ABSTRACT

PHYSICAL AND BIOCHEMICAL PROPERTIES OF PORCINE MUSCLE AS AFFECTED BY SOME ADRENAL HORMONES AND POST-MORTEM GLYCOLYTIC PARAMETERS

by Elton David Aberle

This study consisted of two separate investigations and included 36 market-weight pigs. The adenylic acid deaminase activity and AMP and IMP levels of porcine longissimus dorsi, gluteus medius, and rectus femoris muscles were determined in Part I. The relationship of these parameters to rate of post-mortem glycolysis and pH fall and to development of pale, soft, and exudative muscle was evaluated. In Part II, the effects of ante-mortem epinephrine injection or of prednisolone plus epinephrine injection upon some physical and biochemical properties of porcine muscle were studied. Rate of pH decline, adenylic acid deaminase and phosphorylase activities, glycogen, AMP, and IMP concentrations, Munsell value, and transmission value were determined in the longissimus dorsi muscle. Munsell value, transmission value, phosphorylase activity, and glycogen, AMP, IMP, inorganic phosphate and ATP levels were contrasted in porcine muscles with fast or slow rates of post-mortem glycolysis among the pigs in the control group.

Specific activity of adenylic acid deaminase was .031, .031 and .027 in the longissimus dorsi, gluteus medius, and rectus femoris muscles, respectively. Adenylic acid deaminase, AMP, and IMP were not significantly different between muscles. Correlation analysis indicated a low positive relationship between rate of post-mortem pH decline and adenylic acid deaminase activity of all three muscles.

Rate of post-mortem pH fall, ultimate muscle pH, Munsell value and transmission value were not significantly altered by either epinephrine or prednisolone plus epinephrine injection. Epinephrine injection resulted in significantly higher ($P < .05$) total phosphorylase activity and slightly elevated phosphorylase *a* activity and % phosphorylase *a*. However, neither total phosphorylase nor phosphorylase *a* activity was related to the rate of post-mortem pH fall or frequency of PSE muscle development. Adenylic acid deaminase specific activity was lower ($P < .05$) in muscle from pigs injected with either epinephrine or prednisolone plus epinephrine.

AMP levels were 1.82, 1.72, and 2.10 u mole/g fresh muscle in control, epinephrine, and prednisolone plus epinephrine treated pigs, respectively, and IMP levels were 2.53, 2.74, and 2.54 u moles/g, respectively. Concentrations of the two mononucleotides were not significantly different between treatments. IMP concentration was highly related to rate of pH fall. Correlation coefficients between IMP and *longissimus dorsi* muscle pH were -.93 and -.94 at 15 and 45 min post-mortem, respectively. Muscle from prednisolone injected pigs contained more glycogen ($P < .05$) at both 15 min and at 24 hr post-mortem.

Muscle with slow glycolysis (pH < 5.65 at 45 min) had lower Munsell values and lower transmission values than fast glycolysis muscle (pH > 5.85 at 45 min). Total phosphorylase and phosphorylase *a* activities were not different between slowly and rapidly glycolysing muscle. IMP, ATP inorganic phosphate and glycogen levels were 3.51 u moles, 1.24 u moles,

25.6 u moles, and 6.27 mg/g fresh muscle, respectively, in muscles with rapid glycolysis. In slowly glycolysing muscles, the IMP, ATP, inorganic phosphate and glycogen levels were 1.54, 2.49, 17.4 and 10.28, respectively. IMP, ATP, and inorganic phosphate differed significantly ($P < .05$) between the two types of muscle.

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By

Elton David Aberle

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

INTRODUCTION

The subject of porcine muscle quality has received considerably more attention during recent years, especially among American and European workers. Muscle quality can be defined as those chemical, physical and structural properties which contribute to palatability of the meat. In the past, much emphasis has been placed upon improvement of the quantitative aspects of porcine production such as muscle to fat ratio, longissimus dorsi muscle area, and wholesale cut percentages while qualitative factors were usually ignored. This may have been due to the fact that pigs are usually slaughtered at a young age and thus are expected to have acceptable eating qualities. Also, the type of processing procedures utilized influence palatability attributes.

With the production of leaner, heavier muscled, earlier maturing porcine animals, problems have arisen in the qualitative aspects of pork production. The meat industry has recently become aware of the pale, soft, and exudative (PSE) condition in porcine muscle. This abnormal condition is characterized by excessive loss of fluids and shrinkage during processing and the muscle is less tender and juicy. Incidence of the PSE condition in porcine muscle has been reported to be as high as 25% during some seasons of the year. Recent research has also indicated that such factors as color, firmness, and texture are related to palatability of meat.

