Biochemical Aspects of Post-Mortem Changes in Porcine Muscle

E. J. BRISKEY, L. L. KASTENSCHMIDT, J. C. FORREST, G. R. BEECHER, M. D. JUDGE, R. G. CASSENS, and W. G. HOEKSTRA

Departments of Meat and Animal Science and Biochemistry, University of Wisconsin, Madison, Wis.

Biochemical studies of post-mortem changes in porcine muscle have established that the post-mortem glycolytic rate can vary markedly. Studies on glycolytic intermediate levels show that a rapid glycolytic rate is associated with high levels of G-6-P and glucose, accompanied by low levels of fructose diphosphate, ATP, and phosphocreatine. These and the other biochemical changes discussed are consistent with the physiological concept that certain pigs are predisposed to rapid post-mortem glycolysis and are in an oxygen-deficient state at the time of death. This concept is further supported by the hormonal relationships elucidated thus far. Additional physicochemical changes in post-mortem muscle are discussed.

IMMEDIATELY after exsanguination, complex biochemical reactions, singly or collectively, regulate post-mortem muscle changes which are associated with the transformation of muscle to meat. The magnitude of these changes has a direct effect on meat quality and appears to be closely associated with the rate of anaerobic glycolysis as well as the pH and temperature in the muscle at the onset and completion of rigor mortis (*8*).

The purposes of this paper are to describe general aspects of post-mortem muscle metabolism, to discuss the interrelationships of certain features of post-mortem muscle metabolism with specific chemical and physical characteristics of the post-rigor muscle, and to clarify further the role of the physiology of the animal in influencing or regulating post-mortem changes in the muscle.

Synopsis of Post-Mortem Changes in Porcine Muscle

pH Decline. The rate of post-mortem glycolysis, or glycogen breakdown through various intermediates to lactic acid, may be estimated by measuring the rate of pH decline. The pH decline patterns (*8*, *14*) are extremely variable in porcine muscle and range from virtually no change or retention of a high value to a decline of approximately two pH units within a few minutes after death (Figure 1). The muscle which undergoes violent post-mortem glycolysis either attains a higher post-mortem temperature or has greater retardation in heat removal than normal muscle owing to the heat from the exothermic reactions producing lactic acid (*13*).

Changes in Glycolytic Intermediates and Nucleotide Levels. The quantity of glycogen stored in muscle at the time of death has been generally recognized to be important in determining post-mortem chemical and physical properties of the muscle only if the glycogen is available or accessible for degradation (*31*) and the enzymes are not inhibited by a decreasing pH (*1*). The attainment of ultimate pH values varying from 5.0 to 5.7 with various quantities of residual glycogen raises a major issue concerning the extent of glycolysis and the conditions under which it is inhibited by pH.

Lawrie, Manners, and Wright (*30*) found that bovine sternocephalicus muscle, which retained a high pH value post-mortem, had large quantities of residual glycogen and showed evidence of shorter external chain lengths than psoas major, which attained lower pH values. Briskey and Lawrie (*10*), more recently, reported that glycogen samples isolated from different bovine muscle at pre-rigor and post-rigor periods were broken down at unequal rates by phosphorylase. Sayre, Briskey, and Hoekstra (*38*) found marked differences in the amount of glycogen present in the muscle of pigs of different breeds. Sayre, Briskey, and Hoekstra (*40*) noted a more severe decrease in both external and internal chain lengths of the glycogen from certain pigs with a slow rate of anaerobic glycolysis. These findings indicated that the branching characteristics of the glycogen molecule might differ under various nutritional or hereditary influences and be a factor in regulation of the rate and amount of post-mortem glycolysis. Yet, on the basis of present evidence, molecular degradation of the glycogen molecule appears to have very little association with the accelerated rate of glycolysis in pale, soft, exudative (PSE) muscle or the amount of residual glycogen remaining in the muscle.

Kastenschmidt and coworkers (*29*) have found that muscles which have a fast rate of anaerobic glycolysis usually have high levels of glucose-6-phosphate (G-6-P) in the muscle at the moment of death. This high G-6-P would be indicative of increased phosphorylase activity. They also noted that muscles which have a fast rate of glycolysis have a higher level of free glucose at death, which may be due to various causes—e.g., increased penetration of glucose into the cell prior to death. In general, higher levels of fructose-1,6-diphosphate

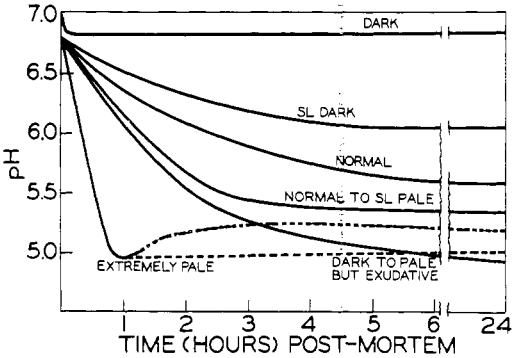

Figure 1. pH pattern *vs.* structural change

Examples illustrate various types of post-mortem pH decline patterns. Measured by probe electrode on the surface (8)

were found in muscle with normal glycolysis compared with muscles with extremes in glycolysis. These workers also found muscle with accelerated glycolysis to have low levels of adenosinetriphosphate (ATP) and phosphocreatine (PC) at the moment of death—which supports the contention of Forrest and coworkers (*20*) that these muscles may have been in an oxygen-deficient, anaerobic state at the time of death.

Rigor Mortis. The most obvious consequence of post-mortem glycolysis is the development of rigor mortis. The rigor mortis process may be considered the combination of the two contractile muscle proteins, actin and myosin, in the absence of ATP, to form the inextensible protein actomyosin.

