D I S C U S S I O N

CONCERNANT LES RAPPORTS PRESENTES AU COURS DES

SESSIONS A et B

(Tome I des compte rendus -- 3 Septembre 1973)

Introductory remarks to Rapport Principal - thème général A

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I would like to begin by outlining once more the fundamental basis of the rigor process in skeletal muscle. I consider this necessary in view of the nature of the contributions to this session, several of which tend to confuse rather than clarify the main problems.

The rigor process consists of all those biochemical reactions which bring about the physical changes in muscle structure and consistency, from the highly extensible, contractile, gel-like state of living muscle to the rigid, non-extensible, non-contractile state of 'dead' muscle. These changes, although complex enough, are far simpler than those which occur in the muscles of a living animal, because during rigor the blood supply to the muscles has of course been cut off and therefore all the changes take place under anaerobic conditions, except on surfaces exposed to air. Thus in the bulk of the musculature the main post-mortem processes are reduced to the steady turnover of ATP and its resynthesis from phospho-creatine and from the reactions of the glycolytic cycle (the phosphoglycerate- and pyruvate-kinase steps). The loss of ATP when these sources of resynthesis are almost exhausted is the immediate cause of rigor stiffening, as discussed in detail in my report. This is indeed the main physical change, accompanied under certain conditions by varying degrees of contracture which, as we have slowly learnt over the past ten years, can have serious effects on the consistency of meat, particularly on its tenderness

The last step in glycolysis is the formation of lactate, accompanied by fall of pH, one H ion being released per lactate ion formed. In normal meat animals, the muscle pH falls from about 7.2 in the living muscle to an ultimate value of 5.4-5.9 in rigor muscle. This pH fall towards the isoelectric point (5.4) of the structural proteins, actin and myosin, is largely responsible for further changes in muscle consistency, particularly for the shedding of osmotically held water from the actomyosin filaments. This causes a change from a firm gel-like consistency to a flaccid one and to a very marked increase in the amount of fluid which can be pressed out of the muscle fibres, mostly through their cut ends. The low pH also brings about denaturation of muscle proteins, particularly the sarcoplasmic proteins, creatine kinase and phosphofructokinase and the myofibrillar proteins of the actomyosin complex, together with proteins of the sarcolemmal membrane. The denaturation is highly pH and temperature dependent, but will nevertheless occur in time under all conditions of storage, however slowly. Its effect on consistency is to increase the shedding of firmly bound water and thus the amount of drip fluid, a phenomenon easily observed during storage of small meat packs in supermarkets.

I must now emphasise the most important point of all from the biochemical point of view: it is the turnover rate of ATP which determines the rates of all the other biochemical reactions during rigor and which also determines the rate and degree of muscle contracture. I will now adduce the reasoning behind this statement:

1. In a closed system of this sort, where the total adenine nucleotide level is constant, no PC nor glycolytic intermediates can break down unless the product of ATP turnover, ADP, is continually produced as acceptor of phosphate from these sources.

2. Even during the fastest observed rates of post-mortem pH fall and of ATP turnover, the glycolytic system is fully capable of keeping up with the demand for ATP resynthesis, providing a supply of PC is present to act as an 'ATP-buffer'. This is proved by the classical experiments of the Cori's who showed that

glycolysis was stimulated after a contraction to more than 200 x the resting rate. Hence it is a waste of time to search for rate-determining steps within the glycolytic pathway itself. This is further proved by the fact that the post-mortem build-up of glycolytic intermediates is always very small, even in extreme cases amounting to less than 8 μmole/g, compared with a 'global' turnover of 150-170 μmole of ATP and a formation of 100 μmole of lactate and of 35-40 μmole of P_i.

3. The experimental proof of points 1 and 2 is provided by Dr. R.K. Scopes' (Scopes, 1973 in press) recent work on reconstituted glycolytic systems, starting with highly purified enzymes. If no ATP-ase was present, but only glycogen, ATP, creatine and phosphate, lactate was produced and PC was formed until all the creatine was phosphorylated, the latter being accompanied by a slight fall of pH of about 0.2 units. When however an ATP-ase was later added, PC was broken down and glycolysis proceeded normally until the ATP and glycogen supplies were exhausted, H ions now being produced in proportion to the lactate formed to give a normal pH fall of about 1.2 units.

Even with large amounts of ATP-ase present the accumulation of intermediates was quite small, showing that none of the glycolytic reactions were truly rate-limiting.