Reports by Ludvigsen (1953), Briskey and Wismer-Pedersen (1961a,b), Bendall and Wismer-Pedersen (1962) and McLoughlin and Goldspink (1963b) indicate that the development of pale, soft and exudative porcine muscle

is related to post-mortem muscle temperature and pH. The PSE condition resulted when a rapid rate of post-mortem glycolysis and pH fall occurred while muscle temperature remained high. Numerous investigations have been conducted into the chemical and biochemical processes which occur in muscle post-mortem; however, the cause of the rapid glycolytic rate and pH drop has not been elucidated.

The rate of anaerobic glycolysis in skeletal muscle can apparently be regulated at several discrete metabolic steps. It has been established that epinephrine affects glycolysis by increasing the activity of the phosphorylase reaction (Cori and Illingworth, 1956; Danforth et al., 1962). It has also been suggested that alterations in the activity of phosphofructokinase may be important in regulating the rate at which hexose monophosphate is converted to lactic acid (Passonneau and Lowry, 1962; Karpatkin et al., 1964). The activity of phosphofructokinase is strongly influenced in vitro by changes in concentration of a variety of cellular constituents such as 5'-adenosine tri-, di- and monophosphate, inorganic phosphate, ammonium ion, potassium ion, and fructose-6-phosphate. Whether these factors are operative in the development of PSE muscle has not been established.

After consideration of these facts, this study was undertaken with the following experimental objectives:

1. To determine if there is a possible relationship between muscle adenylic acid deaminase activity, mononucleotide (AMP and IMP) levels of muscle, rate of post-mortem glycolysis and procine muscle quality.

2. To study the effects of ante-mortem epinephrine and prednisolone injection upon post-mortem chemical events, muscle properties, and enzymatic activities.
3. To determine the muscle concentration of some cellular constituents which are believed to affect glycolytic enzyme activity and thus alter glycolytic rate.

REVIEW OF LITERATURE

Relationship of Post-Mortem Changes to Development of PSE Muscle

The muscles of living porcine animals are moderately dark in color, firm in texture, and dry in appearance (Briskey, 1964). Upon death of the animal, anaerobic conditions develop and the biochemical and physiological reactions which then occur largely govern the ultimate quality characteristics of the muscle. The magnitude of change is a result of rate of post-mortem anaerobic glycolysis and pH decline and temperature of the muscle at the onset and completion of rigor mortis.

Normal muscle. Wismer-Pedersen and Briskey (1961a) stated that when post-mortem anaerobic glycolysis occurred at an intermediate rate, requiring 6 to 12 hr for complete lactic acid production and 4 to 6 hr for completion of rigor mortis, the muscles were normal in color, moderately dry, and moderately firm. These muscles had desirable characteristics for fresh or processed meats. More recently, Sayre and Briskey (1963) found that muscle color and juice retaining properties were not greatly affected if pH was above 5.7 and temperature below 35°C at rigor mortis onset.

In normal muscle, approximately 28% of the total nitrogen can be extracted as sarcoplasmic protein (soluble in 0.03M potassium phosphate, pH 7.4) and 40% as myofibrillar protein (soluble in 1.1M potassium iodide in 0.1M potassium phosphate) as reported by Sayre and Briskey (1963). Bendall et al. (1963) found that creatine phosphate had fallen

to 50% of initial concentration at 1 hr post-mortem in normal muscle while adenosine triphosphate (ATP) level reached 50% of initial concentration at 2 1/2 hr post-mortem. Normal muscle also shows a slight but gradual decrease in water holding capacity (Briskey, 1964) which quite likely is associated with the onset of rigor mortis and disappearance of ATP. This is supported by a postulation of Hamm (1960) that the minimum in hydration corresponds to the maximum in muscle rigidity. The normal drop in pH resulting from anaerobic glycolysis also is responsible for some decrease in water retention. Hamm (1960) suggested that two-thirds of the decrease in hydration in bovine muscle is due to the loss of ATP and one-third to pH fall.

Pale, soft and exudative muscle. The early work of Ludvigsen (1953) showed that extremely low pH values (5.3 to 5.5) were achieved at 45 min post-mortem in the muscles of some Danish Landrace pigs while other pigs had pH values of 6.8 to 7.0. Those muscles with low pH were abnormally light colored and quite exudative. He termed the condition "muscular degeneration".

