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Histochemistry of Anaerobic Glycolysis in Meat Animals

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ABSTRACT

This review explains how p*H* measurements of pork and beef are dominated by the post mortem metabolism of one histochemical type of myofibre with alkaline myosin ATPase (fast contraction) and few mitochondria (an anaerobic specialization). Using glycogenolysis to identify the source of the lactic acid that acidifies muscles post mortem, these myofibres are involved in a variety of commercially important phenomena, from PSE (pale, soft, exudative) pork to electrical stimulation of beef to increase productivity.

Keywords: Anaerobic glycolysis, Meat p*H*, Meat quality.

INTRODUCTION

When muscle is converted to meat, the sliding thick and thin myofilaments that used to power muscle contraction become locked together in rigor mortis. Lacking oxygen from the circulatory system, many myofibres start to obtain energy from their stored glycogen if they have any, and they accumulate lactate because it cannot be taken to pyruvate to create acetyl CoA without oxygen. Lactate spreads easily between myofibres. This acidifies the muscle, and this decline in *p*H is one of the major factors determining meat quality.

There are two conventional ways to examine the acidification that anaerobic glycolysis creates in meat. Most commonly, technicians push a glass or solid state *p*H sensitive electrode into the meat. There is a lot of history here, from the Danish inventor of the *p*H scale, Søren Sørensen, to Arnold Beckman's electronics that have dominated the world markets for *p*H meters [1]. But they all rely on a reference electrode somewhere, and meat does not always have an appropriate conductivity between the calomel electrode (silver/potassium chloride) and the reference electrode. Wet meat may give repeatable results, but dry meat may not. An older way to measure acidification in meat was to emulsify a meat sample in a fluid, taking care to avoid the problems of dilution and inactivating any further glycolysis [2].

Both methods integrate the activities of countless individual myofibres to produce an average. But they do not reveal what is happening at the cellular level where different histochemical types of myofibres may doing different things at different times depending on temperature, neural activity, levels of stored glycogen and applied treatments such as electrical stimulation [3].

In this review, the primary objective is to explain what may be happening at the cellular level with anaerobic glycolysis in meat. There are a few situations where meat may be dominated by a single histochemical type of myofibre in adult animals, but only after birth or hatching (as in the case of superficial parts of chicken breast muscle). So, to fully understand anaerobic

glycolysis in meat we must get down to the cellular level, and this requires histochemistry – a combination of microscopic histology and biochemistry.

FROM WHOLE MUSCLES TO HISTOCHEMISTRY

Post mortem glycogenolysis in bovine myofibres was detected histologically by Robertson and Baker in 1933 [4]. Soon after slaughter, Best's carmine stained the glycogen between myofibrils, but this was lost in later samples because of post mortem glycogenolysis. The significance of this discovery was not obvious at the time – Nobel laureates Albert Szent-Györgi and Hans Krebs were still busy discovering and proving the citric acid cycle, and there were no p*H* meters capable of measuring meat p*H* directly.

Here is an informal introduction to set a background for a story to come. In the late 1800s, muscle physiologists had tickled the surfaces of frog muscles with stimulatory electrodes and found that myofibres with a red colour had slower and weaker contractile responses to pulling up a weight attached to the whole muscle than pale myofibres. This started the whole nomenclature of red and white myofibres.

This did not make much sense when applied to higher vertebrates, most of which have visually red myofibres with some exceptions like the white myofibres of chicken breast muscle. A further puzzle was that the powerful pale myofibres in frogs had small plate like neuromuscular junctions while the slower redder myofibres had expanded grape like junctions.