Accepting therefore that the true rate-determining step in glycolysis in dying muscle is the ATP-ase activity, we know that this activity, like the true resting ATP-turnover rate in living muscle, is only about 1/300th of the maximal activity during a living contraction. Because the post-mortem rate is so low, it becomes very difficult to identify with certainty the ATP-ases which are responsible. This is particularly so because any distburbance to the intact muscle is liable to trigger Ca-release from the sarcoplasmic reticulum and thus stimulate the powerful contractile ATP-ase of actomyosin. For example, lowering the temperature below 10°C has this effect in most muscles, often also causing cold-contracture, as discussed in the main report. Even the preparation of thin muscle strips from an intact muscle, particularly if they

are immersed in a fluid medium, will lead to a doubling of the turnover rate. For this reason I am doubtful whether the methods employed by Dr. Hamm and collaborators in paper A5 really help us very much in identifying the resting ATP-ases.

Another approach to the problem is to consider the temperature coefficients ($Q_{10°}$) of the various ATP-ases known to exist in muscle, and compare them with the $Q_{10°}$ of 1.75 for the rigor process in the temperature range 25-35° where there are no complications due to contracture. The contractile actomyosin ATP-ase, stimulated by Ca-release, and also the ATP-ase which drives the Ca-pump itself both have $Q_{10°}$ values of 3.9 or higher and hence can be ruled out as being active in the resting muscle, except at extremely low levels. On the other hand, myosin ATP-ase in the presence of Mg ions only (as in resting muscle) has a coefficient of about the right order (1.8) and moreover, shows at 35° a specific activity of about 1/250th of that of the actomyosin enzyme, similar to the relative activity during rigor. Hence this enzyme alone would account for all the observed activity in normal resting muscle. At present therefore it seems the most satisfactory suggestion.

Apart from the nature of the true slow resting ATP-ase, we are presented with another major problem, that of the exceptionally high post-mortem rates of ATP-turnover which occur during cold-contracture and also during rigor in the muscles of PSE-prone pigs. It is thought that both of these are due to release of Ca-ions from the Ca-pump in amounts sufficient to just trigger the actomyosin ATP-ase, but there is no actual proof that this is so. The only really acceptable proof might come from injection into the muscle fibres of the Ca-sensitive protein, aequorin, which emits a flash of blue light as it sequesters Ca ions. The method has been used successfully in the study of the very early stages of contraction in the large muscle fibres of some crustacea and mollusca, but has not yet been adapted for use with the much smaller fibres of mammals.

I consider that this problem of the ATP-ases in post-mortem muscle is most important of all, since a method of controlling the ATP-ase activity would automatically enable us to control many aspects of meat quality, particularly cold-contracture and the PSE condition.

I do not wish to speak in any more detail about the cold-shortening problem itself, except to say that I do not consider it any longer profitable to carry out elaborate studies to prove that muscle shortening causes meat toughening. This point is now adequately established. It is more important in my opinion to establish the relation between the degree of cold-contracture and the muscle pH. For example, would the beef muscles described in my report show any 2nd phase cold-contracture if the pH had been allowed to fall below 6.5 before cooling? Equally important is to discover the real reason for the toughening which occurs when cold-shortened muscle is cooked. This is certainly not due solely to myosin-actin overlap in the shortened muscle.

Another aspect of meat science of utmost importance is control of the pre-mortem condition of the animal, particularly the avoidance of undue stress before slaughter of such very stress-prone animals as pigs. This involves deep study of their physiological peculiarities and of the hormonal imbalance which gives rise to them. This problem is under intense investigation at our Institute, but it is yet too early to say more than that the thyroid hormones are certainly implicated.

Finally I would like to turn back to a theoretical aspect of muscle glycolysis. It has been an article of faith for many years amongst workers in our laboratory, particularly Dr. E.C. Bate-Smith, Dr. R.K. Scopes and myself, that Claude Bernard's original discovery in 1870 of the breakdown of glycogen to lactate was correct. In other words that all the glycogen disappearing post-mortem could be accounted for chiefly by lactate appearance and to a minor degree by concomitant appearance of glucose to the extent of about 9% of the glycogen and of known phosphorylated glycolytic intermediates to the extent of about 4%. It therefore came as a shock to read paper A5 by Dr. Hamm and his

collaborators, where it is suggested that this is not so, but that in some cases more than 20% of the glycogen disappearance cannot be accounted for. For this reason I was at some pains to analyse the values for beef muscles in table 1 of paper A5 and I found, after allowing for formation of 7-9% glucose, that the mean value for conversion of glycogen to lactate was 95.8% with a standard deviation of ± 30%. It is obvious that with such a large standard deviation this mean value cannot differ significantly from 100%! I would therefore beg Dr. Hamm to look into the question of possible errors in the glycogen, lactate and glucose determinations, the other intermediates being of little or no consequence. I think this is more important than searching for a new intermediate which in some cases seems to amount to more than 10 µmole/g muscle. Such a quantity would certainly not have escaped detection by modern analytical methods.

This brings me to a general plea to workers in meat science, particularly in the biochemical field, to scrutinise most carefully not only their analytical methods but the whole planning of their experiments. I ask them to bear in mind that they are dealing with a still living system, geared to react to much more subtle changes in external and internal environment than the crude treatments often handed out to it.