Wismer-Pedersen (1959) reported that porcine muscle, in which a very rapid fall in pH post-mortem was recorded, possessed lower water holding capacity. Significantly lower pH values at 40 min post-mortem were also noted by Briskey et al. (1959a) in muscles which ultimately became PSE, even though the values were normal at death. A rapid decrease in pH to values of 5.5, and in some cases to 5.1, at 1 1/2 hr post-mortem while muscle temperature remained at 36 to 41°C has been observed (Briskey and Wismer-Pedersen, 1961a).

Concurrently with the accelerated fall in pH, Briskey and Wismer-Pedersen (1961a) found a rapid decrease in labile phosphate compounds. Lactic acid production and liberation of ammonia were especially rapid and approached completion in the first 2 hr post-mortem. Liberation of ammonia was accompanied by a sharp reduction in ATP (Bendall and Wismer-Pedersen, 1962). The rapid liberation of ammonia and reduction of ATP can be taken as indicative of the onset of rigor mortis during the initial 2 hr period as described by Bendall and Davey (1957). Recently, Kastenschmidt et al. (1966) found that ATP level was already lower at 2 min post-mortem in pork muscle with more rapid rates of glycolysis when compared to muscle with slower glycolytic rates. Briskey et al. (1962) and Bendall et al. (1963) next demonstrated by means of extensibility measurements of muscle strips that muscles which became PSE exhibited a very rapid development of rigor mortis. Rigor mortis onset was reported to occur when ATP concentration reached 30% of the initial level (Bendall et al., 1963).

Bendall and Wismer-Pedersen (1962) stated that all the characteristics of pale, soft and exudative porcine muscle could be artificially induced by allowing normal muscle to pass into rigor mortis at 37°C. This was not consistently observed by Briskey (1964). The latter author found that when one side of the carcass was chilled normally while the other side was allowed to pass into rigor at 37°C, a wide range of muscle structures and appearances was noted, regardless of the holding temperature. Bodwell et al. (1966) reported that subjecting pork carcasses to

a 37°C treatment during rigor onset did not consistently produce PSE muscle. Thus, it would seem that considerable variation exists among animals in their susceptability to development of PSE muscle in response to high temperature and low pH at the onset of rigor mortis.

Protein Extractability of PSE Muscle

Wismer-Pedersen (1959) demonstrated a direct relationship between rate of pH fall and water holding capacity of the muscle. He also found that muscle with low water holding capacity had reduced protein solubility in 0.6M KCl. The postulation was that denaturation of actomyosin during accelerated pH drop could be responsible for lower protein solubility and that the denaturation would be reflected in lower ATPase activity of PSE tissue. However measurement of ATPase activity showed no close relationship to water retention.

Bendall and Wismer-Pedersen (1962) reported that washed myofibrils obtained from watery muscle had lower water retention at low ionic strength and lower extractability at high ionic strength than the myofibrils from normal muscle. Moreover, the washed myofibrils from watery muscle had a higher protein content than similar normal myofibrils. It was the authors' conclusion that the additional protein of the watery myofibrils was denatured sarcoplasmic protein which had been deposited upon and firmly bound to the myofibrillar protein. They indicated that the layer of denatured sarcoplasmic protein was bound sufficiently strongly so as to make the myofibrillar proteins resistant to high ionic strength extraction and

low ionic strength hydration. Bendall and Wismer-Pedersen (1962) also presented histological evidence for the presence of denatured sarcoplasmic proteins precipitated upon the myofibrils.

The results of Bendall and Wismer-Pedersen (1962) were corroborated by McLoughlin (1963) and McLoughlin and Goldspink (1963b). The extractability of sarcoplasmic and myofibrillar proteins from exudative post-rigor muscle was reduced at low and high ionic strengths, respectively, and the solubility change was related to attainment of low pH values soon after death. McLoughlin and Goldspink (1963a) also reported that the pale color of exudative muscle was an effect of rapid glycolysis and low pH while muscle temperature remained high. Acceptable color of post-rigor muscle could be maintained if temperature was reduced to about 30°C before the pH approached 6.0 (Goldspink and McLoughlin, 1964).

