At least 2 mg of pure aflatoxin D_1 was collected between 320 and 340°, scraped from the slide, and weighed on a micro balance. The mass spectrum of this sublimate (Figure 5) indicates a molecular ion of 286 and other prominent peaks at masses 229, 243, and 257. Figure 6 is a photograph of a portion of this sublimate.

DISCUSSION

In view of the highly carcinogenic properties of aflatoxin B_1, information on breakdown products of detoxification is much needed. This information must necessarily be obtained on extremely small samples because of the scarcity of aflatoxin B_1 and the subsequent difficulty in obtaining enough of the ammoniation products for characterization. Fractional microsublimation effectively separated two new compounds from the major reaction product, aflatoxin D_1, and the micro-retrieval technique described was a major factor in obtaining enough of these compounds for molecular weight determinations.

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Effect of Chemical Treatments Causing Rapid Onset of Rigor on Tenderness of Poultry Breast Meat

A. Waheed Khan

Postmortem treatment with either calcium chloride, dinitrofluorobenzene, or iodoacetate and aging at 35° as compared to 20° and antemortem treatment with epinephrine were used to investigate the effect of pH and adenosine triphosphate levels immediately after slaughter on isometric tension development and shear force of pectoralis major muscle of male chickens. These treatments either minimized postmortem glycolysis and synthesis of ATP, or accelerated glycolysis and ATP breakdown, and caused the muscle to go into rigor sooner than normal after death. Onset of rigor within 1 hr after slaughter and shorter than normal interval of time between death and maximum rigor development caused a higher isometric tension and toughness in meat. These damaging effects were minimal in muscle having high pH. The relation between rapid onset of rigor, pH, and tenderness is discussed.

Earlier studies on factors causing variation in tenderness in similar muscles from comparable animals have shown that pH values of 6.2 or lower and onset of rigor within 1 hr after slaughter caused toughness in poultry (Khan and Nakamura, 1970) and beef (Khan and Lentz, 1973). The work was extended to study whether this toughness is caused by rapid depletion of adenosine triphosphate (ATP) or by the acid condition produced immediately after slaughter. Since anaerobic glycolysis is involved in both lactic acid formation and ATP synthesis, it is difficult to separate the adverse effects on tenderness of these two chemical activities under normal conditions. However, this difficulty can be overcome by controlling the biochemical processes involved in postmortem glycolysis and in ATP synthesis and depletion. A study of these activities under controlled conditions would provide a more direct evidence on the involvement of accelerated glycolysis and onset of rigor in the development of changes in tenderness.

This paper describes tests made on pectoralis major muscles in which (a) the regeneration of ATP by phosphokinase was minimized by 2,4-dinitrofluorobenzene treatment (Dydynska and Wilkie, 1966), (b) the regeneration of ATP by adenylate kinase and anaerobic glycolysis was minimized by iodoacetate treatment (Padieu and Mommaert, 1960), (c) the rates of postmortem glycolysis and ATP depletion were accelerated by $CaCl_2$ treatment (Campions et al., 1971) or by raising the aging temperature, and (d) postmortem glycolysis was minimized by antemortem epinephrine treatment (DeFremery and Pool, 1963; Khan and Nakamura, 1970). Postmortem changes in pH, content of adenosine nucleotides, and isometric tension development were measured and shear force determined.

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EXPERIMENTAL SECTION

Tests were made on pectoralis major muscles excised from well-rested male chickens (Leghorn, pathogen free from a single flock, live weight 2–3 kg) immediately after slaughtering and bleeding. Thirty-six of these birds were administered sodium pentobarbital (25 mg/kg body weight) in the thigh muscles 15 min before slaughtering to minimize struggling at death and to obtain muscle tissue having a pH value and high-energy phosphate level comparable to that in live muscle. Six birds were administered epinephrine 12–16 hr before slaughtering as described earlier (Khan and Nakamura, 1970) to obtain muscle having high ultimate pH. Excised muscles were cut along the fibers into strips, 1 cm square in cross section and about 6 cm in length. Strips from adjacent locations were used for measuring changes in rigor tension, shear force, adenosine nucleotide content, and pH. All measurements were made on duplicate samples from the same muscle, and all experiments were repeated three times.

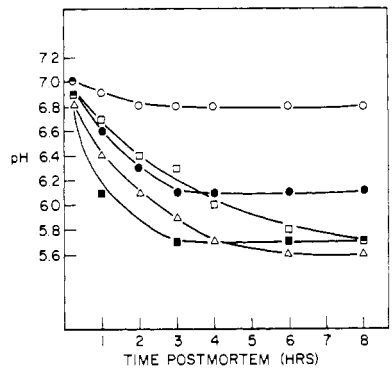

Figure 1. Typical postmortem pH changes in muscle aged in isotonic NaCl solution at 20° (□) and at 35° (△), in isotonic $CaCl_2$ solution at 20° (■), and in iodoacetate (○) or dinitrofluorobenzene (●), both at 20°.

