Conclusions

Meat flavor appears to be determined by the relative amounts of the various compounds, as well as the type of compounds, present or formed in the nonvolatile and volatile fractions when meat is heated. Most processes to which meat is subjected cause compositional changes in one or both of these fractions, which result in flavor alterations.

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TENDERNESS OF CHICKEN

Relationship between Chemical Properties and Tenderness of Poultry Muscle

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Experiments were undertaken to study the fate of metabolites involved in muscular contraction [adenosine triphosphate (ATP), phosphorylcreatine, glycogen, and lactic acid] during the post-mortem period when tenderness is changing. Chickens were subjected to severe mechanical feather-plucking immediately post mortem (a treatment known to induce toughness). The disappearance of ATP, the chemical reaction most closely linked with the onset of rigor mortis, was accelerated. Other prerigor treatments (freezing and thawing, elevated temperature, excising or cutting the muscle, electron irradiation, and exhaustive electrical stimulation) that accelerated ATP and glycogen disappearance as well as the onset of rigor mortis, also induced toughness that was only partially reversed by prolonged aging. When post-mortem glycolysis was minimized by epinephrine injections, sodium iodoacetate injections, or rapid cooking, the meat was tender without aging. Since these treatments accelerate rigor mortis, it is the acceleration of post-mortem glycolysis, not the acceleration of rigor mortis, which induces toughness.

MANY INDUSTRIAL, university, and government laboratories have actively pursued research on meat tenderness. In addition to its economic importance, tenderness, as a research area, is intensely interesting to the biochemist and the biophysicist. The study of meat tenderness covers the transition of muscle from the living state to the dead state, a period which includes rigor mortis. During this period, the physical properties of muscle tissue change profoundly as do the levels of many of the biochemical compounds in muscle. Research in this area has produced, and should continue to produce, fruitful correlations between

the biochemical changes of muscle with its physical properties.

The Western Regional Research Laboratory began a study of the processing variables which influence poultry tenderness about 10 years ago. This project has shown that the steps of the poultry processing operation requiring strict attention by the processor to ensure optimum tenderness are (10, 13) scalding, feather-plucking, and aging. Scalding and feather-plucking, although separate steps in the processing line, are interrelated since very mild scalding conditions yield birds that require more severe feather-plucking conditions (and vice

versa). In general, increasing the severity of scalding, either by raising the temperature of the scald water or by prolonging immersion, or increasing the severity of the feather-plucking treatment, will increase the toughness of the cooked meat. The third step, aging, is essential for the development of tenderness, although the time period involved is much less than that for beef. In contrast to beef, which may require 10 to 20 days for development of optimum tenderness, poultry achieves maximum tenderness within 12 to 24 hours.

This research program, in addition to studying the influence of processing var-

iables on the quality of the final product, has included study of the biophysical and biochemical changes in poultry muscle from the moment of death to the time when tenderness changes are complete. The biochemical changes, and their relation to tenderness, are discussed in the sections that follow.

Rate of Onset of Rigor Mortis in Normal Birds

Effect of Scalding and Feather-Plucking. Experiments performed on rabbit muscle by Erdös *(8)* and confirmed by Bate-Smith and Bendall *(2–4)* showed that the chemical event most closely linked with the onset of rigor mortis is the disappearance of adenosine triphosphate (ATP). Since the author's studies had shown that severe scalding or feather-plucking conditions increased the toughness of normal poultry, the determination of the effect of such treatments on the time of onset of rigor mortis, as measured by the disappearance of ATP, was of interest. For this experiment, two groups of 11-week-old cockerels (eight birds each) were killed, scalded at 53° C. for 50 seconds, plucked, and eviscerated under commercial conditions, with the exception that one group was plucked by hand and the other with a single-drum feather-plucker. After chilling in ice slush for $2\frac{1}{2}$ hours post mortem, each bird was sampled for ATP *(Pectoralis major* muscle) by the method of Griswold, Humoller, and McIntyre *(9)*. This method measures inorganic phosphate and phosphate esters in tissue extracts by differential acid hydrolysis. The experiment was repeated once at a scalding temperature of 60° C. The results are summarized and presented in Table I.