Measurement. Thus, the time course of changes toward inelasticity or rigidity associated with rigor mortis can be measured by use of a specially designed rigormeter apparatus (*12*). This rigormeter (Figure 2) has a solenoid cell, energized by a cyclic timer to release and apply the load in a direction longitudinal to the vertically mounted specimen. A lever attached to the specimen loading systems transmits the extensibility and elasticity of the specimen to the armature of a differential transformer. The AC output signal is rectified and transferred to a DC recording microammeter. Three phases of the rigor process are recorded (Figure 3). The delay phase during which there is virtually no change in extensibility is associated with high levels of ATP and PC. The onset phase, representing a continuous reduction in extensibility, can be correlated with lower levels of ATP and CP. When all extensibility is lost, the muscle is considered in full rigor which is termed completion phase.

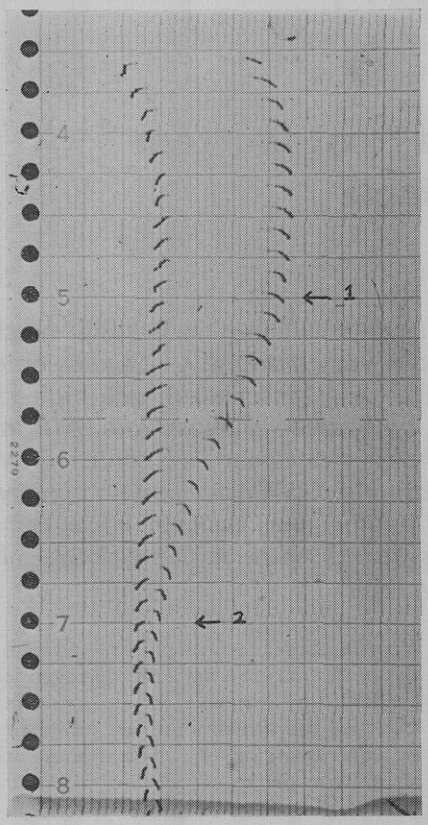

Figure 3. Typical rigor pattern strip chart

Point 1 marks the end of the delay phase. Point 2 marks the end of the rapid phase. Chart speed = 8 divisions per hour (12)

Association with Sarcomere Length. Excised muscles usually shorten during rigor mortis (*24, 34, 36*). Recently, however, Sink and coworkers (*42*) were able to correlate the duration of the delay phase of rigor mortis (excised strip) with the extent of contraction, as measured by sarcomere length, in the intact post-rigor muscle. Sarcomeres were short (approximately 1.6 microns) in muscles which had presumably undergone a 30-minute delay phase of rigor mortis. However, when the delay phase of rigor mortis was estimated to be long (150 minutes), the sarcomere shortening that occurred was much less, giving a sarcomere length of approximately 2.0 microns (Figure 4).

Tension due to positioning of the carcass shown by Herring, Cassens, and Briskey (*23*), as well as the condition of the antagonistic muscles, may also influence sarcomere length; however, any lengthening of the delay phase may result in retention of longer sarcomeres in the post-rigor stage (Figure 5). The possible association of anaerobic glycolysis with post-mortem contraction appears to be an important area in need of clarification (*4, 8, 16*). It is of further importance since post-rigor contraction state appears to be related to tenderness of the muscle (*14, 24, 35*).

Association with Stimulatory Response. In further studies on the rigor phenomenon, Forrest and coworkers (*18*) described a technique based on muscle response to electrical stimulation which is useful in predicting, within a few minutes after the time of exsanguination, the time course of rigor mortis, rate of post-mortem glycolysis, and ultimate color-morphology rating (Figure 6). The response of an excited

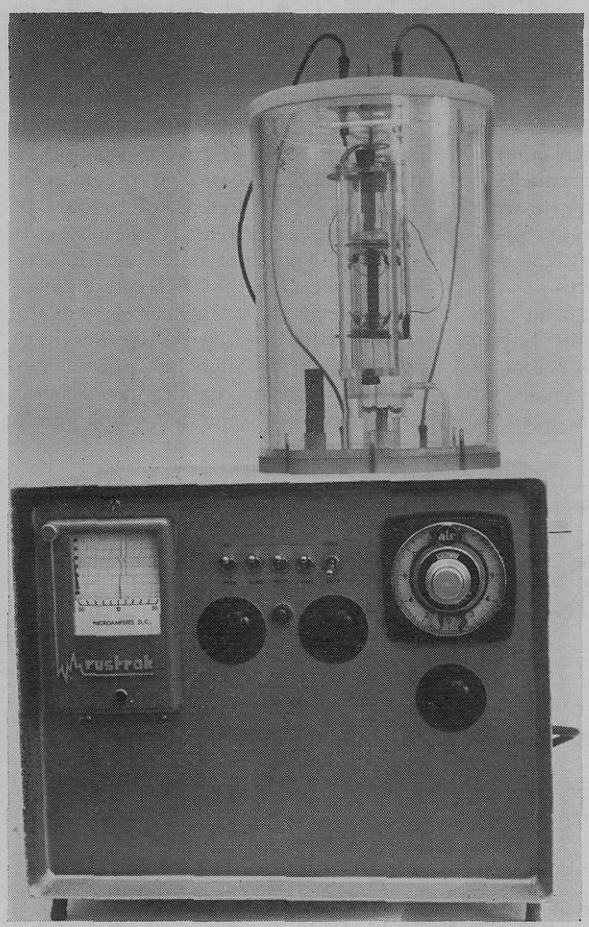

Figure 2. Rigormeter apparatus (8)