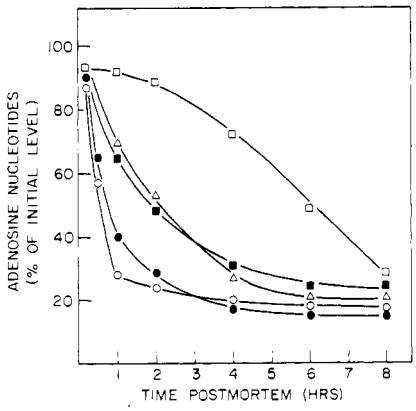

Figure 2. Typical postmortem changes in adenosine nucleotide content during aging in isotonic NaCl solution at 20° (□) and at 35° (△), isotonic $CaCl_2$ solution at 20° (■), and iodoacetate (○) or dinitrofluorobenzene (●), both at 20°.

Tests to determine the time course of rigor mortis and shear force were made on the same sample. The elapsed time after death before the onset of rigor and the extent of rigor tension that developed were determined by measuring changes in isometric tension as described earlier (Khan, 1974). The method consisted of suspending muscle strips in test or control solution and monitoring tension changes by means of an isometric transducer connected via a strain-gage amplifier to a recorder. In this study, muscle strips were suspended for 20 hr at 20° in isotonic NaCl solution (40 mg/sample, control) or in isotonic $CaCl_2$, 5 m*M* dinitrofluorobenzene (DNFB), or 5 m*M* iodoacetate (IA) solutions (test samples). All solutions were adjusted to pH 6.9–7.0 with NaOH and contained 50 ppm of tetracycline to prevent bacterial growth. Some tests were also made on samples kept in isotonic saline solution at 35° to determine the effect of higher temperature.

For shear force measurement, the samples were removed from the tension measuring apparatus after 20 hr and cooked to an internal temperature of 82–85° as described earlier (Khan and Lentz, 1965). Cooked samples were then cut into strips, 50 × 100 mm in cross section, and a total of 8–10 shear force measurements were made using a texture press system equipped with a meat shear cell.

The progress of glycolysis was followed by measuring the pH, and the depletion of ATP was followed by measuring the conversion of adenosine nucleotides to inosinic acid (Khan and Frey, 1971). In the latter test, the concentrations of both ATP and ADP were determined rather than the ATP level alone, because ADP acts as an acceptor of energy rich phosphates stored as phosphocreatine or generated by the glycolytic cycle.

RESULTS AND DISCUSSION

Typical changes in pH and in the content of adenosine nucleotides during the first 8 hr postmortem in samples treated with $CaCl_2$, DNFB, and IA and in untreated samples aged in isotonic saline solution at 20 and 35° are shown in Figures 1 and 2. In control samples that were aged in isotonic NaCl solution at 20°, the rates of pH decline and of adenosine nucleotide depletion were characteristically slow. Aging at 35° and $CaCl_2$ treatment both accelerated these changes. As indicated by the ultimate pH values (Table I), epinephrine and IA had the largest inhibitory effect on postmortem glycolysis; DNFB produced a considerably small effect. All treatments caused rapid depletion of adenosine nucleotides as compared to the control samples.

The development of rigor tension in chemically treated muscles was directly related to the depletion of adenosine nucleotides, but not to changes in pH (Table II). Irrespective of pH changes, the muscle started to develop tension when its adenosine nucleotide level dropped below 60% of the original concentration, and attained a maximum tension when its adenosine nucleotide level dropped below 30% of the original concentration. Development of rigor tension occurred at pH values of 6.6 or over in epinephrine and IA treated muscles and at pH values of 6.2 or lower in control or $CaCl_2$ treated muscles.

Tension began to develop in all chemically treated samples within 1 hr after slaughter as compared to 5–6 hr after slaughter in control samples (Table II). Results shown in Table II and in Figures 1 and 2 as well as those reported elsewhere (Dydynska and Wilkie, 1966; Khan and Nakamura, 1970; Padieu and Mommaert, 1960) indicate that epinephrine, DNFB, and IA treatments minimized phosphokinase and myokinase activities and/or postmortem glycolysis, and hence the synthesis of ATP. This loss of ATP synthesis, coupled with the normal breakdown of existing ATP in the muscle by intrinsic enzymes, hastens the depletion of ATP reserves and consequently hastens the onset of rigor. $CaCl_2$ treatment accelerated ATP breakdown and caused rapid onset of rigor. These results indicate that factors which maintain postmortem synthesis of ATP and inhibit ATP breakdown would help in preventing rapid onset of rigor.