Since the level of ATP measured immediately post mortem is in the range of 4.5 to 5.0 mg. per gram, these experiments demonstrate that, with the milder scalding conditions, rigor mortis was well established in the machine-plucked birds when it was just beginning in the hand-plucked birds. At the higher scalding temperature, the results were similar except that rigor mortis occurred more rapidly. These experiments were the first demonstration that treatments which are known to increase toughness also cause a more rapid onset of rigor mortis.

Thaw Rigor. Thaw rigor, the rapid development of rigor mortis that occurs when prerigor muscle is frozen and thawed, has been recognized for many years. The effect of thaw rigor on both the time of onset of rigor mortis and the toughness of the cooked meat was investigated. For this experiment, paired *Pectoralis major* muscles were used to avoid the wide variation that exists between birds. Paired muscles from the same bird are remarkably alike in their tenderness if they have undergone identical treatments.

## Table I. Effect of Scalding and Feather-Plucking Conditions on ATP Breakdown in Chicken Breast Muscle

	ATP Concentration, Mg./Gram		
Scalding Conditions	Hand-plucked	Machine-plucked	P^a
53° C., 50 sec.	2.6	0.6	<0.001
60° C., 50 sec.	1.6	0.6	<0.001

[a] Probability that difference is due to sampling error.

To measure the effect of thaw rigor on the rate of onset of rigor mortis, ATP and glycogen levels in the breast muscles of a freshly slaughtered chicken were followed. The muscles were excised rapidly and placed in polyethylene bags. One muscle was frozen immediately in a dry ice–ethanol bath and stored at −23° C.; the other muscle was held at 13° to 14° C. (tap water) and sampled periodically for ATP and glycogen. Glycogen was determined colorimetrically with anthrone *(16)* with the reagent stabilized by addition of 1% thiourea *(15)*. The frozen muscle was sampled within two days for ATP and glycogen, then thawed in tap water and sampled periodically. The results, presented in Figure 1, show quite clearly that freezing and thawing prerigor muscle markedly accelerates the net breakdown of glycogen and ATP and, hence, hastens the onset of rigor mortis.

Next, the effect of thaw rigor on the toughness of the subsequently cooked meat was determined. For this experiment, 23 cockerels were divided into two groups of 11 or 12 birds each. They were killed by bleeding, and the breast muscles were removed immediately and sealed in individual polyethylene bags. One muscle from each bird in the first group was frozen rapidly in a dry ice–ethanol bath, thawed immediately in running tap water, and aged with the remaining muscles from both groups for 24 hours at 2° C. Just before cooking, one muscle from each bird in the second group was frozen and thawed identically. The muscles were then clamped between two duralumin plates (2.3 mm. thick) which were held together by machine screws with wing nuts. The plates were maintained $^3/_8$ inch apart by tubular spacers fitting around the screws. For cooking, the muscles were immersed in boiling water for 30 minutes, then cooled in running tap water. In order to provide strips with uniform cross-section for shear resistance measurements, a section 1-inch wide and parallel to the fibers was cut from each muscle. The strips were sheared in a Warner-Bratzler shear force apparatus.

The control muscles, aged but not frozen, had shear resistance of about 8 pounds, as did the experimental muscles
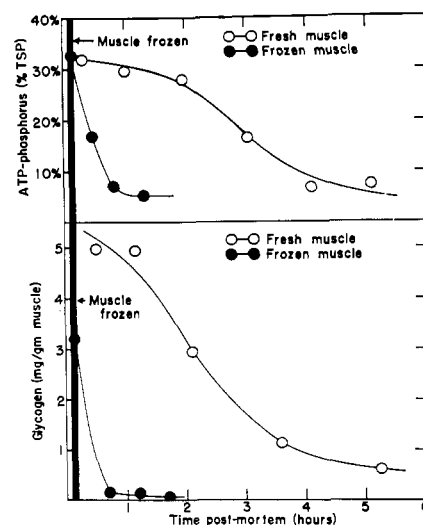
Figure 1. Effect of freezing and thawing fresh muscle on rates of ATP disappearance and glycogen breakdown

ATP-phosphorus concentration expressed as a percentage of the total acid-soluble phosphorus (TSP)

which had been aged for 24 hours prior to freezing and thawing. On the other hand, the experimental muscles which had been frozen and thawed immediately post mortem and then aged for 24 hours had shear resistance of about 16 pounds. This result is significant at the 0.5% probability level. Since the effect of freezing and thawing aged muscle is negligible, the toughening effect of prerigor freezing is due, apparently, to the rapid onset of rigor mortis.