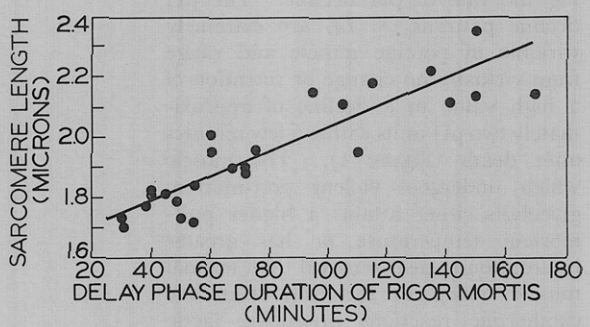

Figure 4. Scattergram showing the relationship between sarcomere length and delay phase of rigor mortis

Correlation coefficient of 0.90 was obtained (42)

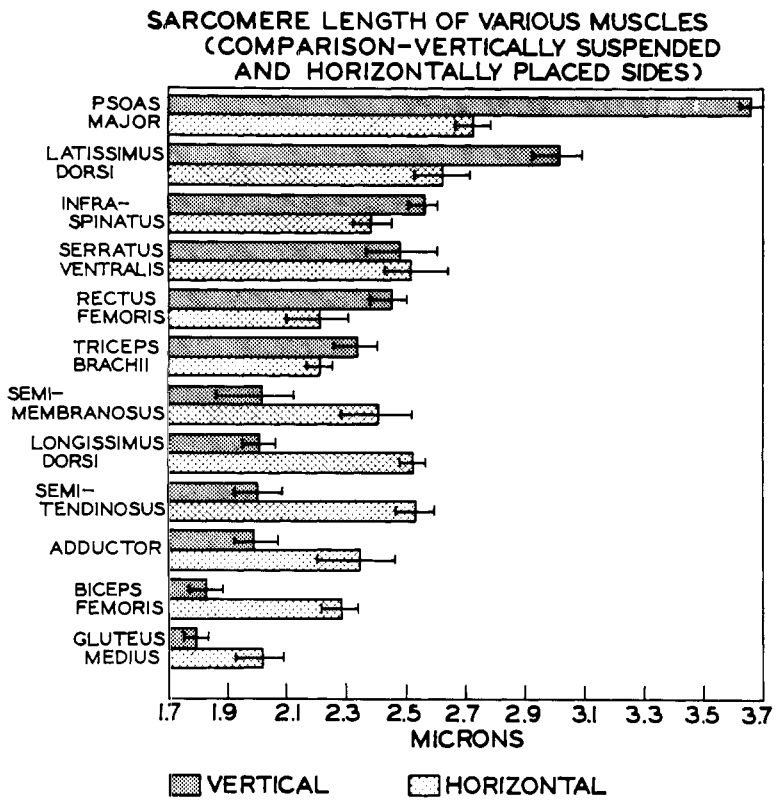

Figure 5. Comparison of carcass position on the ultimate sarcomere length in several muscles (23)

muscle to electrical stimulation was highly associated with post-mortem muscle properties. The excitability threshold (lowest voltage at which contraction resulted) was high in those muscles which had a short time course of rigor mortis, fast rate of post-mortem glycolysis, and pale, soft, and exudative (PSE) ultimate color-morphology. Those muscles with a long time course of rigor mortis, slow rate of post-mortem glycolysis, and normal color-morphology rating had low excitability thresholds. The duration of contractility was longer in this type of muscle. Multiple regression analysis indicated that up to 87% of the variability in color-morphology rating could be predicted by combining the various parameters of muscle response to electrical stimulation. Karpatkin, Helmreich, and Cori (*28*) have reported a significant buildup of lactic acid in muscle as a result of electrical stimulation. Hallund and Bendall (*21*) have recently reported a long-term effect of stimulation resulting in an acceleration of the rate of pH decline over several hours in electrically stimulated muscles. At a much earlier date, Denny-Brown (*17*) used response to electrical stimulation to identify muscles with predominantly red (lower stimulation frequency required to produce tetany) fibers compared with those with predominantly white fibers.

Association of Sarcomere Length with Dark Fiber Content. Normally dark or highly pigmented muscles (*9*) have a slow rate of glycolysis (*13*) and are subsequently more resistant to the development of the PSE condition (*8*). In a recent study by Beecher and coworkers (*3*) of the percentage of dark fibers in various muscles, the highly pigmented muscles with a high percentage of dark fibers also had longer sarcomeres than the poorly pigmented muscle (Figures 7 and 8). The longer sarcomeres in the red muscles may either contribute to or result from the slower post-mortem glycolytic rate (*25*). Even though further work is required to establish the collective effect of post-mortem changes on post-rigor sarcomere lengths, it is still of great interest to the area of meat quality that light colored, inactive muscles which have the shortest post-rigor sarcomers show the severest PSE conditions. A definitive study to compare the sarcomere length of PSE *vs.* normal muscle from the same muscle of different animals has not yet been completed. Some light colored muscles (*2*) are more sensitive (faster rate of glycolysis) to reactions and struggle associated with death than some dark colored muscles.

Post-Mortem Change and Protein Solubility. The solubilities (*22*) of sarcoplasmic (low ionic strength) and myofibrillar proteins (high ionic strength) are markedly altered by post-mortem conditions of pH and temperature (*11*, *37*) in the muscle. To obtain muscle protein extractability consistent with in vivo composition, it is essential to freeze the muscle samples immediately after death in a cryogenic liquid. Loss of protein solubility is closely associated with rate of glycolysis and fluid-retaining properties of the post-rigor muscle. Low pH (<5.7) and high temperature (>35° C.) during the first 2-hour period post-mortem or at the onset of rigor mortis contributed to loss in solubility of the sarcoplasmic fraction (Figure 9). However, the extractability was decreased to 55% of the original value when pH was low and temperature high at the onset of rigor mortis. McLoughlin (*33*) also showed that, as pH at 45 minutes post-mortem decreased, solubility also decreased. The rapid loss in solubility of the sarcoplasmic fraction that contains myoglobin obviously plays a major part in the development of the PSE condition. Scopes and Lawrie (*41*) reported that creatine phosphoryltransferase in pig muscle sarcoplasmic proteins is affected markedly by the high temperature–low pH combination.