The next jump forwards in understanding of this subject required the invention of cryostat methods. Small strips of muscle were frozen in liquid nitrogen, trimmed while frozen, then sectioned transversely while frozen in a cryostat (a refrigerated microtome). This enabled some outstanding organic chemists to develop histochemical methods to reveal mitochondria and fast and slow contracting myofibres based on their myosin ATPase activity. Thus, within the visually red muscles of higher vertebrates was a mixture of histochemical types of myofibres, where even the fast contracting fibres were red with some myoglobin. The visually white muscles like chicken breast muscles had all fast contracting myofibres once they matured after hatching. But all the myofibres in the major postural muscles of mammals had plate like neuromuscular junctions. Thus, the major muscles of higher vertebrates like cattle, sheep and pigs had no really slow myofibres like those in frogs. But there was still a differentiation between myofibres with plate like neuromuscular junctions– some were specialized for fast, powerful contractions while others were specialized for slower but more sustainable contractions. All had some mitochondria and could store glycogen, but only the fast ones had a high level of enzymes for anaerobic glycolysis, and only the slow ones stored triglyceride droplets for sustainable aerobic activity.

This was an embarrassing time in biological science communication. Many researchers who examined cryostat sections of skeletal muscle proposed their own nomenclature that their competitors would not follow. The problem is still with us today, leaving the rest of us to attempt a clarification of what histochemical types of myofibres we are writing about as we attempt to explain the histochemistry of glycogenolysis in meat. Figure 1 shows a simple nomenclature for histochemical types of bovine myofibres, following the oldest terminology of red and white myofibres.

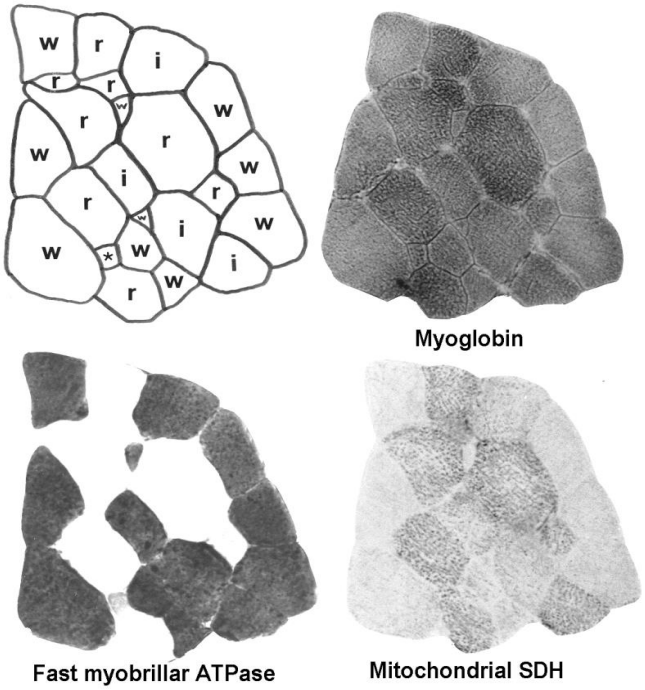

Fig. 1: A simple nomenclature for histochemical types of myofibres in beef. White myofibres (w) are fast contracting with alkaline myofibrillar ATPase and few mitochondria while red myofibres (r) are slow contracting with acid stable myofibrillar ATPase and many mitochondria. All myofibres may have myoglobin, but it is strongest in red myofibres. There are intermediate myofibres (i) with fast contraction and a medium content of mitochondria.

READING TRANSVERSE SECTIONS

In Figure 1 there are intermediate myofibres (i) that are in the process of changing their histochemistry. Depending upon animal age and weight and the requirements for muscle contraction, postural muscles get more red myofibers by conversion from white myofibers. Other muscles may convert red to white myofibers to gain power [5]. In Fig. 1, there is a small myofibre marked with an asterisk (*). This is a tapered ending of a myofibre embedded in endomysial collagen. In adjacent serial sections it appeared as a normal diameter myofibre. Thus, reading transverse sections of muscle to measure glycogen content is difficult. An easy solution is to ignore intermediate myofibres and tapered endings because, at this plane of sectioning, their glycogenolysis will not contribute very much to overall lactate production and muscle p*H.*

MEASURING GLYCOGENOLYSIS

If identifying histochemical types myofibres is difficult, then measuring their glycogen content as it disappears to lower muscle p*H* is a greater challenge. The basic problem is that the glycogen in a rested animal immediately after slaughter is located in the sarcoplasm between myofibrils. Measuring glycogen photometrically in myofibres is difficult because of the white light transmitted through the myofibrils (distributional error in photometry). There are ways around this problem by measuring at two wavelengths, one at the absorbance maximum of the Schiff-reagent and one to assess the unabsorbed white light, then comparing the two mathematically [6].