Other Stimulatory Treatments. In addition to the conditions described above (severe scalding and feather-plucking, thaw rigor), several other stimulatory treatments were applied to poultry breast muscle in the prerigor state. These treatments included variation in the environmental temperature (from 0° to 40° C.), exhaustive electrical stimulation, muscle excision (compared with the muscle remaining on the carcass), and electron irradiation (2×10^6 rads from a linear accelerator). Each treatment accelerated post-mortem glycolysis, the disappearance of ATP, and, consequently, the onset of rigor mortis. Parallel studies, in which the toughness of the meat was determined after aging for 24 hours, showed that these treatments increased also the shear resistance, and, hence, toughness. These experiments support the hypothesis that treatments which hasten the onset of rigor mortis inhibit tenderization.

Elimination of Post-Mortem Glycolysis

Ante-Mortem Subcutaneous Injection of Epinephrine. The experiments reported in the preceding section provide conclusive evidence that an acceleration of the onset of rigor mortis in normal

chickens renders the meat appreciably tougher than control meat after a 24-hour aging period. In these experiments, the onset of rigor mortis was accompanied by ATP disappearance, glycogen breakdown, and lactic acid accumulation. These data were not sufficient to decide which of these three phenomena was responsible for the harmful effects of rigor acceleration. If post-mortem glycolysis were eliminated or inhibited, there would still be a rapid ATP disappearance and an acceleration of rigor without glycogen breakdown or lactic acid accumulation. The information obtainable from such an experiment should pinpoint the source of the toughening reaction. One applicable experimental approach was developed by Radouco-Thomas and coworkers (14) who prevented post-mortem glycolysis in several mammalian species with an ante-mortem subcutaneous injection of epinephrine. Under suitable conditions, this treatment depletes the muscle glycogen prior to slaughter (5).

To study the effect of glycogen depletion on the tenderness pattern of poultry, an experiment was conducted with light turkey hens (6.0 pounds average weight) in seven groups of eight birds each. All birds were fasted 18 hours prior to slaughter. When feed was removed, each bird in three groups received a subcutaneous injection of epinephrine (1.5 mg. per kg.). The birds were slaughtered and processed under simulated commercial conditions, aged in ice slush for various periods, packaged in plastic bags, frozen in a blast freezer, and then sawed in half along the keel. One half from each bird was thawed rapidly in tap water and used to determine the pH of the breast muscle. The other half was cooked from the frozen state in vegetable oil at 110° C. to determine the shear resistance of the breast muscle. The pH serves as a check on the effectiveness of the treatment. The average pH of all control birds was 5.90 (standard deviation σ = 0.12), but that of the epinephrine-treated birds was 6.94 (σ = 0.13). The results are summarized in Table II.

Treated birds, aged for times inadequate for normal birds, were as tender as fully aged control birds. In other experiments, measurements of ATP concentrations in muscle from epinephrine-treated birds showed a marked acceleration of the breakdown of ATP and, consequently, a more rapid onset of rigor mortis. Thus, the toughening of meat that occurs when normal birds undergo a rapid onset of rigor was attributed to accelerated post-mortem glycolysis and not to accelerated breakdown of ATP.

Similar experiments have been conducted with chicken fryers. In all cases, prevention of post-mortem glycolysis by the administration of epinephrine has resulted in meat which was tender immediately post mortem.

Table II. Injection of Epinephrine and Tenderness of Turkey Breast Muscle

| Aging Time in Ice Chill,[a] Hours | Mean Shear Resistance, Pounds | | p[b] |
	Epineph-rine-treated	Con-trol	
0	8	22	<0.005
1	7	30	<0.001
2	8	27	<0.005
24	...	9	...

[a] Processing time was 35 to 40 minutes.
[b] Probability that difference is due to sampling error.