Before closing my remarks, I would like to add a tribute to Professor Jean Hanson who died suddenly and tragically in the first week of August from a rare form of meningitis. Her loss will be deeply felt in the field of muscle biology to which she contributed so much, and particularly amongst her colleagues in King's College London, where she had just assembled an excellent team of muscle biologists.

Her major contribution was of course her collaboration with Dr. Hugh Huxley which produced the sliding filament theory of contraction in 1953. This is perhaps the most important and revolutionary concept introduced into muscle biology since Engelhardt and Liubimova's discovery of the ATP-ase properties of myosin in 1939 and Szent-Gyorgyi's discovery of actin in 1943.

We all knew her as Jean, the lively, enthusiastic and irrepressible Jean. I well remember her presentation of the sliding filament theory to a meeting in Leeds in 1953, which was attended by all the leading lights in the muscle field, such as Dr. Astbury, Professor Weber, Dr. Bailey and many others. This young woman stood up in front of them all and began her talk with remarks to the effect that 'we can now for the first time present a complete theory of how a muscle contracts'. Imagine the electrifying effect of this statement on an audience amongst which at least ten conflicting and rather muddled-headed hypotheses were current at that time. Well, that was Jean's nature and all honour to her for her courage and forthrightness in expressing it. We shall all miss her, not least because at the time of her death she was on the point of contributing to another major breakthrough in the muscle field.

A 0 Thème général: Biochimie du muscle post mortem

§ Q.- from HAMM

1) On page 6 you mentioned that diffusion of P_i , splitt off from ATP, to the sarcoplasma causes start of the glycolytic sequence. I wonder if P_i released from ATP really starts glycolysis; there is a lot of P_i in the muscle tissue before breakdown of ATP. Is not the disappearance of ATP the process which starts glycolysis e.g. by activating the phosphofructokinase step ?

R.- I think both P_i release and ATP disappearence are involved in triggering glycolysis, but the most important step is release of ADP to act as acceptor.

2) Has anybody studied the effect of agents, which specifically activate or inhibit membrane ATPases, on the cold shortening phenomenon ?

R.- I do not know much about work on membrane ATPases, except for the well known effect of ouabain on the Na/K pump in some membranes .

3) How do you explain the shortening of fibres during the first phase of cold shortening without splitting of ATP (Fig.3) ?

R.- ATP is split (and resynthesized) continously throughout the first phase of cold-shortening as shown by the disappearence of PC and appearence of lactate (see fig. 3 and 4).

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§ Q.- from CASSENS R.G.

Would muscle in the intact carcass follow more closely the pattern of cold shortening for loaded or unloaded strips of muscle ?

R.- In the intact carcass, cold shortening of sarcomeres takes place in a very irregular fashion. The first sarcomeres to shorten will develop enough tension to stretch the relaxed ones in any particular fibre, but as the latter begin to shorten those which shortened earlier may already themselves have started to relax again . Thus, the tensions which are developed are geater than those in unloaded strips and less than those in heavily loaded strips.

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§ Q.- from HENRY M.

Le Dr BENDALL a fait allusion au rôle éventuel des hormones thyroïdiennes sur la qualité de la viande. Que sait-on, en 1973, du rôle des hormones de la corticosurrénale notamment de l'aldostérone ? (suite au travaux de 1958 de HENRY M. et de PASSBACH et al de 1968) .

R.- I do not think we are yet in a position to assess the relative importance of the hormones of the thyroïd and the suprarenal glands to the problem of PSE meat. The thyroïd is certainly implicated, but its site of action has certainly not been identified .

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§ Q.- from KOTTER

Daß Anionen in Zerkleinertem Fleisch den I.P. von Muskelproteinen senken und damit (ohne Veränderung des aktuellen pH - Wertes) auf das Eiweiß einen der pH-Erhöhung analogen Effekt haben ist bekannt. Wie verlaufen Toten -starre und Reifung, wenn in schlachtwarm zerlegte Fleischstücke mit einem Injektat Anionen (Z.B. Cl-Ionen) eingebracht werden.

It is generally known that anions in the commimited meat decrease th I.P. or muscle proteins and (without changing the veal pH) effect proteins in the same way as pH increase. What will be to the process of rigor and ripening be like when in the slaughter warm meat we inject anions (e.g. Cl) ?

R.- I do not know the answer to this question. Perhaps Dr KOTTER will perform an experiment to find out .

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thème A O (suite)

§ Q.- from SCHUT J.

When the contraction phase n°1 is brought about by the decreasing activity of the calcium pump at low temperature, resulting in a high Ca^{++} concentration in the intrafibrillar space, how can one explain the subsequent relaxation ?

