Muscle biology, post mortem muscle biochemistry and consumer meat acceptance

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INTRODUCTION

Regarding meat, a challenge for the ninety's is to know how research can assume survival in a consumer driven industry. It is claimed that there are at least three factors influencing the perception consumers have of meat out of any ethical considerations. They are : first quality considerations in relation with the sensorical properties of meat, second safety according to the growing awareness of the relationship between nutrition and health and last but not the least the value for money which highligts the critical importance in a very concurrential food market of product of constant and certified quality. Quality variability is without any doubt one of the major problem red meat industry has to overcome, which requires as a top priority that control of the post mortem changes be achieved, i.e. the early post mortem period and the ageing phase.

1 - EARLY POST MORTEM CHANGES

The most important changes in the immediate post mortem period are the onset of rigor mortis and the related pH changes. The best part of the basic knowledge in this field is due to the works of the British researchers and especially BENDALL , (BENDALL, 1973). During the 1980's, very little advances have been made in this area at least from a basic point of view. However the matter is far from being exhausted. For instance, if the metabolism of glycogen and energy-rich compounds is rather well understood, it is not the case for many structural changes which are of primary importance for technological and eating qualities, such as water holding capacity, colour, tenderness and juiciness. The extreme case of PSE meat, which has been so much studied during for the last thirty years gives a good example of that. Buchter and Zeuthen showed as soon as 1971 that PSE meat is tougher than normal meat. An attractive hypothesis could be that the myofibrillar structure is much contracted, because rigor mortis sets up at high temperature in PSE muscle. In fact, the sarcomere length is even slightly higher in PSE meat than in normal pork (HONIKEL, 1977). Now, it seems that the toughness of PSE meat is due to the fact that the structural changes associated with meat ageing, do not occur in PSE muscle (ESTRADE et al., 1991). But why ?, to our knowledge, very few is known about the long-term effects of early post mortem pH changes on meat ageing.

Post mortem pH changes and rigor onset closely depend on intrinsic and extrinsic conditions affecting the muscle tissue at the time of death. Intrinsic conditions are defined by metabolic capacities (fiber typing), biochemical status (energetic potential, oxygen debt), physico-chemical conditions (temperature, pH). Extrinsic conditions involve : nervous stimuli (from central or peripheral origin), circulating hormones, metabolic capacities of other organs. Both intrinsic and extrinsic conditions are chiefly determined by genetic and environmental factors. The latter are essentially the slaughter conditions, the rearing conditions playing a major role only in rather old animals such as beef cattle .

1. Genetic factors

We will take examples in the pig species, because meat quality genetics is better known in pigs than in the other red meat species. As rearing conditions are more and more standardized in pig production even in free range pigs, the variation in muscle composition and metabolism in a given slaughter pig population is mainly from genetic origin, at least in the small age range where most animals are killed. As in all meat animals, the major part of this variation is determined by polygenes and the polygenic regulation of meat quality traits will not be controlled until the genome mapping of meat animals will not be established. However, a large part of the variability in some technological qualities is explained by two major genes, i.e. the halothane sensitivity (HAL^n) gene and the RN^- gene (normal alleles are respectively HAL^N and rn^+). Indeed, these two genes explain the largest part of the individual variation within some pig breeds, and of the

breed variation in some European pig populations (SELLIER, 1988).