Effect of Sodium Iodoacetate on Tenderness of Chickens.
Enzyme-inhibiting levels of sodium iodoacetate were used to determine how this reagent might affect the need for aging to achieve tenderness. Glycolysis was thus eliminated a second way—namely, by inhibiting the enzyme phosphoglyceraldehyde dehydrogenase (12). Although this method of eliminating glycolysis differs in principle from the epinephrine method, both methods are accompanied by a very rapid onset of rigor, and, of course, a higher ultimate pH than normal.

For this experiment, fasted chicken fryers (eight birds per group) received an intravenous injection of 200 mg. of iodoacetic acid per kg. (as the sodium salt) 3 to 6 minutes before slaughter. The birds were frozen at the times indicated in Table III, and the pH and shear resistance of the muscles determined as previously described, except that the cooking temperature was 130° C. The average pH of all control birds was 5.74 (σ = 0.12), but that for the iodoacetate-treated birds was 6.76 (σ = 0.25). Table III shows that inhibition of post-mortem glycolysis by an intravenous injection of sodium iodoacetate is essentially as efficient in yielding tender meat without aging as is the prevention of glycolysis by injection of epinephrine. In this experiment, however, aging reduced the shear values of control birds significantly below those of treated birds.

Tenderness of Chicken Muscle Cooked Very Rapidly Post Mortem.
As a third method of avoiding or minimizing glycolysis, normal chicken muscle was cooked so quickly that glycolysis would not have time to proceed very far. Processing steps, which required 30 to 40 minutes in the other experiments, were changed so that the time interval between the initial throat cut and cooking was $1\frac{1}{2}$ to 2 minutes. After the chicken was killed, the breast muscles were excised rapidly. One muscle from each of twelve birds was clamped between metal plates and placed immediately in boiling water where it reached an internal temperature of 85° C. in 3.1 minutes. The

Table III. Injection of Iodoacetate and Tenderness of Chicken Breast Muscle

| Aging Time in Ice Chill,[a] Hours | Mean Shear Resistance, Pounds | | p[b] |
	Iodo-acetate-treated	Con-trol	
0	10	31	<0.005
24	10	5	<0.001

[a] Processing time was about 30 minutes.
[b] Probability that difference is due to sampling error.

muscles were cooked and sheared as previously described. These shear values were compared with shear values for paired control muscles held at room temperature for 1 hour before cooking. The muscles cooked 2 minutes after death had a mean shear resistance of 12.4 pounds. The shear resistance of the paired controls cooked 60 minutes after death was 17.2 pounds. This difference is significant at the 1% level. The results clearly show that chicken muscle is more tender if cooked quickly, immediately post mortem, than if aged for 1 hour. Quantitatively, this result is not so clear-cut as those from the first two methods used to avoid glycolysis. That is, fully aged muscle, which usually has a shear resistance in the range between 4 and 7 pounds, would be still more tender than the quickly cooked muscle. The average pH of the meat cooked rapidly post mortem was 6.55. Thus, an appreciable amount of glycolysis occurred, even in the short processing and cooking times employed.

Influence of Death Struggle on Energy Sources of Muscle

Post-Mortem Breakdown of N-Phosphorylcreatine and ATP. The role of post-mortem glycolysis in determining ultimate tenderness has focussed attention on the net breakdown of the energy sources N-phosphorylcreatine (PC) and ATP, particularly as influenced by the extent of the death struggle. In one experiment, twelve $3\frac{1}{2}$-pound chicken fryers were divided randomly into two groups of six birds each. Each bird in one group received an intravenous injection of pentobarbital (25 mg. per kg.). When the bird was unconscious, it was slaughtered by bleeding. Both breast muscles were excised immediately, placed in polyethylene bags, held at room temperature, and sampled periodically for PC and ATP. Each bird in the second group was stunned electrically during slaughter to prevent struggling but otherwise received no anesthesia. PC was analyzed by the method of Eggleton, Elsden, and Gough (7), using the gentle acid hydrolysis conditions recommended by Barker, Ennor, and Harcourt (1). The results are shown in Figures 2 and 3.
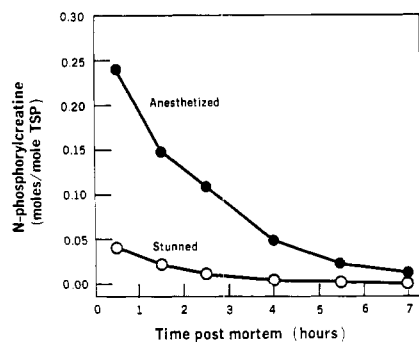
Figure 2. Post-mortem breakdown of N-phosphorylcreatine in chicken breast muscle
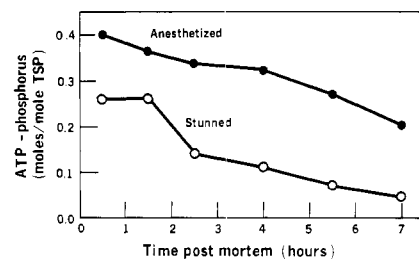

