, 5 mg per gram. Added to this there is a powerful system of fermentation which can rapidly replace the phosphate of the ATP used up. The system of oxidation, however, is very poorly developed. Consequently the muscle will only be able to work for a short time but will be able to work violently. The wing will be unfit for a long-distance flight for which it is not intended. Once the animal has reached its goal, the poor oxidative mechanism will have time to clear away the lactic acid formed. Fermentation does not need structure, being performed by soluble proteins, thus the contractile matter is not embedded in the solid structure of oxidative mechanisms. This results in the muscle having no color; it will be "white," since actomyosin is colorless. The muscle will be rather fibrous, dry and not juicy after the denaturation of actomyosin which occurs in cooking.

The rabbit also has "white" meat, somewhat similar to the chicken breast. This is because it lives in holes, and does not run long distances for safety. If pursued, it gives up rapidly, though it can run a short distance rather fast.

As an example of the other extreme we can take the rabbit's cousin, the hare, an animal generally known for its ability to run long distances. A rich store of ATP is of no use in a long run and fermentation in itself would lead to an accumulation of lactic acid which would paralyze the muscle. The only system which can provide the energy in a protracted fashion is oxidation, which can keep pace with the energy-demand if there is an adequately powerful oxidative mechanism present. As mentioned before, oxidation is linked to the solid state and so in those animals which use their muscle for prolonged work, we find the actomyosin embedded into the solid oxidative mechanism. The result will be that after denaturation of its proteins, the muscle will not fall easily into fibers but will remain more solid and elastic. It also will be more juicy, owing to extractive substances of the oxidative machinery. There will also be an additional quality: color. The whole oxidation depends on the oxygen supply. The oxygen supply is not quite continuous, since the contraction of the muscle partially shuts down the capillaries. The oxygen supply is thus intermittent, while the oxygen demand is continuous. To bridge the gap there is a buffer between the two, a small storage of oxygen. The substance capable of storing oxygen is a dyestuff, closely related to hemoglobin. This "myoglobin" is rather dark in color. In water-fowl which have to stay under water for long periods the quantity of this myoglobin is especially large, hence the very dark color of the meat of the wild duck. In mammals living on dry land the color mostly runs parallel to the other qualities mentioned, the elasticity and juicy nature. The more the muscles are made for prolonged performance, the more exceptive this performance, the more pronounced are the qualities discussed. The beef is somewhere half-way up the scale. Its meat is not very dark, much lighter than that of the horse which accordingly can easily outstrip the bull in running but is darker than the rabbit's.

These are not merely theoretical considerations. They bring us close to problems of every day life, such as that of the "dark cutter." No adequate explanation has hitherto been given for the fact that a certain percentage of the beef which is cut has a dark meat. This color does not diminish its food value, but makes the meat less attractive and causes considerable financial loss to the seller who has to exhibit his produce. To my knowledge no successful systematic studies have yet been performed on this subject. I can only give my own guess as an explanation: that the dark cutters are genetically different from the rest, representing a strain of animals fitted by Nature for longer runs. This agrees with the observation that this dark meat, as I am told, is more elastic and rubbery. If this problem should be

studied, first of all the nature of the dark color must be ascertained – a fairly simple job. I expect the color to be due to an increased amount of myoglobin. If these expectations are born out of experience, then as the next step in solving this problem, some method should be found to recognize the dark cutter in vivo. Then the genetics of this quality should be studied and the breeding of dark cutters controlled.

As a third important point I mentioned at the beginning of this paper the physiological state of activity. This is a point which also deserves our closest consideration because along these lines, I think, considerable improvements could be achieved in meat production. Regarding function we have all had one important experience: our muscles become hard when they contract. The muscle which was soft and plastic at rest becomes hard when it enters into activity. The difference is enormous and we take it as a natural matter of fact. It means that there are enormous differences in the physical constants of muscle according to the state of functional activity and these differences in physical constants must at the same time mean equally great differences in culinary value. Let us consider the chemical mechanism of these changes, elucidated in my laboratory. As I mentioned before, the contractile matter is actomyosin, a complex of actin and myosin. However, at rest, the two proteins are not joined together, but are present dissociated as free actin and free myosin. The two proteins have a great affinity but are pushed apart by electric repulsive forces. These repulsive forces are due to ions and ATP. The latter is adsorbed to myosin and gives it a charge which repels actin. Myosin is a very soft, almost liquid gel and so is actin. When they unite to form actomyosin, a new substance is formed which has new qualities. In solution the change we observe is an enormous increase in viscosity. If the two proteins are present in fairly high concentration, as is the case in muscle where there is 8% myosin and 2% actin, on union they form a stiff and hard gel. If ATP is present this gel goes over into a new modification in which its particles are shorter. This is muscular contraction. What brings actin and myosin together in vivo is the wave of excitation which momentarily disturbs the balance of charges and with it the balance of attractions and repulsions between actin and myosin.

In contracting muscle the ATP is split very rapidly, as the energy-need of motion demands. But ATP is also split, though at a much slower rate, in resting muscle. If there is oxygen present, it is re-synthesized and its concentration is kept constant, but if there is no blood-flow there is no oxygen and the ATP must disintegrate. This happens after death. The consequence of this disappearance of ATP will be that the repulsive forces between actin and myosin diminish and the two proteins eventually get together and form the stiff and hard actomyosin. There being no ATP present now this actomyosin will not contract but remains stiff and hard. This condition is called rigor mortis. It sets in spontaneously a few hours after death. If the muscle is frozen immediately after death and then thawed again, it contracts energetically, splits all its ATP, and then remains hard and contracted. Thus we can have, depending on the concentration of ATP and the pretreatment of muscle, a soft and plastic structure or a hard, rigid structure and there is no need for me to point out the importance of these changes for nutrition. If the muscle is stored for some time after death, the rigor spontaneously dissolves again. This is not due to the resynthesis of ATP but to commencing autolysis, most probably to the disintegration of the actin filaments which are built of small globular molecules. The forces holding these actin globules together into filaments are not very well known yet. We know that ATP is involved in the process in which the globules are linked together to form filaments. We know also that the forces which hold

the actin globules together can be broken rather easily and once the actin filament disintegrates into globules the stiff actomyosin gel must soften up again. Thus we have a whole cycle of physical states from a soft and tender muscle to a hard, stiff, inelastic one and back again. It would seem to be of the greatest importance for the meat industry to study these states systematically and then apply the experience to the best advantage.

Before closing my lecture I would like to mention one more problem, though I am unable to make any definite statements about it, and this is tenderizing. Before thinking of tenderizing I would first use the natural cycle outlined above to produce the most tender, palatable meat. Once the natural cycle is known one could try to speed it up or retard it and fix the most propitious conditions. This does not mean that nothing more could be done. It has been shown in my laboratory lately that the myosin molecule is rather easily split by proteolytic ferments into six fragments. Such a splitting must entail the liquefication of actomyosin and thus an increasing tenderization of meat. It has also been shown that in the absence of ATP the actin filament disintegrates rather easily and irreversibly, which must have a similar effect. We showed also that the disintegration of the actin filament can be brought about readily by charges, conferred on actin by ions. So the possibilities of achieving tenderization by artificial means are rather good. To arrive at this goal, however, we have to know more about the forces which hold the actin filament or the myosin molecule together, and this demands systematic basic study. Once we are better informed about the nature and intensity of these forces which hold the actin filaments and myosin molecules together then very probably means can be found to break and destroy them. It is an experience of every-day life that it is easier to destroy than to build and thus tenderization, involving such destruction of intermolecular links, should not belong to the realm of the impossible.