11. *HAL*^{n} gene

The *HAL*^{n} gene is presently well-known to be responsible for porcine stress syndrome (PSS), PSE meat proneness and superior muscular development (EIKELENBOOM & MINKEMA 1974). It was believed for around 15 years that it would be possible to use it without problem in a beneficial way, because it was found to be recessive for both defects but additive for muscularity. So a strategy of crossbreeding was developed in most European countries from 1978-1980 in order to produce heterozygotes combining the advantages of the two homozygotes for muscularity and meat quality. Consequently, the challenge was to find a reliable technique to identify the *HAL*^{n} recessive allele even in heterozygotes in order to manage it in pig populations. Nowadays, this strategy is questionable because there are more and more reports in favour of an additive inheritance of the *HAL*^{n} allele for meat quality (for a review, see Sellier, 1988) ; SATHER et al. (1989) even found that this allele might become dominant in heavy pigs. Anyway, it appears more necessary than ever before to get tools in order to control or even, if necessary, to eradicate this gene.There is a striking parallelism between the history of research on halothane sensitivity and that of the research on some humane genetic diseases, as Duchesne myopathy or mucoviscidosis, which also are due to recessive genes. Decades of research on the physiological and biochemical mechanisms of these diseases have not led to any significant progress from a practical point of view. Molecular biology techniques recently allowed identification of the corresponding genes and led to the understanding of their expression. Similar techniques can be applied to the problem of halothane sensitivity (ARCHIBALD, 1987).

12. RN$^-$ gene

The RN$^-$ gene unfavourably affects the processing yield of cooked meat products (NAVEAU, 1986) and probably the muscle protein content (MONIN, 1989). For comparison, it decreases technological yield of cooked ham by around 5-6 %, vs. 2-3 % for halothane sensitivity (SELLIER, 1988). It seems that the RN$^-$ gene acts by increasing the muscle glycogen content, especially in white muscles. Consequently meat presents a low pH and a high residual glycogen content. It is easy to detect the RN$^-$ gene on living animals by determining glycogen on a muscle biopsy because this allele is dominant. So it seems possible to get rid of it by selection on a few generations in the populations where it is undesirable.

The studies on the RN$^-$ gene revealed the importance of glycogen for meat technological properties out of the well-known effect on meat pH. A high content of residual glycogen, directly induces a marked decrease in processing yield of cured-cooked meat products (FERNANDEZ al., 1991). The involved mechanisms are not yet known.. Moreover, as residual glycogen occasionally constitutes as much as 6 % of the dry matter in muscle, it is likely to have some effect on eating quality, especially flavour. Indeed, significant correlations have been found between glycogen content and flavour intensity in dry ham (BUSCAILHON et al., 1991). Clearly the role of muscle glucids in determining meat quality needs reevaluation.

2. Slaughter conditions

Slaughtering **can be considered as the most important practice in the meat industry, since it is the only process which all animals must pass through before consumption**. At least two aspects are worth of consideration in slaughtering : the ethical problem and the process efficiency in terms of cost as well as of resulting meat quality. Regarding the ethical problem, more and more people ask for serious guaranties as far as animal welfare is concerned. Regarding efficiency, there is no doubt that tremendous gains of productivity have been made in some decades. Obviously no comparable progress has been made in the "quality" of the process, and its effects on meat quality.

It is necessary to distinguish two steps in the slaughtering process : the preslaughter period corresponding to the transportation of the animals and the lairage in the abattoir and the slaughter sensu stricto. These two steps differ by their consequences on meat quality as well as by their implications for animal welfare. Here we will limit the discussion to the problems relative to meat quality.

The influence of slaughter conditions on meat quality have been matter of many studies (for reviews, see GREGORY, 1987). Already in the 1930's, Callow reported the increase in meat pH resulting from preslaughter stress (CALLOW 1938). Although many reports are contradictory, some points are now well established :

- preslaughter stress induces a decrease in muscle glycogen, which can lead to DFD meat occurence especially in male animals as young bulls or boars. The biochemical mechanism is well known but the physiological causes of individual variations in reaction intensity are by far less well understood (for a review, see TARRANT, 1988). At the time of killing, it is well established that if struggling is prevented, the post mortem pH fall is slowed down in pigs and goats (HEFFRON et al., 1974), but not in cattle (LISTER & RATCLIFF 1970) . The role of muscular contractions is obvious. In contrast, the role of catecholamines is controversial. Probably there is some interaction between both, since catecholamines potentiate muscular contractions.There is no doubt that the physiological and biochemical mechanisms of the reaction to stress and particularly to slaughter need to be investigated in depth. In the preslaughter period, the body temperature variation has received very little attention despite its importance for meat quality, as shown by GARIEPY et al. (1989). In a given group of pigs, differences in muscle temperature as great as 4 °C can be observed soon after slaughter (LETANG, personal communication) ; such temperature variation is likely to deeply affect ultimate meat quality.