R.- I am not able to offer a good explanation of the relaxation phase. I think we must remember, however, that cold shortening involves only a few sarcomeres at any one time , because the contracture develops very little force compared with the potential maximum calculated from the ATP-turnover rate. Thus Ca^{2+} can have been released only to a very minute extent as the temperature fell . Relaxation may occur because the Ca pump can regain control and pump back these few Ca^{2+} ions .

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§ Q.- from LACOURT A.

1) Comment expliquez-vous que les fibres de type "contraction lente" présentant in vivo une contraction inférieure à celle des fibres "contraction rapide", semblent d'après vos résultats, développer une tension plus importante lors du cold shortening ?

R.- I do not think there are any hard and fast rules relating degree or speed of cold-contracture either to-a) the muscle contraction(type, i.e. fast or slow; or b) to the colour, i.e. myoglobin content. We have found all kinds of variations in beef anf pig muscles, as table IV of my report clearly shows.

2) A votre avis, comment se déclanche la deuxième contracture au froid. Le réticulum sarcoplasmique intervient-il à ce niveau dans le muscle ?

R.- I think phase 2 contracture is akin to rigor shortening. The latter seems, according to WHITE (1970), to occur as the ATP in the neighbourhood of the contractile filaments falls to very slow levels (less than 0,1 µ./mole/g muscle). Under these conditions it seems that Ca^{2+} ions are not obligatory for contraction, but rather that the troponin complex loses its repelling effect, and cannot any longer prevent actin myosin interaction .

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§ Q.-from EIKELENBOOM G.

Isn't it the ratio between ATP and its hydrolysis products which determines the glycolytic rate ? If this is true, other factors as the ATPases might have an effect on the glycolytic rate .

R.- The rate of both PC breakdown and of glycolysis is primarily determined by the free ADP level. This in itself can be calculated from the known amounts of PC, creatine and ATP present at any particular stage . At pH 7,1 (resting condition), the free ADP level is ~ 30 n moles/g, rising to ~ 80 n mole/g at pH 6,5 .

/A 2/

§ Q.- from DUMONT B.L.

Peut-on préciser le rôle de l'exercice sur la rapidité de l'évacuation, par le sang, du lactate hors du muscle ?

R.- Lactate disappears very rapidly from the muscle even during exercice .

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thème A 2 (suite)

§ Q.- from LEEST J.A.

1) It is generally accepted that stress just before slaughter has an adverse effect on meat quality because it enhances the anaerobie breakdown of glycogen in the muscle post mortem. How can it therefore be explained that in the biopsy samples from stressed pigs no significant correlations exist between glycoglytic metabolites and meat quality parameters while these do exist in samples from non-stressed animals ?

R.- The time-lag between biopsy taking and slaughtering .

2) Are data available on the metabolite content in the muscles after slaughter and on pH and rigor value ?

Yes, there are. The level of the metabolites is mostly higher (100 %) than the biopsy ante mortem. The relation between these parameters and the post mortem metabolites were better .

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§ Q.- from BENDALL J.R.

Would Dr SYBESMA agree that the temperature , at which the biopsy samples are kept , is the most important factor determining the glucose-6-P level ?

R.- In my opinion the experiments gave too less information wether keeping time or temperature are the most decisive factor .

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§ Q.-

In beiden Vorträgen wurde über den Einfluß verschiedener analytischer Daten auf die Qualität des Fleisches berichtet; Dabei wurde nicht auf die untersuchten Qualitätsmerkmale näher hingewiesen. Würden Sie bitte kurz erläutern, um welche Merkmale des Fleisches es sich hierbei handelte ?

R.- 45 minutes post mortem measurement of temperature
pH
rigor

24 hours post mortem
- visual examination (scoring)
- transmission value measurement (Hart)
- reflectance (Göfo measurement) .

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§ Q.- from MONIN G.

Au cours de vos expériences , avez-vous remarqué des différences de teneurs en intermédiaires glycolytiques du muscle liés à des facteurs génétiques ou d'élevage (environnement, mode d'alimentation etc ...) ?

R.- In other experiments we have noticed differences in genetical background in respect of glycolytic intermediates .
The relation between the intermediates and environment, feeding level etc.. was less clear .

/ A 3/

§ Q.- from RUBIN

In paper A 4 the preferred voltage is 300 .
What is the frequency ?
In both papers A 3 and A 4 the final meat quality does not seem to be affected by the conditions used during stunning, except of course for blood splashing. In view of the results reported, and in numerous previous papers, are the authors prepared to recommend conditions for electrical stunning which are satisfactory from the point of view of both technology and quality ? If not, can they suggest definitive experiments so that we can dispose of the subject once and for all .