Onset of rigor within 1 hr after slaughter and a shorter than normal interval of time between the death and maximum rigor development increased isometric tension and toughness, except in high pH meat (Table I). High pH appears to lessen the deleterious effects of rapid onset of rigor on tenderness. For example, epinephrine-treated samples (pH 6.9–7.1) as compared to samples aged at 35° (pH 5.7–5.9) developed tension sooner after death, but had significantly ($P = 0.01$) lower shear force. Since under normal conditions, drop in pH and onset of rigor occur concomitantly in meat (DeFremery and Pool, 1960), a rapid rate of glycolysis would lead to rapid onset of rigor, in addition to low pH, and consequently cause toughness. The rate and extent of glycolysis in meat can be assessed from its within 1 hr postslaughter and ultimate pH values (Khan and Lentz, 1973).

The results indicate that both the within 1-hr postslaughter pH and the ultimate pH values have a marked effect on rigor changes and tenderness. On the basis of this effect, muscles can be characterized as follows.

(a) Muscle Having High pH Value (6.9–7.1) within 1 hr Postslaughter and Low Ultimate pH (5.7–5.9) Value. This muscle undergoes the largest proportion of glycolysis postmortem, has a delayed rigor, develops a minimum of rigor tension, and is generally tender. Because of its low

Table I. Effects of Various Chemicals and Temperature on Rigor and Tension Development, pH, and Shear Force of Poultry Breast Meat[a]

Treatment	No. of birds	Aging temp, °C	Time postmortem, hr: Onset of rigor	Time postmortem, hr: Full rigor	Ultimate pH	Max tension, g	Shear force, kg
Sodium chloride (control)	9	20	5–6	7–9	5.7–5.9	12.0 (4.0)	2.9 (0.2)
Calcium chloride	6	20	<1	2–3	5.5–5.7	17.6 (3.1)	3.4 (0.6)
Dinitrofluorobenzene	6	20	<0.5	1–3	6.0–6.2	31.1 (6.5)	8.0 (1.2)
Epinephrine	6	20	<0.5	1–2	6.9–7.1	20.8 (2.0)	2.3 (0.2)
Iodoacetate	6	20	<0.5	3–5	6.6–6.8	40.2 (8.0)	7.4 (1.2)
Sodium chloride	9	35	1–3	3–4	5.6–5.8	18.4 (3.8)	3.7 (0.4)

[a] Standard deviation is shown in parentheses; *t*-test analysis showed that differences between control and test samples were significant at 1% or lower level.

Table II. Effect of Various Chemicals on the Development of Rigor, Concentration of Adenosine Nucleotide, and pH in Poultry Breast Meat[a]

Treatment	Time postmortem, hr: Onset of rigor	Time postmortem, hr: Full rigor	Adenosine nucleotides, % of original concn: Onset of rigor	Adenosine nucleotides, % of original concn: Full rigor	pH: Onset of rigor	pH: Full rigor
Sodium chloride (control)	5–6	7–9	57 (2)	21 (4)	6.1–6.2	5.7–5.9
Calcium chloride	<1	2–3	49 (5)	17 (5)	6.0–6.1	5.6–5.8
Dinitrofluorobenzene	<0.5	1–3	54 (4)	20 (3)	6.4–6.6	6.0–6.2
Epinephrine	<0.5	1–2	55 (3)	21 (4)	6.9–7.1	6.9–7.1
Iodoacetate	<0.5	3–5	50 (5)	18 (5)	6.6–6.8	6.5–6.8

[a] Only muscle having 15-min postslaughter pH values of 6.8–7.1 were used. Aging temperature was 20°. Data show the range of values for six different birds. Standard deviation is shown in parentheses.

ultimate pH, this muscle has a desirable color and is expected to undergo minimum microbiological changes during storage. The control samples used in this study fall in this category.

(b) Muscle Having Low pH Value (6.2 or Lower) within 1 hr Postslaughter and Low Ultimate pH (5.7–5.9) Value. This muscle undergoes the largest proportion of its glycolysis during slaughter or early postmortem, develops rigor sooner after death, develops a higher rigor tension, and is generally tough. Conditions causing low postslaughter pH have been described earlier (Bendall, 1966; Briskey, 1964; Khan and Nakamura, 1970; Ma and Addis, 1973; McLoughlin, 1970). Some of these conditions were simulated in the present study by the use of $CaCl_2$ and DNFB.

(c) Muscle Having High pH Value (6.9–7.1) within 1 hr Postslaughter and High Ultimate pH (6.9–7.1) Value. This muscle comes from animals that have undergone physical or chemical treatments which lower the glycogen level at the time of death or minimize postmortem glycolysis. It develops rigor sooner after death, and is generally tender (DeFremery and Pool, 1963; Khan and Nakamura, 1970). It has a dark red color and a high water holding capacity. Meat from birds treated with epinephrine falls into this category.

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