At the time of death muscle tissue environment undergoes drastic changes such as :

- an increase in circulating hormones, especially catecholamines ; strong muscle contractions during stunning and often during bleeding ; progressive setting of anoxia ; increase in muscle temperature, which can reach more than 1 °C in a few seconds during electronarcosis ; pronounced acidification of blood and muscle tissue in case of carbon dioxide stunning.

Each of these phenomena may affect the biochemical changes in muscle tissue during and after death, especially glycogenolysis and ionic translocations. Ionic translocations, particularly of calcium ions, act in the early times on the rate of glycogenolysis and on the delay of rigor onset. They could also have long-term effects on activities of proteolytic systems, which are sensitive to ionized calcium concentration and osmotic changes.

One question is central for the understanding and the control of the effects of slaughter on post mortem evolution of muscle tissue and subsequent meat quality : to what extent each of the above-cited factors, i.e. hormones, anoxia, temperature, acidification, do influence the *rate* and the *extent* of the physical and chemical changes ? The answer to this question conditions the improvement of slaughter techniques, by indicating on what point(s) the efforts must first be focused. In the past, as pointed out above, a lot of studies dealing with slaughter methods have given contradictory results, and so did not lead to reliable conclusions. To our opinion, that is because it was not possible to individualize the effects of factors which constantly interacted in studies carried out on whole animals. Studies at the tissular and even cellular level would help to solve such problems. Regarding the former, use of perfused isolated tissue was proposed and really allowed to study independently the effects of catecholamines and contractions on the peri mortem and post mortem changes in muscle (MIRI et al., 1991). Control of cell environment could even be better achieved using myofibres bundles or isolated myofibres. **Various techniques are now available to investigate changes affecting physicochemical traits in intact cells.** To our knowledge, their application in the field of meat research has been so far limited essentially to studies of myofilament lattice spacing (OFFER & KNIGHT 1988). Their use to study metabolic changes or ionic translocations would help to get a better understanding of the biochemical events responsible for the conversion of muscle into meat.

2 -POST MORTEM AGEING AND MEAT TEXTURE

Meat tenderisation occurs in all muscles of all meat animal species irrespective of their composition especially their collagen content. In any case, improvement in meat tenderness is of myofibrillar origin. Moreover the mechanisms and the causes of this improvement in meat tenderness are probably always the same resulting in an overall weakening of myofibrils but the causes of this weakening are still not clearly

established and the very large variability between animals and muscles in ageing rate not yet explained.

Knowledge of the structural and biochemical changes responsible for meat texture improvement are of prime importance for at leat two reasons. First it is impossible to study ageing mechanism if we cannot measure the structural modifications responsible of the texture changes. Second, for pratical purposes, the availability of an ageing index, simple enough to be applied on a large scale, could be used to manage the variability observed in the extent of meat ageing.

2.1 - Post mortem changes and related development in meat texture

Myofibrillar structural and biochemical changes occurring in post mortem muscle have been well documented (KOOHMARAIE 1988; OUALI, 1990). However only few of them seems to be directly related to the improvement in meat tenderness. A possible explanation of this would be a misunderstanding of the myofibrillar structure and of how this structure works. This failure might be probably partly overcome by reconsidering these changes in the light of the recently new proposal dealing with the distribution and the function of proteins within the sarcomere (POLLACK, 1990). Localisation of some proteins especially tropomyosin and troponin T could be quite different from that originally expected. So distribution of troponin T for instance, a protein which is extensively degraded in post mortem muscle, would led to change our statement about its contribution to the post mortem decrease in meat toughness. (Fig. 1).

2.2. Mechanisms responsible for meat tenderization

The meat tenderising process probably involved two sets of mechanisms. The first to be established was proteolysis of muscle proteins, and it is still considered as the primary mechanism of meat tenderization. The second set of mechanism is physicochemical in nature and mainly concerns the large increase in postmortem muscle osmotic pressure (OUALI, 1990).