The solubilities of sarcoplasmic and myofibrillar proteins were determined by Sayre and Briskey (1963) at the time of slaughter, onset of rigor mortis, completion of rigor mortis and 24 hr after death in porcine muscles exhibiting a wide range of physiological conditions during the initial post-mortem period. Those proteins soluble in 0.03M potassium phosphate, pH 7.4 were designated sarcoplasmic. Subsequent extraction with 1.1M potassium iodide in 0.1M potassium phosphate, pH 7.4, yielded the myofibrillar proteins. Muscle protein solubility was greatly altered by both the temperature and pH condition which existed at the onset of rigor mortis or during the first few hr after death. Sarcoplasmic protein solubility at 24 hr was decreased to 55% of the value found at 0 hr in muscle groups which exhibited high temperature, $> 35°C$, and low pH, 5.3

to 5.5, at the onset of rigor mortis. In contrast, only a 17% reduction in sarcoplasmic solubility was observed in groups with high pH, > 6.0. It should be noted that when rigor mortis onset occurred between pH 5.7 to 5.9, and < 35°C, sarcoplasmic protein solubility was reduced only 12%. Myofibrillar protein solubility ranged from no reduction during the first 24 hr after death when pH remained high at onset to a 75% reduction in muscle with low pH and high temperature at onset of rigor mortis (Sayre and Briskey, 1963). The 24 hr pH seemed to have only a minor influence on protein solubility. Muscle protein solubility appeared to be one of the major factors affecting the juice-retaining properties of muscle.

Scopes (1964) studied the solubility characteristics of myofibrillar and sarcoplasmic proteins in various induced and simulated post-mortem conditions. Sarcoplasmic proteins were readily denatured at 37°C and pH values below 6.0 and at higher temperatures more independently of pH. Denaturation of sarcoplasmic protein in situ was associated with decreased myofibrillar solubility in 1.0M KCl. Starch gel electrophoresis indicated one major and several minor proteins which were specifically denatured by conditions of low pH and high temperature; the major protein was identified as ATP-creatine phosphotransferase.

Factors Which Influence Meat Hydration

Muscle proteins and water. Hamm (1960) compiled a very comprehensive review of the basic concepts of muscle hydration. He stated that the "true hydration-water" of muscle is the amount of water that attaches to

protein by monomolecular and multimolecular adsorption. This water amounts to about 4 to 5% of the total water in the muscle tissue and it is bound directly to certain hydrophilic groups, which include polar groups such as carboxyl, amino, hydroxyl and sulfhydryl groups. The physical properties of fixed, bound water are different from those of "free" water. The bound water has a lower freezing point, a lower vapor pressure, and a lower dissolving power than normal water.

Most of the water in muscle is free water, chemically speaking (Hamm, 1960). It freezes at the same temperature as normal water, has the same solvent power, and does not seem tightly bound to the protein molecules. Most of the free water can be considered as mechanically immobilized by the network of cellular protein membranes and protein filaments. Hamm concluded that changes in the water holding capacity of meat are not due to any changes in the true hydration water fixed to the polar groups of the protein.

Hamm (1960) further stated that in contrast to the tightly bound water, the amount of free water immobilized within the tissue is strongly influenced by the spatial structure of the muscle tissue. Tightening this spatial structure or network of proteins decreases immobilized water and increases easily expressible water and that loosening the protein structure has the opposite effect. This so-called stereo effect (Hamm, 1959) is extensively influenced by changes in protein charges, by attraction or repulsion of charged groups. Adjustment of pH to certain values or the presence of certain ions greatly affects the spatial arrangement and therefore affects water holding capacity. Hamm (1959) defines water holding

capacity as the ability of meat to hold fast its own or added water during the application of any force (pressing, heating, chewing, grinding, etc.).

The influence of pH on meat hydration is a typical example of the importance of protein net charge (Hamm, 1959). The isoelectric point of muscle is about pH 5.0. At this point, the net charge of muscle proteins is at a minimum. Therefore, at this pH, meat hydration also has a sharp minimum. The pH of normal meat is about 5.5, which is close to the isoelectric point, and consequently water holding capacity is low.

Divalent cations and electrolytes. Hamm (1959) reported that in addition to hydrogen ions, divalent cations, which are naturally present in muscle, are also important in meat hydration in spite of their relatively low concentration. Magnesium (25 mg/100 g) and calcium (5 mg/100 g) were found to decrease water holding capacity of muscle. Removal of part of the calcium from the muscle by treatment with a cation exchanger resulted in a strong increase in water holding capacity at pH values above the isoelectric point. The increase in hydration by removal of divalent cations was thought to be a typical case of the stereo effect, whereby removal of metallic crosslinkages freed more charged groups for water binding. Swift and Berman (1959) found an inverse relationship between water retention and either magnesium or calcium content in a study of the factors influencing water retention in 8 bovine muscles.