PSE PORK

PSE pork used to be called watery pork when it first became a commercial problem in Europe and the UK in the 1960s, but then it became linked to three other commercially important attributes [7]. Pork with an abnormally low p*H* is very pale (P), unusually soft (S), and exudative (E), a fluid loss that causes problems commercially. Commercial problems are a great stimulus to research, justifying government and industrial funding, and finding the cause of PSE pork started a major field of investigation. The ultimate cause of PSE in pork was later found to be calcium ion activation from the sarcoplasmic reticulum [8], the genetic basis of which enabled it to be bred out of commercial pigs. But much of the research on lactate production and anaerobic glycogenolysis in meat is still valid. The p*H* of meat has profound importance everywhere, not just in pigs with a mutation in their ryanodine receptors.

One might expect photometric measurements of glycogenolysis in normal pigs to show a steady decline after slaughter in all histochemical types of myofibres starting with some glycogen. Eventually this expectation holds true, but some red myofibres starting with relatively low glycogen actually increase their glycogen for a while, as if they are taking up the lactate from surrounding myofibres and using it for glycogen synthesis, otherwise difficult to explain in a muscle without a blood supply [9]. At the cellular level, things may be more complex than revealed by a p*H* probe.

The distribution of glycogen shown in Fig. 3 is quite uniform before post mortem glycogenolysis, even to the extent that it provides a convenient way to study the proliferation of myofibrils in growing pigs [10].

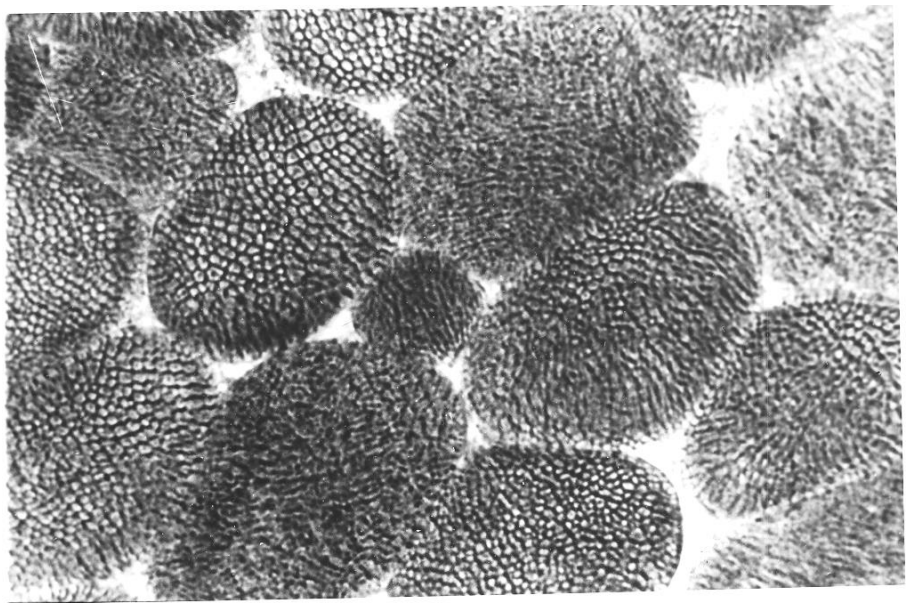

Fig. 2: Glycogen in the sarcoplasm between myofibrils stained by the periodic-acid Schiff reaction.

But the story does not stop with taking glycogenolysis down to the cellular level. At the sub cellular level, inside myofibres, there are some unexplained things happening. This research is going back a long way, before image analysis methods were readily available. In 1990, the best

way to scan a microscope image of glycogen in a myofibre was to use an electromechanical scanning stage [11]. Why does glycogenolysis start at the edge of the myofibre under the sarcolemma? Perhaps some pigs have a glycogen storage disease and the central cores of myofibres lack glycogenolytic enzymes? Does this have any connection to unexplained variations in meat quality in pigs [12]?