R.- The frequency used in paper A 4 was 50 hertz (Cycles/Sec). This is too wide a question to be dealt with adequately in the short time available; it would be desirable to seek the views of the meeting on this matter, and to debate it at length. (Reference may be made to the work of McLoughlin who has demonstrated that stunning leads to a lowered initial pH, faster glycolysis, lower pH ult. and poorer meat quality compared to the same parameters in pigs slaughtered without stunning. Also to the work of LISTER and RATCLIFF read at Bristol 1971 which showed a similar improvment in the same parameters when pigs were not stunned, but narcotised with a lethal dose of Mg^{++} . Thus it appears to be inherent in the electrical stunning process that some deterioration and loss of meat quality occurs as a result of stimulated adrenergic activity .

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§ Q.- from BORZUTA K.

Is the time 3 seconds of application of current enough to stun well the pigs?

R.- Pigs vary widely in their individual response to standard stunning procedures; some animals will recover a degree of activity within 3 seconds of stunning , while others exhibit a deeper narcosis and are flaccid up to 15 seconds or more (at the point of bleeding). We observed no overall differences in these respects in the experimental pigs stunned either for 3 seconds or 8 seconds or with high or low frequency current .

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§ Q.- from DEVEREUX J.

Is the pig sufficiently stunned after application of current for 3 seconds to allow sticking to be carried out without the animal showing signs of recovery ?

R.- see answer to Dr BORZUTA K. (above) .

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§ Q.- KIDNEY A.J.

In the high frequency experiments was the current used of square wave type (as demonstrated at our Meeting in Hungary) or ordinary sine wave ?

R.- Sinusoidal current was used .

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§ Q.- DUMONT B.L.

Quelle peut être la nature du "stress" réalisé par votre aiguillon électrique ?

R.- The nature of the stress induced by electrical stunning is a matter that requires physiological investigations , existing knowledge is considerable but time is too limited to permite a summing up of this matter. Suffice it that adrenergic activity ensues , which can exert a major effect upon post mortem biochemical changes and meat quality .

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thème A 3 (suite)

§ Q.- from JUL M.

Was any differences observed in the nature of the contractions ? Did 3 seconds at 50 cycles give adequate stunning (Danish experiences suggest that it does not) .

R.- See answer to question by Dr BORZUTA (above) .
The fit induced by electrical stunning is similar in its effects-withdrawe of the hind limbs and tonic contraction of the forelegs which are held out stiffly during and immediately after passage of the current - when using high or low frequency current .

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§ Q.- from FROUIN

La fréquence de 1300 cycles est-elle la fréquence optimum ou a-t-elle été prise au hasard ?
Autrement dit, l'auteur connait-il la relation continue entre le temps requis et la fréquence ?

R.- The experimental work concerned the use of two frequencies only, and two periods of stunning. We do not have data to enable a curve to be constructed, relating these stunning parameters to haemorrhage .

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§ Q.- from SYBESMA

a) since the standard deviations of most of the quality characteristics in group 7 B (table 2) were larger than in 7 A, would n't it be more likely that the stress in fact resulted in more PSE and DFD like carcasses ?

b) It would be useful to discuss whether the meat quality parameters used in this study are the most appropriate ones .

R.- It is true that the SD values were wider in groups B than in groups A. But no examples of PSE were seen in either group ; and DFD meat was not significantly more evident in one group rather than in the other. The parameters examined - drip and color- were examined because these are quality features which are of prime importance in retailing meat .

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§ Q.- from ROZIER

En dehors des conditions particulières du courant électrique, avez-vous étudié l'influence de la durée entre l'anesthésie et la saignée sur la fréquence des hémorragies musculaires ?

R.-The influence of the delay period before sticking (bleeding) is generally considered to play a major role in regard to haemorrhaging .

In the experiments described in this paper, the influence of this delay was only examined in conjonction with long time of stun (8-9 seconds) with low frequency (50 c/ s) current : under these conditions the incidence of haemorrhage in the shoulder joints was so high - 80 % - that increase of the delay period, from 12 seconds to 15 seconds produced no further effect .

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§ Q.- from TATULOV
From your paper, can we draw the conclusion that the frequency of haemorrhage depends on the method of electrostunning and it does not depend on the pre-slaughter treatment of the animals ?

R.- This does appear to be the logical conclusion deriving from the results of this work .

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thème A 3 (suite)

§ Q.- from BARABES P.

Auch in Ungarn beschäftigt man sich mit der elektrischen Betäubung.Unsere Ergebnisse sind auch Ahnliche, wie die in dem A 3 und A 4 Vortrag erwähnt wurde. Aber soll man berken , dass wir statt der höheren Frequenz, nur mit 200 HZ, aber mit der viereckigen Form arbeiten. Hier wurde die höhere Frequenz vielleicht mit dem steilen Auf- und Ablauf des Impuls ersetzt . Neben der 300 V maximal-Spannung verwenden wir auch eine Strombegrenzung. ach meiner Meinung hat es grosse Bedeutung .