Adenosine triphosphate, an organic polyphosphate, has the ability to produce a higher water holding capacity. The post-mortem drop in muscle hydration is proportional to the decomposition of ATP to ADP, AMP and

finally to IMP (Hamm, 1959). Hamm (1959) proposed that ATP has an effect similar to the cation exchanger. Because of its ability to form strong complexes with alkaline earth metals, ATP ties up part of the divalent ions, probably the magnesium. Breakdown of ATP releases the cations since AMP and IMP are much less able to form the necessary complexes. Hamm (1959) reported that at pH 7, the structural proteins of bovine muscle immediately post-slaughter contained less bound magnesium than those of rigor or post-rigor muscle. Sherman (1961) indicated that polyphosphates and citrate influenced water retention due to their relatively high ionic strength and to their influence on muscle pH. These salts function primarily to form strong complexes with alkaline earth metals. They eliminate divalent cations in meat, especially magnesium ions.

The addition of sodium chloride increased the water holding capacity of meat quite strongly (Hamm, 1959). This effect was due to the influence of chloride ions rather than to sodium ions. Salt crosslinkages may have been split off by binding of chloride ions and there was an increase of meat hydration of both net charge effect and stereo effect.

Temperature-pH relationship to meat hydration. Briskey and Wismer-Pedersen (1961a) found that muscles which underwent a sharp decrease to pH 5.1 at 1 1/2 hr post-mortem, and a subsequent elevation to 5.3 to 5.6 as the carcasses cooled, were exudative. Bendall and Wismer-Pedersen (1962) stated that the immediate cause of wateriness in muscle was the combined effects of high temperature (37°C) and low pH upon the muscle proteins in agreement with Wismer-Pedersen and Briskey (1961b). Bendall and Wismer-

Pedersen (1962) also concluded that the depression and elevation of pH values reported by Briskey and Wismer-Pedersen (1961a) was due to the effect of temperature on the pK's of ionizable groups of the proteins and buffering substances. Therefore, they refuted the suggestion that the depression and elevation of pH was a factor in the development of exudative porcine muscle.

Bendall et al. (1963) reported further on the rates of ATP and creatine phosphate disappearance in relation to pH and onset of rigor-mortis in muscle samples removed from the carcass at slaughter and subsequently held at a constant temperature of 37°C. Muscle which eventually became exudative showed a more rapid ATP turnover and a shorter lag period to the onset of rigor mortis. The authors could not explain the reason for the differences in rates and were unable to provoke more rapid changes by varying ante-mortem treatment. It was noted that muscle which was allowed to go into rigor at a constant temperature of 37°C always became watery and pallid in appearance. The authors concluded that the reason for pale, watery muscle was a combination of high temperature and low pH and that this could be prevented by rapid cooling to below 30°C.

Muscle location and hydration. Swift and Berman (1959) studied water retention and associated factors in 8 bovine muscles. The results indicated that the relative order of the muscles followed a pattern with regard to water retention and pH. The serratus ventralis from the rib and chuck and the rectus abdominus from the plate and flank had the highest pH and water retention values while the semitendinosus and semimembranosus from the round had the lowest values.

Using a modification of the Grau-Hamm procedure for determination of free moisture, Urbin et al. (1962) reported significant variation in free moisture due to location in the cross-section of the longissimus dorsi muscle. The medial portion of the l. dorsi had significantly lower free moisture values than did the lateral portion.

Topel et al. (1966) reported on variation in juice retaining properties between and within certain selected porcine muscles. Among the ham muscles studied, the biceps femoris showed the lowest juice retaining properties while the rectus femoris had the greatest variation in juice-retaining properties between proximal and distal positions within the muscle.

Glycolytic Enzymes and Metabolites in Relation to the Development of PSE Muscle

Phosphorylase. Because it had been suggested that phosphorylase activity could influence the rate of glycogen breakdown (Cori, 1956), Wismer-Pedersen (1959) studied phosphorylase activity in 6 psoas major muscles which had different rates of post-mortem pH fall in an attempt to ascertain the reason for the accelerated lactic acid production. The fresh tissue was macerated in 0.04M phosphate buffer, pH 7.4, containing 0.001M versene and 0.02M sodium fluoride. The macerate was incubated at 37°C for 30 min with an amount of glycogen corresponding to 1% of the weight of the meat. An indirect relationship was found between the 45 min pH and the amount of glycogen decomposed, which suggested that phosphorylase activity was increased in muscles with lower pH. The author

also postulated that increased phosphorylase activity may have been a consequence of adrenalin release, since adrenalin is known to increase phosphorylase activity (Cori and Illingworth, 1956).