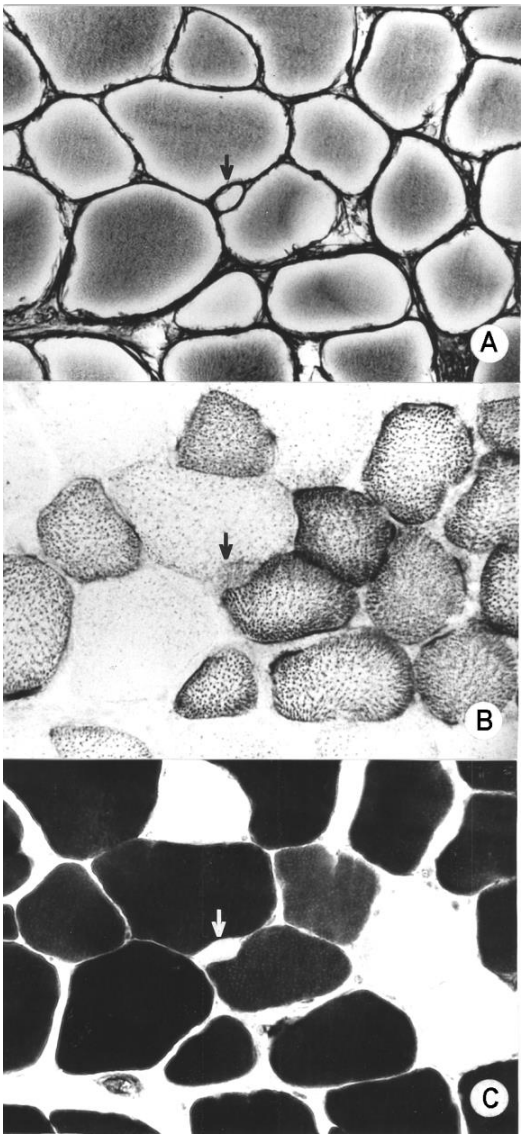

Fig. 3: Serial transverse sections where myofibre outlines are shown by silver staining of endomysium (A), mitochondrial content is shown by a reaction for SDH (B), and slow contracting myofibres are shown by acid stable ATPase (C). The arrow shows the tapered ending of a myofibre unrelated to the myofibres to which it is attached.

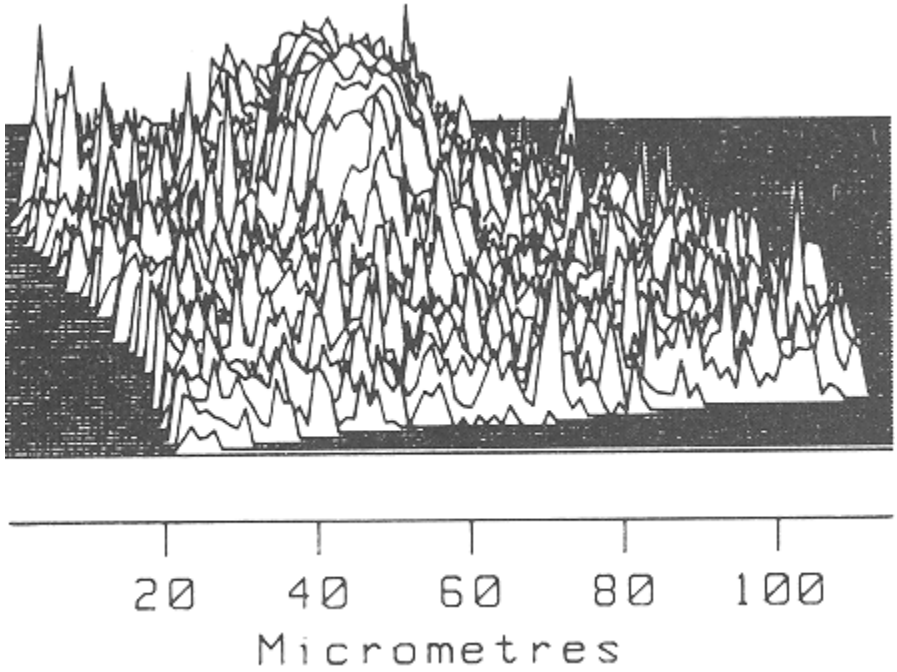

Fig. 4: Scanning stage photometry of PAS-stained glycogen in a porcine myofibre during glycogenolysis. The height of the peaks shows the absorbance of PAS-stained glycogen.