Myofibrillar protein showed no loss in solubility under conditions of slow pH decline regardless of temperature at the onset of rigor mortis (Figure 9). When low pH (<5.7) developed at a high temperature (>35° C.), less than 50% of the 0-hour fibrillar protein was extractable at the onset of rigor mortis, and only 25% was extractable after 24 hours. Bendall and Wismer-Pedersen (*5*) postulated that the development of PSE muscle results in only a denaturation and loss of solubility of sarcoplasmic proteins, and inferred that it is the subsequent precipitation of these sarcoplasmic proteins on myofibrillar proteins which renders them somewhat insoluble to a high ionic strength buffer. While much more needs to be known on the alterations in muscle proteins post-mortem, it seems pertinent that a surface freezing of the skin with liquid nitrogen (*6*, *7*) accelerates cooling rate, prevents the development of PSE muscle, and preserves the solubility of both the sarcoplasmic and myofibrillar protein fractions.

Color and Gross Morphology. The physical manifestation of the above-mentioned chemical changes is easily visualized (Figure 10). A relatively slow rate of glycolysis resulting in moderately low pH and low temperatures is associated with normal muscle color and gross morphology (*8*, *13*, *39*). These muscles are grayish-pink to red in color, moderately firm in structure, and moderately dry in appearance (normal). If pH remains high, or at least if it is

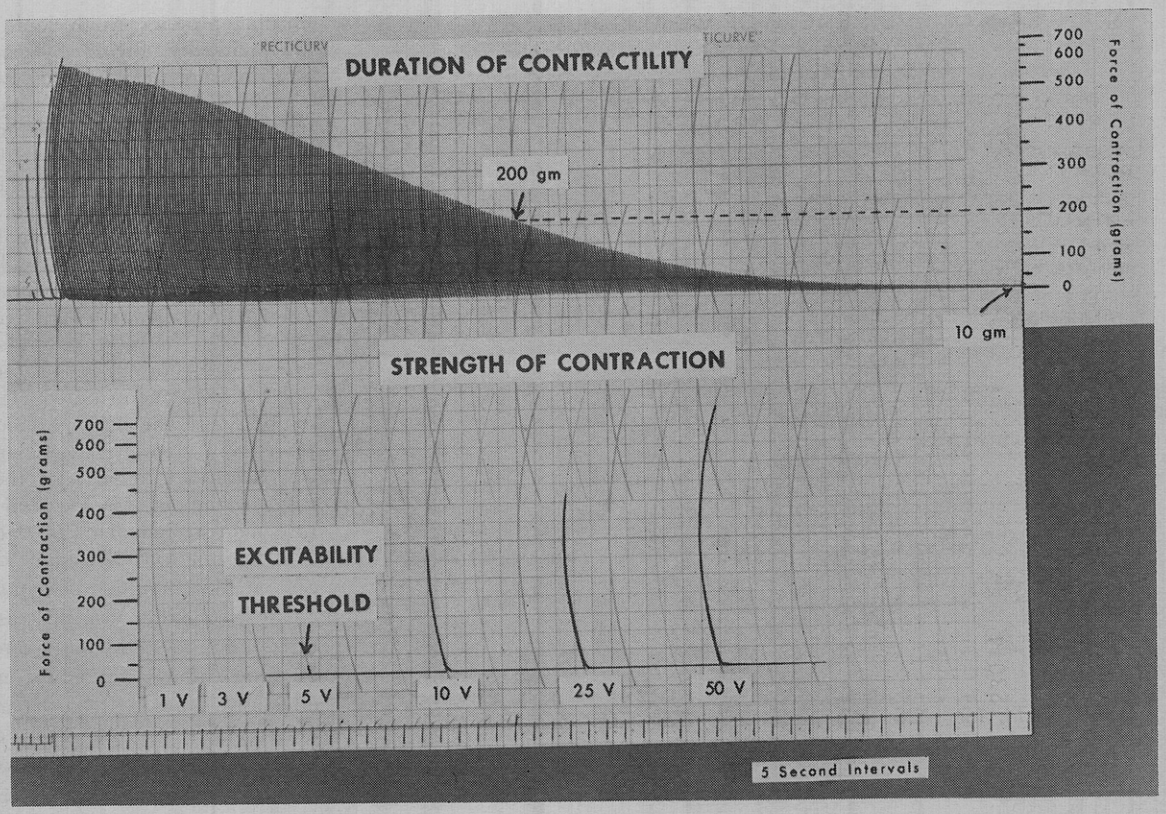

Figure 6. Typical electrical stimulation record of a 1 × 1 × 6 cm. strip of *longissimus dorsi* muscle

Conditions for strength of contraction: single shocks of 0.1-msec. duration. Conditions for duration of contractility: 50 volts, two shocks per second, and 0.1-msec. duration. Points arbitrarily chosen were the force 200 grams and 10 grams (18)