R.- M. Barabas 's observations are particulary interesting as they refer to use of square wave current, of which we at present have no direct experience. Preliminary data on the effectiveness of square wave current in stunning the animal and in reducing haemorrhage was given by Szücs et al at the 9th Meeting in Budapest (1963). It would be of value to know whether subsequent experience in Hungary has established the optimal voltage, amperage, frequency, and time of stun in relation to degree of narcosis of the pig, and quality of the meat. Safety factors for operators are also important when voltages as high as 350 are used .

/ A 5/

§ Q.- from BENDALL

Please, reply to my question :
Are sure that the low % recovery of glycolytic intermediate and products (i.e., lactate + glucose etc...) in terms of glycogen disappearence is not due to analytical errors ?
(See table 1)

R.-(from the secretary)
On trouvera la réponse à cette question dans le commentaire que le Dr HAMM a fait à propos des remarques du Dr BENDALL dans son introduction.

Note du secrétariat : these comments partly reply to the above question of Dr BENDALL .
Comments of Dr Hamm on Dr Bendall's introductory remarks concerning the paper of Hamm, Dalrymple and Honikel .

In all the samples investigated in this work and in more recent experiments, 20 to 40 % of glycogen which had disappeared, have not been converted to lactate. This percentage varies from muscle to muscle. As we mentioned on page 80(first words), one of the samples showed exceptional values and should not be regarded. Even if we regard all metabolites of the glycolytic circle and also free glucose, only 65 to 88 % of the glycogen which disappeared were converted to lactate and the other metabolites. Of course, we looked on the error in the determinations of glycogen, lactate, glucose and hexosephosphates but the errors are quite small and do not explain the discrepancy observed. A part of the discrepancy is due to the formation of free glucose. It might be difficult to identify the remaining 10 to 30 % because this fraction can consist of many different compounds and not only by just one, which, as Dr Bendall points out, cannot have escaped detection by modern analytical methods.
The rate of glycolysis in muscle post mortem depends on the rate of breakdown of ATP. Insofar I agree completely with Dr Bendall. We did not mention it because everybody working in this field should know that. It is another question,however, in which way glycolysis is coupled with ATP hydrolysis and at which steps glycolysis is controlled. From the results of us and other investigators it can be concluded that the phosphofructokinase step is controlling the rate of glycolysis. Of course, this step is controlled by the ATP concentration in the tissue. In meat research we should look not only on fresh meat but also on processing of meat. By certain additives, e.g. by inorganic diphosphate, the rate of ATP breakdown in comminuted tissue post mortem is increased but the phosphofructokinase step is blocked and, therefore, the normal post mortem glycolysis is changed. Now let me come to our experiments in which we immersed muscle stripes in different media. If I undertand Dr Bendall's introductory remarks right,he thinks, that the immersion of stripes leads to unphysiological, artificial conditions. Our slices of 1,5 to 2 mm thickness contain a certain proportion of intact muscle fibres which were surrounded by salt solutions of physiological concentrations similar to the ones in a whole piece of muscle. As our experiments show, this immersion in different media did not affect the ATP breakdown in the same manner. There are differences. Alkali salts and tris solutions show very small differences whereas Mg or Ca salts cause remarkable effects. We want to empasize that we have an in vitro system which always lacks the real conditions within the intact cell. But like all scientific approaches to the problems in the intact cell (including Dr Scope's experiments with artificial mixtures of enzymes and substrates) they give hints to the real conditions. Dr.Bendall, too,used muscle stripes submerged in a bath for his experiments on cold shortening. These are also in vitro conditions from which conclusions could be drawn .

Comments of Dr Hamm (suite)

Ergänzende Bemerkunlengen zum Beitrag Hamm/Dalrymple/Honikel (Paris)

In order to ensure that the ions and the molecules in the incubation medium are penetrating into the cells we checked the intake of EDTA and of the inhibitor quinidine sulfate by the muscle slices. For this experiment we measured the concentration in the muscle at 6 hours after death in comparison to the concentration used in the incubation medium. Within the cells the same concentrations of EDTA and quinidine sulfate were found as in the incubation medium.
The incubation experiments reported in our paper were carried out in 0.10 to 0.17 M tris buffer. These conditions provided a fairly constant pH of about 7 during the whole time of incubation . As we recently found, the effect of certain cations on the ATPase activity in muscle slices is much more pronounced if 0.05 M tris buffer or no buffer are used , i.e. if the pH drops during the breakdown of ATP as it is normally the case in the muscle post mortem. As the slide shows, with NaCl and also KCl (not indicated in this table) no significant difference in the rate of ATP hydrolysis between buffered and unbuffered media could be observed. With $MgCl_2$, however, a rapid fall of pH caused a more rapid breakdown of ATP than at nearly constant pH . This effect is even more obvious with $CaCl_2$. $CaCl_2$ in the incubation medium buffered with 0.1 M tris caused a breakdown of ATP within 7 hours ; with 0.05 M tris , where the buffering effect is very weak, the ATP disappeared within about 2.5 hours. The rapid decrease in pH seems to stimulate the ATPase-acitvating effects of $CaCl_2$ and -to a lesser extent- of $MgCl_2$.