- Enzymatic mechanisms

Basically, any proteinase located inside muscle cells could be a potent contributor to meat tenderization and must be taken into account in this context. Of the different muscle proteolytic systems described in the literature, only two -the acidic lysosomal proteinases and the calcium dependent neutral proteinases have received much attention from meat scientists. It is generally assumed that their concentration in live muscles are muscle type dependant with the higher content in the oxidative slow twitch fibers (OUALI, 1990; OUALI and TALMANT, 1990).Their activities in live tissue have to be highly regulated and this might be controlled through at least two different ways: (1) at the level of the expression of their genes, i.e. at the transcriptional and/or the post-transcriptional levels and (2) at the level of the potenetial activity of the synthetized enzymes through positive (activators) and negative (inhibitors) effectors.

Concerning the transcriptional level, many studies dealing with structural gene analysis for either calpains (HATA et al. 1989) or cathepsins (FERRARA et al. 1990) have been carried out. The relationship between different structures are explored and informations on their genetic controlled expressions are just beginning to emerge . Thus major efforts are nedeed in this research field to elucidate the control mechanism of such constitutive genes and the regulation of the post transcriptional steps.

Control of the enzymatic activity was ascribed to proteinase inhibitors present in all cells and tissues of mammalian species (BARRETT et al. 1985). How inhibitor-enzymes interactions are modulated in vivo remain an unsolved issue. Postmortem, this control is probably less efficient and proteinase efficiency might be assessed through measurement of the enzyme-inhibitor ratio. Regarding muscle proteinase inhibitors only few data are available. The presence of lysosomal proteinases inhibitors was reported in rabbit and bovine and skeletal muscle. Calpastatin is the endogenous protein inhibitor acting specifically on calpain . The findings reported for different species (OUALI & TALMANT 1990) show that the meat conditioning rate may be correlated negatively to muscle calpastatin content when no relationships exist between meat aging rate and calpain concentrations. Current investigations (ZABARI et al. 1991) clearly show that the equipment of muscle in cysteine proteinase inhibitors other than calpastatin is very complex and can affect meat aging rate.

In vivo the proteolytic potential can be changed by hormonal treatment: b-agonists have been reported to alter activity of intracellular

proteolytic enzymes including calpains and cathepsins (KRETCHMAN et al, 1989) probably in the last case, by a direct action on muscle cells (BECHET et al. 1990). Thus the use of this molecules increasing muscle protein deposition could induce negative effects on *post-mortem* proteolysis. If the proteolytic potential differences observed between species and within a specie with age are generally correlated to meat conditioning rate, the high intensity of this potential cannot explain the absence of proteolysis in *masseter* muscle (OUALI & TALMANT 1990). The sensitivity to proteolysis of myofibrils from various muscles could be an other parameter influencing the meat aging rate.

- physicochemical mechanisms

Over the last decade, the main new matter for debate is the possible contribution to the weakening of myofibrills of the osmotic pressure which remarquably increased in post mortem muscle. Thus, in all bovine muscles investigated osmotic pressure raised rapidly after death and may be twice as high as in live tissue (WINGER and POPE, 1980-81). Such ionic strengths are high enough to weaken the myofibrillar structure and to make it more sensitive to proteolysis.

2.3 Biological variability in meat tenderising rate and intensity

The rate and the intensity of the tenderising process of meat are highly variable, greatest variability being observed between animals and within animal, between muscles. As previously shown (GANN and MERKEL, 1978) the rate of this process is faster in fast twitch fibers. As the possible causes of the variability noted between animals of similar age, breed and sex are still unknown we will focus on the possible origins of the muscle effects. In this respect, the three major points of interest are the variability in: (1) levels of enzymes and inhibitors, (2) sensitivity to proteolysis of intracellular proteins and (3) osmotic pressure changes.