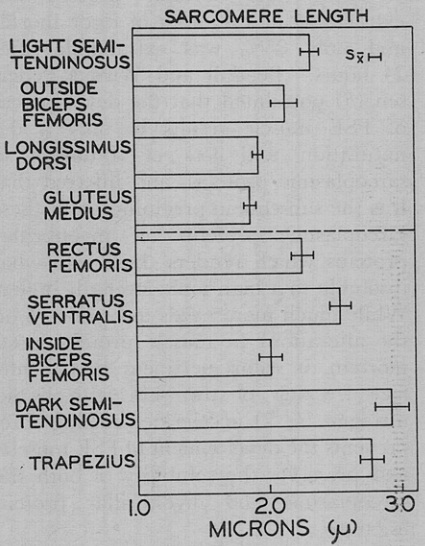

Figure 7. Relationship of sarcomere length to dark fiber content

Light muscles listed above dashed line; dark muscles below (3)

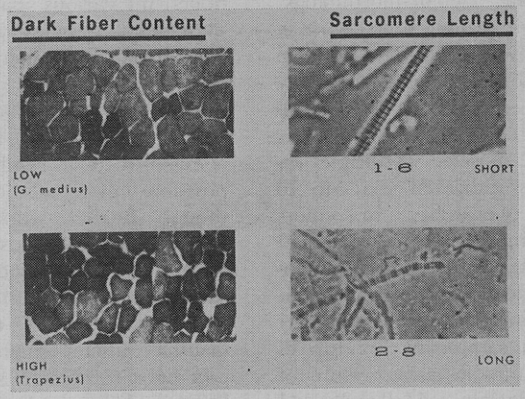

Figure 8. Photomicrographs of muscle sections illustrating the relationship of sarcomere length to dark fiber content

Stain for dark fibers is Sudan black B (3)

retarded in rate of decline, muscles remain dark red in color, firm in structure, and dry in appearance (DFD). Conversely, a rapid rate of pH decline resulting in acid conditions at a high temperature, is associated with the development of pale, soft, exudative muscle (PSE). Exposure of animals to elevated environmental temperatures immediately antemortem gives rise to rapid post-mortem muscle glycolysis in some breeds of porcine animals but not others (8). Under normal conditions, the combination of low pH and high temperature in muscle immediately post-mortem usually is associated with PSE muscle, although some biological variation does exist.

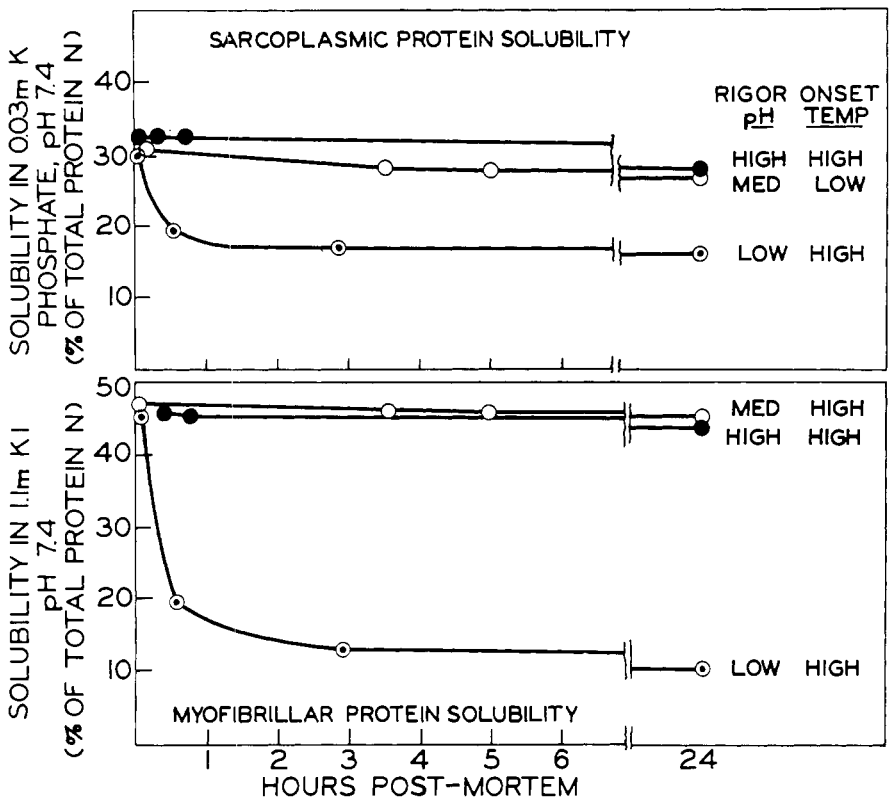

Figure 9. The effect of pH and temperature conditions at rigor onset on sarcoplasmic (above) and myofibrillar (below) protein solubility

Conditions at rigor onset are given on the right (37)

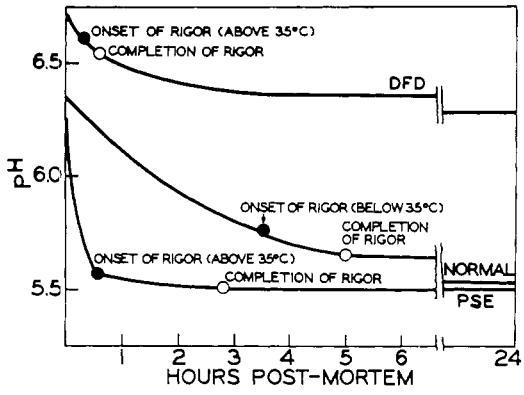

Figure 10. pH and rigor mortis as related to muscle color and gross morphology

The three patterns listed are typical of many observed. Conditions are listed along each line (8)

Synopsis of the Influence of Animal Physiology on Post-Mortem Changes in the Muscle

Animal Variation. In a study of 30 Poland China pigs selected to represent equally two boars (several highly related sows), Judge, Grummer, and Briskey (*27*) noted the offspring from the two boars differed markedly in ultimate meat quality. The pigs from boar 1 (B1) had muscles which were PSE ($P < 0.05$), whereas those from boar 2 (B2) had normal musculature. Likewise, the B1 pigs had larger *longissimus dorsi* muscles than the B2 pigs. The B1 pigs also had smaller thyroid glands ($P < 0.05$) and higher $PB^{131}I$ values (% plasma ^{131}I) than the B2 pigs. While these observations do not constitute a heritability study, they still, nevertheless, would seem to implicate genetics in the development of the low meat quality characteristics of pale, soft, exudative musculature.