GLYCOGENOLYSIS IN BEEF

In porcine muscles the embryonic arrangement of myotubes and secondary myofibres is retained post natally in the pattern of red and white myofibres [13], although nobody has ever explained why. But in bovine muscles, as in most other mammals, this clear pattern is soon lost after birth, leaving us looking at an almost random patter of different histochemical types of myofibres (although red myofibres still tend to be near the central axis of their fasciculi).

The dominant pattern of glycogenolysis in beef is from white myofibres (Fig. 5).

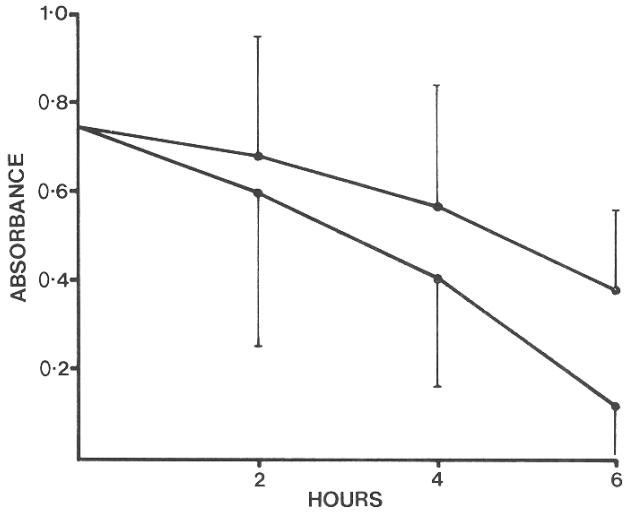

Fig. 5: Decline in absorbance corrected for distributional error of PAS-stained glycogen in white myofibres (alkaline ATPase and low SDH) in beef sternomandibularis muscles at 22° C (upper line) and 40° C (lower line). Vertical bars are standard deviations.

Intermediate myofibres contribute a little less and red myofibres much less to lactate production and lowering of p*H* (because they start with less glycogen). These results were obtained in excised muscle samples wrapped to maintain humidity and exclude oxygen; otherwise transected motor axons would have caused random patterns of glycogenolysis in motor units [3]. It is always difficult to experiment with excised muscle samples trying to guess how they might have behaved if they were still in an intact carcass.

The data shown in Fig. 5 follow what would be expected from first principles – glycogenolysis is slower at a low temperature than at a higher temperature. However, this does not hold true if temperatures are lowered below 5° C, below which temperature glycogenolysis is activated by the accumulation of adenosine monophosphate which then activates phosphorylase *b* [15]. At the cellular level, histochemistry shows that this phenomenon originates from white myofibres with strong alkaline ATPase and weak SDH [16].

Another topic where an informal introduction is required, concerns electrical stimulation during the conversion of muscle to meat. Skeletal muscles in meat animals are prone to contracting as they develop rigor mortis, particularly at a low temperature. This creates a permanent decrease in sarcomere length, and with thin and thick myofilaments locked together, the meat may become very tough. In the meat industry, avoiding sarcomere shortening by slow refrigeration is a risk for microbial spoilage and reduces productivity. Thus, electrical stimulation is widely used method to minimize refrigerated storage times. The question to be answered here is what histochemical types of myofibres respond to post mortem electrical stimulation? It is no surprise to find that fast-contracting white myofibres with alkaline ATPase and few mitochondria gave the strongest glycogenolytic response to post mortem electrical stimulation [17].

CONCLUSION

Measuring meat p*H* with electrodes is an integral method of meat science, generating countless research papers where p*H* is then correlated with nearly all aspects of meat quality – colour, tenderness, taste. etc. The data reviewed here show that post mortem glycolysis and meat p*H* are nearly always determined by the activity of white myofibres with alkaline ATPase and few mitochondria.

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