An interesting effect is obtained with fairly high concentrations of $CaCl_2$. 0.35 M $CaCl_2$ exhibited a very rapid breakdown of ATP . The pH in the same sample, however, stays fairly constant (pH 7.0 to 6.9) over the whole 24 hours period of incubation. Dr Dalrymple observed in our laboratory that concentrations of 0.1 M $CaCl_2$ and higher shut off the breakdown of glycolysis in the cell. Therefore, no lactate is produced. This means that no ATP can be resynthesized by glycolysis. So, this experiment indicates the amount of ATP in the cell at a time shortly after death and the breakdown by an ATPase. According to this result, the drop of pH in muscle post mortem is mainly due to glycolysis .

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§ Q.- from LACOURT

Pensez-vous qu'il puisse exister d'autres voies de réduction du NAD que la glycogénolyse et le cycle de Krebs (en particulier le " α glycogéno-phosphate shuttle system " ?

Pensez-vous que ce système puisse fonctionner de façon notable dans le muscle post mortem ?

R.- We have not yet looked on the importance of such systems post mortem .

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§ Q.- from KREOZER

Sie berichten, daß 0,05 M $MgCl_2$ und 0,03 M $CaCl_2$ einen stark aktivierenden Effekt auf die ATP-Hydrolyse ausüben. Wurde schon der Einfluß ähnlicher $ZnCl_2$ - Koncentrationen auf die ATP-Hydrolyse geprüft ?

R.- Wir haben diesen Einfluß noch nicht studiert. Die Wirkung von Zn^{++} - Ionen auf die ATPase-Aktivität isolierter myofibrillärer Proteine (Myosin,Actomyosin) wurde von anderen Autoren untersucht. Studien über die Wirkung von Zn^{++} auf den ATP-Abbau im Muskelgewebe post mortem sind mir nicht erinnerlich .

/ A 7/

§ Q.- from LEEST J.A.

1) In the experiments a considerable difference was found in the development of rigor between slaughterhouses. Is there any indication to what extent slaughtering procedures may be responsible ?

R.- Differences between slaughterhouses and the origin of the pigs can not always be separated. There is evidence that the regime before and during slaughtering (transport , stunning are of importance) .

2) From table 2 can be seen that all rigor classes are present in all four quality score groups. Can you give us information about the relationship between rigor value (measured appr. 45 minutes post mortem) and meat quality score ?

R.- Les extrêmes se touchent (PSE and DFD) .

.- Stiffness can occur in carcasses which give all kind of quality . However most stiff carcasses produce PSE meat .

/ A 9/

§ Q.- from SYBESMA

The data suggests that PSE meat might be less tender .
Would it be possible by starting the very quick chilling at a much earlier moment post mortem, to improve tenderness (by slowing down post mortem metabolism) without causing decrease in tenderness by cold contracture .

R.- We have found that PSE meat is less tender than normal meat and less tender than DFD meat.
In our experiments, reported in the paper, there was a time lag of two hours between bleeding and chilling will be much shorter. I cannot predict the results.

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§ Q.- JUL M.

Was it established if the splitting of the carcasses in itself had any influence on toughness ?

R.- This is not established directly .
From other experiments, carried out by us, it appeared that the chilling rate was significantly increased by splitting of the carcass, so I suspect that there will be an effect on tenderness .
The rish for cold shortening is less for a whole carcass.

/ A 10/

§ Q.- RANKEN M.D.

Measurements of electrical conductance using a probe have been shown to be related to toughness in turkeys (RANKEN & SHRIMPTON,1968; HAIGH & STADELMAN 1970). The measurements are difficult to interpret but very easy to make. They are instantaneous and non-destructive. Have you considered this possibility ?

R.- It would be interested to see whether relationship do exist in pigs, particularly in view of the indication presented in paper A 9 (Dr MOERMAN) that PSE meat might be more tough .

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§ Q. LEEST J.A.

1) Rigor value only shows a relatively weak, but nevertheless significant correlation (0,32 **) with percent transmission. Is the influence of this variable in predicting ultimate meat quality so small, that it is not useful to measure it ?

thème A 10 (suite)

If not, this seems to be in contradiction to the importance that is attributed to the rigor value by SYBESMA and his co-workers as a useful source of information .

R.- I think it is useful to measure rigor with the method according to SYBESMA. In our study appr. 60 % of the carcasses which were in rigor at 45 minutes post mortem turned out to be PSE at 24 hours post mortem . By carrying out a pH measurement on these preselected carcasses one can increase the accuracy of the prediction and also discriminate between PSE and DFD meat .