-Muscle variability in enzymes and inhibitors content

As aforementioned the concentration of the two most investigated proteolytic systems, namely lysosomal cathepsins and calcium dependent proteinases or calpains, decrease as muscle contraction speed increase. Such a distribution does not fit with the rate and extent of meat tenderising which were shown to increase as muscle contracted faster (OUALI, 1991). Conversely, a good relationship was observed with the calpain/calpastatin ratio and a similar relationship could be expected for the lysosomal system. The enzyme/inhitor ratio might be considered as a good index of the post mortem potential proteolytic efficiency.

-Muscle variability in protein susceptibility to proteolysis

When submitted to proteolysis by either cathepsins D, B, L or papaïn purified slow myosin isoforms as well as sarcoplasmic protein fractions purified from slow twitch muscles exhibited a much lower susceptibility than the fast isoform (DUFOUR et al., 1989; OUALI, 1990). The present finding fits well with the observed rapid postmortem damage of the myofibrillar structure in type II muscles (GANN and MERKEL, 1978).

- Variability in osmotic pressure of postmortem muscles

Osmotic pressure or osmolality is proportional to the concentration of soluble low molecular weight compounds irrespective on whether these are electrically charged or not (WINGER and POPE, 1980-81). In postmortem muscle, as pH fell down, osmotic pressure increased exponentially and reached its maximum value at the completion of the rigor process. This behaviour is a common feature for all bovine muscles investigated so far and probably for all meat animal species. The maximum value attained is highly muscle type dependent (OUALI, 1990) and is positiviely correlated with the muscle contraction speed. The maximum osmolality achieved for type I muscle and type II muscles are equivalent to ionic strengths in the range 0.23 to 0.30. Such ionic strength values are high enough to cause important weakening of myofibrils and to improve the efficiency of indigenous proteinases (WU and SMITH, 1987).

CONCLUSION

The aim of this contribution was mainly to stress how fundamental research in the field of muscle biology and related post mortem muscle

biochemistry is critically required by a meat industry facing new challenges stemming primarily from new consumer requirements. If we are already aware of many of the chemical changes taking place post mortem in the muscle tissue and their effects on the physical properties of meat we are not yet fully capable to integrate our data into coherent structures allowing extrapolations either toward basic understanding or practical applications.

Indeed with our present knowledge we are not even able to anticipate (in terms of meat quality attributes most of the consequences of quite simple changes in early post mortem processing mainly because the very high variability of the muscle characteristics in the live animal which has a strong impact on the biochemistry of the post mortem conversion process. No actual progress will be achieved, allowing predictive modelling of the post mortem changes and so optimization of many processes and individual practices until we get a better understanding and a quantification of the impact of the biological variability of muscle traits on the control of the biochemistry of the post mortem changes.

REFERENCES

ARCHIBALD, A.L. (1987): A molecular genetic approach to the porcine stress syndrome. Curr. Top. Vet. Med. Anim. Sci. "Evaluation and control of meat quality in Pigs" : 343-358.

BARRETT, AJ.J., (1985): The cystatin : small protein inhibitors of cysteine proteinase. In Intracellular protein catabolism (E.A. Khairallah, J.S. BOND and J.W.C. BIRD, eds) A.R. Liss. New York. 105-116.

BECHET, D. ET AL (1990): Cimaterol reduces cathepsin activities but has no anabolic effect in cultured myotubes. Am. J. Physiol., E 822-E827.

BENDALL, J.R. (1973). Post mortem changes in muscle. In : Structure and function of muscle, ed. H. Bourne, Academic Press, New York : 243-309.

BUCHTER, L. and ZEUTHEN, P. (1971). The effect of ageing on the organoleptic qualities of PSE and normal pork loins.In : Condition and meat quality of pigs for slaughter, Pudoc, Wageningen : 247-254.

BUSCAILHON, S., et al (1991):Relationships between tissue composition and sensory qualities of dry cures ham. 37th ICOMST, Kulmbach.

CALLOW, E.H. (1938): Muscular fatigue and pH. Ann. Rep. Food Invest. Board (London) : 57.

DAVEY, C.L. (1983): Postmortem chemical changes in muscle-Meat aging. Recip. Meat Conf. Proc. 36, 108-115.