Thyroid and Adrenal Gland Characteristics. In view of the apparent relationship between an animal's ability to withstand stress and its resistance to post-mortem development of the PSE muscle condition, Judge, Briskey, and Meyer (*26*) designed experiments to measure certain parameters of thyroid and adrenal gland secretions in pigs of the Poland China and Chester White breeds. Poland China pigs with PSE muscles had elevated levels of serum $PB^{131}I$. Although no marked differences ($P < 0.05$) were evident in urinary excretion levels of 17-ketosteroid (17-KS), 17-OH-corticosteroid (17-OHCS), and catecholamines when the animals were grouped on the basis of muscle color-morphology and rate of pH decline, trends appeared for all of these parameters. Additionally, positive correlations were found between 17-KS level and muscle color-morphology rating and 17-OHCS level and delay phase (rigor mortis) duration. Collectively, these data give support to the postulation of Ludvigsen (*32*) that pigs which ultimately have PSE musculature may have some degree of deficiency in adrenocortical hormone production. More recently, Cassens and coworkers (*15*) conducted a histochemical study of adrenal glands from pigs of the Poland China and Chester White breeds. Poland China animals had significantly greater amounts of sudanophilic masses in the zona reticularis than did the Chester White animals. When breeds were combined, a significant correlation (−0.32) was found between zona reticularis masses and 45-minute post-mortem pH. The suggestion was made that the masses in question were indicative of a degenerative process.

These findings may implicate metabolic rate, ability to adapt, blood flow, and oxygen supply.

Heart and Respiration Rates. The stress of a warm environment frequently results in death of pigs (breeds or strains) which show the greatest susceptibility toward developing the PSE muscle condition (*8*). Conversely, pigs of other breeds or strains are capable of withstanding heat stress (*38*, *39*) and retaining normal color and gross morphology of the muscle post-mortem. A warm environment prior to slaughter caused rapid post-mortem pH decline in the Poland China and Hampshire muscle, whereas Chester White pigs had the capacity to withstand heat, metabolize muscle glycogen, achieve a normal or high ultimate pH, and retain a normal musculature. In view of these findings, Forrest and coworkers (*19*) studied the physiological parameters of heart and respiration in an attempt to determine the characteristics of animals which were resistant to heat treatment. Since heart and respiration rates are associated with blood flow and oxygen

supply to the muscle, it was suggested that the initial state of, as well as changes in, these physiological parameters immediately prior to exsanguination, may be associated with the post-mortem properties of the muscle. When there was very little change in heart rate owing to treatment, the muscles were essentially normal. However, when the animals showed drastic increases in heart rates, which, in addition to other factors, reflected a greater need for oxygen during warm treatment, the post-mortem pH decline was extremely rapid and muscles became PSE.

Blood Characteristics *vs.* Muscle Properties. More recently, Forrest and coworkers (*20*) subjected Poland China and Chester White pigs to a warm environment and measured the PO_2, PCO_2, and pH of the venous blood before and after treatment. Heart and respiration rates were also determined before the animals were placed in the chamber and at 5-minute intervals during the time the animals were in the chamber. The controls were held in the same chamber without heat. A blood sample was collected anaerobically from the jugular vein of each animal before and after the 30-minute period in the environmental chamber for determinations of PCO_2, PO_2, and pH. The animals were exsanguinated immediately after collection of the last blood sample. pH values were taken at various intervals post-mortem and the muscles were also scored subjectively for color and gross morphology at 24 hours post-mortem.

The muscles from the control animals of both breeds were nearly normal with the exception of one Poland China which became pale, soft, and exudative. The Poland Chinas from the warm treatment had rapid rates of pH decline and were all extremely PSE, whereas the Chester Whites had normal *longissimus dorsi* muscles. In the treated Chester White pigs, the heart rates were only slightly higher than pre-treatment levels; however, in the Poland China pigs the heart rates showed sharp and continuous increases in heart rate. Conversely, the respiration rates continually increased in Chester White pigs, but in Poland China pigs the respirations increased sharply and then declined rapidly. In the Poland Chinas of the warm treatment group, the PCO_2 increased significantly while PO_2 decreased markedly. Conversely, the Chester Whites decreased in blood PCO_2 during warm treatment and showed a slight increase in PO_2.

Conclusions

Biochemical studies have established that porcine muscles can vary markedly in glycolytic rate. A rapid glycolytic rate is associated with high levels of G-6-P and glucose as well as low levels of fructose diphosphate, ATP, and phosphocreatine. Remaining to be elucidated are direct causative factors of the very rapid decline in ATP in PSE muscle. Whether or not a myofibrillar ATPase is operative post-mortem in these muscles is still questionable. In this connection, observations on the relationship of stimulatory response to rigor mortis and the association between sarcomere length and rigor mortis are interesting and would merit further study.

While it is established that myofibrillar and sarcoplasmic protein solubility are markedly diminished by conditions of low pH and high temperature at rigor onset, further work on myofibrillar protein interactions would be of value.

In the area of physiology, it is established that blood oxygen, CO_2, and pH are markedly changed in stressed animals which eventually exhibit rapid post-mortem glycolysis. Blood arteriovenous difference data on individual muscles is necessary before the concept of oxygen debt in PSE muscle can be established. Additionally, the hormonal state of the animal becomes an important consideration.