Figure 3. Post-mortem breakdown of ATP in chicken breast muscle

The marked difference in initial PC concentration (approximately 80% of the estimated resting level) must be due to the muscular activity during slaughter of the stunned birds. This lower level of PC apparently explains the consistently lower levels of ATP in stunned birds, since PC is a phosphate donor for the metabolic resynthesis of ATP.

Slaughter Conditions and Properties of Chicken Breast Muscle. Since anesthesia delayed the disappearance of PC and ATP, an experiment was conducted to determine the effect of anesthesia on the tenderness of aged meat. Twenty-four $3\frac{1}{2}$-pound chicken fryers were divided randomly into three groups of eight birds each. Each bird in one group was slaughtered by an outside neck cut without electric stunning or anesthesia. Birds in the other two groups were slaughtered as described in the preceding experiment. One *Pectoralis major* muscle was removed from each bird immediately post mortem and analyzed for glycogen. The carcass was held at room temperature for 1 hour without further processing, and then placed in a 2° C. room for 24 hours. The second *P. major* muscle was then excised, clamped between metal plates, cooked, and sheared as previously described. The data are summarized in Table IV. The time to onset of rigor mortis was estimated from manipulation of the neck and legs.

The results of the previous experiment, in which PC and ATP levels were markedly reduced after an active death strug-

gle, have been confirmed with respect to the initial level of glycogen. As expected, the greater breakdown of glycogen during struggle resulted in a more rapid onset of rigor mortis and tougher aged meat. With regard to tenderness, the only statistically significant difference was between struggling and anesthetized birds. The shear values of the struggling birds indicate that they would be judged as tender by a trained taste panel. However, these birds received no further processing or stimulatory treatment following slaughter. In addition, the muscles that were subsequently cooked remained on the skeleton for 24 hours before they were excised just prior to cooking. The reports of de Fremery and Pool on poultry (6) and Locker on beef (11) showed that cutting a muscle loose from the carcass before the onset of rigor mortis, thus allowing it to shorten, is an additional factor that increases toughness in aged meat.

Conclusions

The work reported here demonstrates that an acceleration of post-mortem glycolysis in young poultry increases the toughness of fully aged meat. Moreover, the elimination or inhibition of post-mortem glycolysis eliminates the need for aging to achieve tenderness. These studies represent a beginning toward an understanding of the reactions between and within the fibrillar proteins that must be fundamental to meat texture. Questions of basic importance which the present work has still left unanswered are:

Why poultry muscle, which is initially tender, should become tough when post-mortem glycolysis occurs; How poultry muscle, which has become tough under the influence of post-mortem glycolysis, becomes tender again during the aging period; Why an acceleration of post-mortem glycolysis inhibits the tenderization that normally occurs during the aging period in poultry muscle.

Until these questions are answered, it cannot be said with assurance that meat tenderization is fully understood nor can optimum control of the tenderization process be achieved.

Acknowledgment

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Table IV.   Degree of Death Struggle and Properties of Chicken Breast Muscle

Slaughter Conditions	Initial Glycogen, Mg. per Gram	Time to Onset of Rigor Mortis, Minutes	Shear Resistance, Pounds
Anesthetized (pentobarbital)	8.4	43	7.3
Electrically stunned	6.0	10	8.7
Freely struggling	3.2	8	9.1[a]

[a] Significantly different from anesthetized ($P < 0.05$).

END OF SYMPOSIUM