DUFOUR, E., et al (1989): Lysosomal proteinases sensitive regions in fast and slow skeletal muscle myosins. Biochimie, 71, 625-632.

EIKELENBOOM, G. and MINKEMA, D. (1974). Prediction of pale, soft, exudative, muscle with a non-lethal test for the halothane-induced porcine malignant hyperthermia syndrome. Tijdschr. Diergeneesk., 99 : 421-426.

ESTRADE, M., ROCK, E. and VIGNON, X. (1991). Ultastructural post mortem changes in myofibrillar structure of normal and halothane sensitive pigs. 37th ICOMST, Kulmbach.

FERNANDEZ, X.,and L. LEFAUCHEUR (1991):Paris Ham processing : technological yield as affected by residual glycogen content of muscle. Meat Sci. 29, 121-128.

FERRARA, M., et al., (1990): Gene structure of mouse cathepsin B. FEBS Lett. 273, 195-199.

GANN, G.L. and MERKEL, A.A. (1978). Ultrastructural changes in bovine longissimus dorsi muscle during postmortem ageing. Meat Sci. 2, 129-144.

GARIEPY, C., AMIOT, J. and NADAI, S. (1989). Ante mortem detection of PSE and DFD by infrared thermography of pigs before stunning. Meat Sci., 25 : 37-42.

GREGORY, N.G. (1987). Effect of stunning on carcass and meat quality in pigs. Curr. Top. Vet. Med. Anim. Sci. "Evaluation and control of meat quality in Pigs" : 265-272.

HATA, A., et al., (1989): Tandemly reiterated negative enhancer like elements regulate transcription of a human gene for the large subunit of calcium dependent protease. J. Biol. Chem. 264, 6404-6411.

HEFFRON, J.J.A., et al. (1974). Post mortem glycolysis in Semitendinosus, Psoas and Longissimus dorsi muscles of captive-bolt slaughtered and anaesthetized Boer goats (Capra hircus). 20th EMMRW, Dublin : 40-42.

HONIKEL, K.O. (1987):The influence of chilling on meat quality attributes of fast-glycolyzing pork muscles. Curr. Top. Vet. Med. Anim. Sci. "Evaluation and control of meat quality in Pigs" : 273-284.

LISTER, D. and RATCLIFF, P.W. (1970): The effects of preslaughter injection of magnesium sulphate on glycolysis and meat quality in

the steer. 16th EMMRW, Varna, 255-263.

MIRI, A. et al. (1991):Use of perfused isolated muscle as studied by 31P NMR to investigate metabolism and post mortem changes in muscles. (in press) Meat Science.

MONIN, G. (1989). Genetic effects on technological qualities of pig meat. 40th Ann. EAAP Meet., Dublin, paper GP3.1.

NAVEAU, J. (1986): Contribution à l'étude du déterminisme génétique de la qualité de la viande porcine. Héritabilité du rendement technologique Napole. Journées Rech. Porcine en France, 18 : 265-276.

OFFER, G. and KNIGHT P. (1988): The structural basis of WHC in meat. Devel. Meat Sci., 4 : 63-243

OUALI, A., and TALMANT, A., (1990): Calpains and calpastatin distrubition in bovine, procine and ovine skeletal muscles. Meat Science, 28.331-348.

OUALI, A. (1990): Meat tenderization: possible causes and mechanisms. A review. J. Muscle Foods. 1, 129-165.

OUALI, A. and TALMANT, A. (1990). Calpains and calpstatin distribution in bovine, porcine and ovine skeletal muscles. Meat Sci. 28, 331-348.

OUALI, A. (1991):Sensory quality of meat as affected by muscle biochemistry and modern technologies. In: Animal biotechnology and quality of meat production. (DEMEYER, D., FIEMS L., and COTTYN B. eds) Elsevier, Amsterdam (in press).

POLLACK, G.H. (1990): Muscles & Molecules. Uncovering the principles of biological motion. Ebner & Sons Publishers, Washington.