2) In your introduction you state that early post mortem detection of potentially abnormal muscle quality is important because it allows the processor to subject these carcasses to a different post mortem treatment .
Do you know wether sorting of pig carcasses on early post mortem measurements is applied in practice ? If yes, how does this sorting take place and what types of meat products are manufactured from the different kinds of carcasses ?

R.- I don't know of a place where these measurements are applied in practice but there exist interest among meat packers for development of methods which can be used.

3) How does management cope with the problem of unpredictable daily variations in meat quality in relation to producting planning ?

R.- This is a question of management. The question is wether these daily variations will remain unpredictable after careful analysis .

4) What is your opinion on the economical importance of abnormal meat quality ?

R.- Dr Leest, who raised the question,has presented some years ago a study in which he estimated the loss to industry to be considerable . Some methods have been developed in meat technology to reduce these losses such as the use of polyphosphates. If however the use of these additives will be restricted the problem will be more serious as it has ever been, since selection in pig breeding is still going forwards towards more muscular animals.

On the other hand there is the related problem of pigs dieing during transport as a consequence of stress. In spite of efforts to minimize the stress their percentage is increasing also each year. POHLCHRISTOPH determined some years ago the economic loss as a result of these deads only in Germany to be 56 millions D M.

/ A 11/

§ Q.- from DUMONT B.L.

Which could be the effect of conformation in the case of your different treatment upon tenderness ?

R.- Increases in conformation, as they relate to increases in muscle-to-bone ratio and/or fatness , would :

a) probably decrease the extent of cold shortening because both would serve to insulate the interior of the muscle and thus minimize temperature shock .

b) probably decrease the effects of vertebral or ligamentum severance, in as much as increases in muscling and/or fatness would serve to support the configuration of the muscles and prevent (in part) the effect of gravity or weighted loads on muscle elongation.

c) have little effect on the tenderizing effects of obturator foramen suspension, expect as the angle across the Quadriceps femoris might be changed. If the angle is diminished the effect would undoubtedly be lessened, allowing greater shortening of sarcomeres in the hind leg and loin and thus decreasing ultimate tenderness.

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thème A 11 (suite)

§ Q.- from BARNES T.J.

Should the freshly dressed beef carcass be placed in chill room immediately, or hung at atmospheric temperature for the first 24 hours before refrigeration in order to obtain a more palatable and tender product .

R.- Our studies indicate that storage of the carcass at 16°C for 16 to 20 hours immediatly post mortem increases tenderness as much as 47 % and as 7 %. Subsequent storage of carcasses at 2°C for 5 to 11 days suggests that this advantage in tenderness diminishes during subsequent aging .

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§ Q.- from DUMONT B.L.

Which was the influence of the different treatments on tenderness of others muscles, specially in the forequarter ?

R.- Numerous additional muscles have been investigated in several of these studies. The effect of vertebral severance in that of increasing the tenderness of the Triceps brachii and Latissimus dorsi. Severance of the Ligamentum nuchae increases the tenderness of most of the neck muscles (e.g. Sternohyoideus and Trapezius) .Suspension via the obturator foramen increases the tenderness of the cushion muscles of the round (e.g. Semitendinosus and Biceps femoris) approximately 15 to 35 %, but decreases the tenderness of the Psoas major and Quadriceps femoris. The latter muscles are tender enough, however, to remain acceptably tender even when some shortening is allowed. Elevated storage temperatures increase the tenderness of those muscles which are less firmly attached and/or less well insulated by fat (e.g. Cutaneous trunci) to a greater extent than is demonstrated for the Longissimus dorsi muscle .

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§ Q.- from PATTERSON J.T.

Have microbiological studies been carried out when the carcasses were chilled at 16°C rather than at 2°C and if so what were the results ?

R.- Yes.

Mesotrophic and psychrotrophic counts have been obtained from the muscle and fat surfaces of carcasses. Chilling of carcasses at 16°C for 16 to 20 hours increases the mesotrophic count, but decreases the psychrotrophic count measured at 24 and 96 hours, respectively. Our present hypothesis is that the high initial temperature increases the rate of mesotrophs and the subsequent competition from mesotrophs delays the growth of psychrotrophs. Extend of round "souring" has been determined and involves less than 1 % of the carcasses investigated. If either problem (surface growth or sour rounds) occurs, control mechanisms are avalaible (rinsing with 200 ppm of chlorine or injection of CO_2 into the acetabulum, respectively) .

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§ Q.- from WISMER-PEDERSEN

Have you found any effect of obturator foramen suspension of cooking loss of the meat on heat processing ?

R.- Although extensive studies have been completed, no increase in cooking loss has been associated with suspension of the carcass by the obturator foramen .