Thus, the problem of biochemical aspects of post-mortem changes in porcine muscle cannot be adequately resolved without some deference to the physiology of the live animal.

Literature Cited

(1) Bate-Smith, E. C., Bendall, J. R., *J. Physiol.* **110,** 47 (1949).
(2) Beecher, G. R., Briskey, E. J., Hoekstra, W. G., *J. Food Sci.* **30,** 477 (1965).
(3) Beecher, G. R., Cassens, R. G., Hoekstra, W. G., Briskey, E. J., *Ibid.*, p. 969.
(4) Bendall, J. R., XIth European Meeting of Meat Research Workers, Belgrade, August 1965.
(5) Bendall, J. R., Wismer-Pedersen, J., *J. Food Sci.* **28,** 156 (1962).
(6) Borchert, L. L., Briskey, E. J., *Ibid.*, **29,** 203 (1964).
(7) *Ibid.*, **30,** 38 (1965).
(8) Briskey, E. J., *Advan. Food Res.* **13,** 89–178 (1964).
(9) Briskey, E. J., Hoekstra, W. G., Bray, R. W., Grummer, R. H., *J. Animal Sci.* **19,** 214 (1960).
(10) Briskey, E. J., Lawrie, R. A., *Nature* **192,** 263 (1961).
(11) Briskey, E. J., Sayre, R. N., *Proc. Soc. Exptl. Biol. Med.* **115,** 873 (1964).
(12) Briskey, E. J., Sayre, R. N., Cassens, R. G., *J. Food Sci.* **27,** 560 (1962).
(13) Briskey, E. J., Wismer Pedersen, J., *Ibid.*, **26,** 197 (1961).
(14) Cassens, R. G., in "Physiology and Biochemistry of Muscle as a Food," E. J. Briskey, R. G. Cassens, J. C. Trautman, eds., University of Wisconsin Press, Madison, Wis., in press.
(15) Cassens, R. G., Judge, M. D., Sink, J. D., Briskey, E. J., *Proc. Soc. Exptl. Biol Med.* **120,** 854 (1965).
(16) Cori, C. F., in "Enzymes: Units of Biological Structure and Function," O. H. Gaebler, ed., p. 573, Academic Press, New York, 1956.
(17) Denny-Brown, D. E., *Proc. Roy. Soc. London* **104,** 371 (1929).
(18) Forrest, J. C., Judge, M. D., Sink, J. D., Hoekstra, W. G., Briskey, E. J., *J. Food Sci.* **31,** 13 (1966).
(19) Forrest, J. C., Kastenschmidt, L. L., Beecher, G. R., Grummer, R. H., Hoekstra, W. G., Briskey, E. J., *Ibid.*, **30,** 492 (1965).
(20) Forrest, J. C., Kastenschmidt, L. L., Judge, M. D., Briskey, E. J., "On the Inability of Certain Porcine Animals to Maintain Physiological Homeostasis during Exposure to a Warm Environment," University of Wisconsin, Madison, Wis., 1966.
(21) Hallund, O., Bendall, J. R., *J. Food Sci.* **39,** 296 (1965).
(22) Helander, E., *Acta Physiol. Scand.* **41,** Suppl. 141 (1957).
(23) Herring, H. K., Cassens, R. G., Briskey, E. J., *J. Food Sci.* **30,** 1049 (1965).
(24) Herring, H. K., Cassens, R. G., Briskey, E. J., *J. Sci. Food Agr.* **16,** 379 (1965).
(25) Infante, A. A., Klaupiks, D., Davies, R. E., *Science* **144,** 1577 (1964).
(26) Judge, M. D., Briskey, E. J., Meyer, R. K., "Endocrine-Related Post-Mortem Changes in Porcine Muscle," University of Wisconsin, Madison, Wis., 1966.
(27) Judge, M. D., Grummer, R. H., Briskey, E. J., "Comparison of Meat Quality from Offspring of Two Boars," University of Wisconsin, Madison, Wis., 1966.
(28) Karpatkin, S., Helmreich, E., Cori, C. F., *J. Biol. Chem.* **239,** 3139 (1964).
(29) Kastenschmidt, L. L., Hoekstra, W. G., Briskey, E. J., "Metabolic Intermediates in Skeletal Muscles with Fast and Slow Rates of Post-Mortem Glycolysis," University of Wisconsin, Madison, Wis., 1966.
(30) Lawrie, R. A., Manners, D. J., Wright, A., *Biochem. J.* **73,** 485 (1959).
(31) Lawrie, R. W., *Biochim. Biophys. Acta* **17,** 282 (1955).
(32) Ludvigsen, J., *Acta Endocrinol.* **26,** 406 (1957).
(33) McLoughlin, J. V., Conf. European Meat Research Workers, 9th Conf., Paper 33, Budapest, Hungary, 1963.
(34) Marsh, B. B., *Biochim. Biophys. Acta* **12,** 478 (1953).
(35) Marsh, B. B., in "Carcass Composition and Appraisal of Meat Animals," Paper 12, D. E. Tribe, ed., C.S.I.R.O., Melbourne, Australia, 1964.
(36) Newbold, R. P., in "Physiology and Biochemistry of Muscle as a Food," E. J. Briskey, R. G. Cassens, J. C. Trautman, eds., University of Wisconsin Press, Madison, Wis., in press.
(37) Sayre, R. N., Briskey, E. J., *J. Food Sci.* **28,** 674 (1963).
(38) Sayre, R. N., Briskey, E. J., Hoekstra, W. G., *J. Animal Sci.* **22,** 1012 (1963).
(39) Sayre, R. N., Briskey, E. J., Hoekstra, W. G., *J. Food Sci.* **28,** 292 (1963).