SATHER, A.P., JONES, S.D.M. and MURRAY, A.C. (1989):Carcass yield and meat quality from pigs with known genotypes at the halothane locus. 40th Ann. EAAP Meet., Dublin, paper GP3.7.

SELLIER, P. (1988): Aspects génétiques des qualités technologiques et organoleptiques de la viande chez le porc. Journées Rech. Porcine en France, 20 : 227-242.

TARRANT, P.V. (1988). Stress de transport chez les animaux de ferme. Rec. Méd. Vét. 164 : 823-833.

WU, F.Y. and SMITH, SB., (1987):Ionic strength and myofibrillar solubilization. J. Anim. Sci. 65:597-608

WINGER, R.J., and POPE, C.G., (1980):Osmotic properties of post rigor beef muscle. Meat Sci., 5:355-369

ZABARI, M. (1991):Fractionation and characterization of proteinase inhibitors from bovine skeletal muscle. 37th ICoMST Meat Science and Technology, Kulmbach

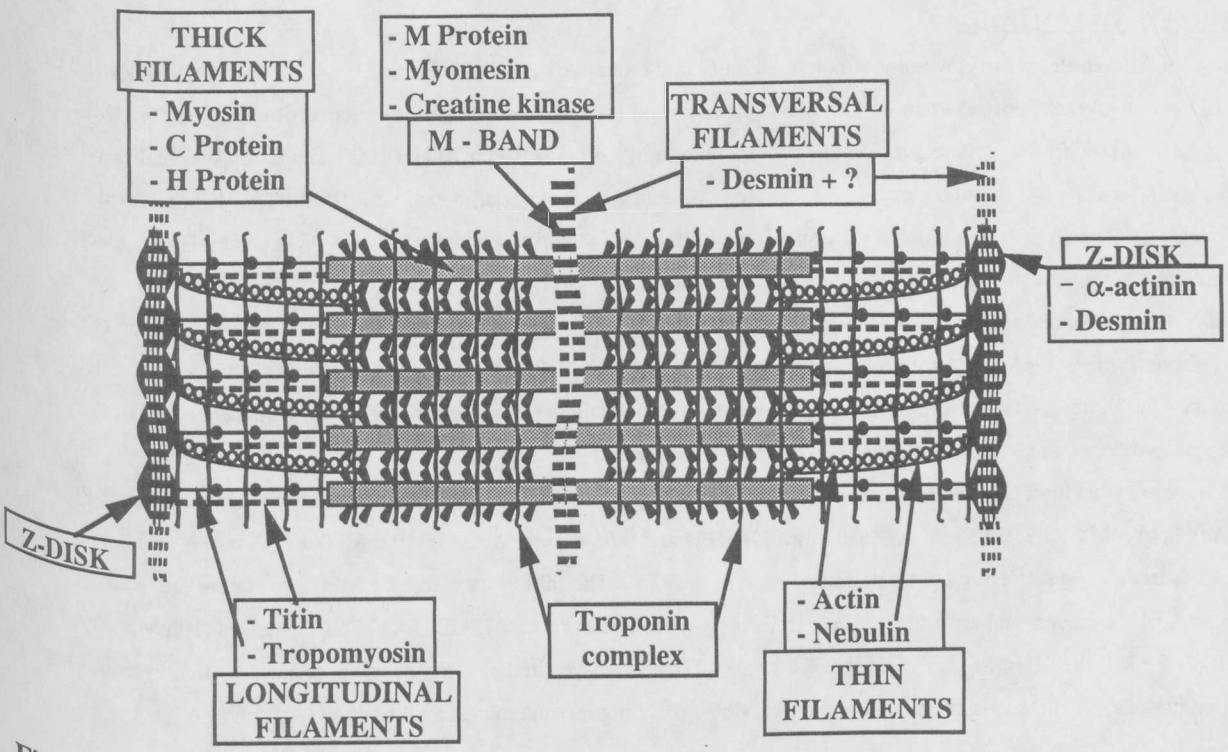

Figure 1 : Schematic model of sarcomere assembly proposed by POLLACK (1990).