(40) Sayre, R. N., Briskey, E. J., Hoekstra, W. G., *Proc. Soc. Exptl. Biol. Med.* **112,** 223 (1963).
(41) Scopes, R. K., Lawrie, R. A., *Nature* **197,** 1202 (1963).
(42) Sink, J. D., Cassens, R. G., Hoekstra, W. G., Briskey, E. J., *Biochim. Biophys. Acta* **102,** 309 (1965).

Received for review October 27, 1965. Accepted February 1, 1966. Division of Agricultural and Food Chemistry, 150th Meeting, ACS, Atlantic City, N. J., September 1965. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by Public Health Service Research Grant EF-81 (C7) from the Division of Environmental Engineering and Food Protection. Department of Meat and Animal Science, Paper No. 430.

FOOD PROCESSING

The Chemistry of Meat Pigments

JAY B. FOX, Jr.

Meat Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, Philadelphia, Pa.

The reactions of the heme pigments of meat, myoglobin and hemoglobin, are important in determining the colors of fresh and cured meats. The effect of the partial pressure of oxygen, the means by which oxidized pigments are re-reduced, and the thermodynamic and electrochemical requirements for the interconversion of the three pigments of fresh meats—myoglobin, metmyoglobin, and oxymyoglobin—are covered. The mechanisms of conversion of the native pigments of meat to the stable red pigments of cured meats are described and the effects of various conditions on the conversion are discussed. The known reactions which produce green heme pigments are related to the development of off-colors and "greening" in both fresh and cured meats.

The chemistry of the color of meat is the chemistry of the heme pigments, myoglobin and hemoglobin, which, in so far as meat color is concerned, are identical in their reactions (Figure 1). The number of reactions are not many, but they are governed or produced by a wide variety of conditions. This paper is concerned with some of the more important of these conditions. The muscle heme pigment myoglobin is the principal but not the whole source of meat color. Even in a well bled piece of meat, hemoglobin, the blood pigment, will comprise 20 to 30% of the total pigment present, sometimes more. Although most of the reactions of the two pigments are identical, several reactions of importance in meat color, such as autoxidation, reaction with nitrite, and denaturation, have different rates for the two pigments. It therefore seems highly improbable that color changes or color intensity in meat with pigment concentration could be correlated without considering the quantities and reactivities of both hemoglobin and myoglobin.

Color Cycle in Fresh Meats

This is a dynamic cycle and in the presence of oxygen the three pigments, oxymyoglobin, myoglobin, and metmyoglobin, are constantly being interconverted. The take-up of oxygen by myoglobin converts the purple reduced pigment to the bright red oxygenated pigment, oxymyoglobin. This process produces the familiar "bloom" of fresh meats; at high oxygen pressures the reaction, as written in Figure 1, is shifted mainly toward the left. The red complex, once formed, is stabilized by the formation of a highly resonant structure; and as long as the oxygen remains complexed to the heme, the pigment will undergo no further color changes. However, the oxygen is continually associating and dissociating from the heme complex, a process which is accelerated by a number of conditions, among them low oxygen pressures. When this occurs the reduced pigment is subject to oxidation by oxygen or other oxidants. It is not exactly known whether the oxidation takes place during association or, as is indicated by the dashed arrow in Figure 1, during dissociation. Regardless of how it is accomplished, there is a slow and continuous oxidation of the heme pigments to the met form. When the meat is fresh, the production of reducing substances endogenous to the tissue will constantly re-reduce the pigment to the purple form, and the cycle continues if oxygen is present. The two areas of

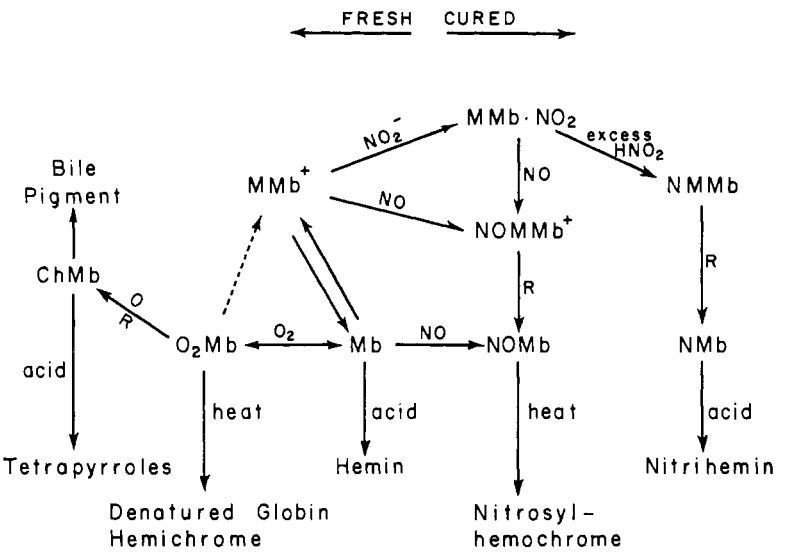

Figure 1. Heme pigment reactions of meat and meat products

ChMb, cholemyoglobin (oxidized porphyrin ring); O_2Mb, oxymyoglobin (Fe^{+2}); MMb, metmyoglobin (Fe^{+3}); Mb, myoglobin (Fe^{+2}); MMb.NO_2, metmyoglobin nitrite; NOMMb, nitrosylmetmyoglobin; NOMb, nitrosylmyoglobin; NMMb, nitrimetmyoglobin; NMb, nitrimyoglobin, the latter two being reaction products of nitrous acid and the heme portion of the molecule; R, reductants; O, strong oxidizing conditions