In a later report, Sayre et al. (1963b) found that total phosphorylase activity was not associated with rate of pH decline post-mortem, glycogen level of fresh muscle or with other ultimate physical properties of the muscle. In addition, phosphorylase activity was not affected by excitement, fasting, or sucrose feeding of the animals before slaughter. In general, most of the phosphorylase activity was in the b form at 10 min post-mortem (Sayre et al., 1963a). However, 3 animals possessed significant quantities of phosphorylase a. No values for the ratio of phosphorylase a to phosphorylase b were given.

It has been recognized that phosphorylase b is active in living tissue only in the presence of adenosine monophosphate (AMP) (Stetten and Stetten, 1960). Since AMP is normally present in very low levels in living muscle, it was suggested that phosphorylase exists in two forms, as the active and inactive functional states. However, it is noteworthy that Morgan and Parmeggiani (1963) have suggested that post-mortem levels of AMP are sufficient for phosphorylase b activity and that activation of the b form appears to be an important factor in the rapid rate of glycogenolysis under anaerobic conditions.

Phosphofructokinase. The implication that phosphorylation of fructose-6-phosphate (F-6- P) was a rate controlling step in glycolysis (Passonneau and Lowry, 1962) and the observation that phosphofructokinase limits the rate of glycolysis in liver flukes (Mansour and Mansour, 1962) led Sayre

et al. (1963c) to investigate phosphofructokinase activity of porcine muscle extracts in relation to PSE development of the intact muscle. Activity of this enzyme was not associated with rate of post-mortem glycolysis or with physical properties of the muscle. Phosphofructokinase activity was not affected by ante-mortem sucrose feeding or heat treatment. While the previous observations would seem to preclude phosphofructokinase as a contributing factor to the development of PSE muscle, the authors recognized that the conditions of enzyme assay may not have been the same as in vivo conditions. Indeed, phosphofructokinase activity in vivo has been shown to be sensitive to a variety of cellular constituents (Mansour, 1963), among them its substrate, fructose-6-phosphate, and AMP, inorganic phosphate, ADP, ATP and ammonium ion (Ozand and Narahara, 1964; Regen et al., 1964).

Lactic dehydrogenase. The data from biopsy samples reported by Briskey and Wismer-Pedersen (1961b) indicated that there were larger pyruvic acid pools in the living tissue of normal porcine muscle than in those which had a rapid post-mortem pH decline. Only minor differences were observed in pyruvic oxidase activities between the two types of muscle. However, muscle that elicited a rapid pH fall had a slower rate of methylene blue reduction (used as a measure of lactic dehydrogenase activity). If the samples showed rapid reduction of methylene blue, they were ultimately uniform in color, dry in appearance and firm in structure (Briskey and Wismer-Pedersen, 1961b). Zinc content was significantly correlated with methylene blue reduction, lactic dehydrogenase being a zinc containing enzyme.

Cassens et al. (1963) confirmed the above results in a study of porcine muscles of varying function. The trapezius muscle, which is considered to be more active and has a slower rate of pH fall post-mortem than does the longissimus dorsi muscle, was reported to have a higher zinc content and higher lactic dehydrogenase (LDH) activity than the longissimus dorsi muscle.

Charpentier and Goutefongea (1964) observed a marked decrease in the activity of lactic dehydrogenase in exudative muscles. Comparison of LDH from normal and exudative pig muscles by starch gel electrophoresis revealed 3 anodic bands much reduced in staining intensity and a fourth anodic band much increased in patterns from exudative muscles. The authors suggested that the LDH pattern might be used as a diagnostic tool on the blood plasma of the live animal.

Glycogen. The amount of glycogen in the muscle at the time of death has been generally recognized to be important in determining post-mortem physical and chemical properties of muscle only if: a) the glycogen is available for degradation (Lawrie, 1955) and b) the enzymes are not inhibited by decreasing pH (Bate-Smith and Bendall, 1949). Briskey and Wismer-Pedersen (1961a) found that muscle which exhibited rapid post-mortem glycolysis had slightly higher glycogen levels than muscle with a normal rate of pH fall. It is recognized, however, that the rate at which glycogen is degraded post-mortem has a greater influence upon ultimate muscle properties than the amount present at the time of death (Sayre et al., 1963a,b; Briskey and Wismer-Pedersen, 1961b).

Lawrie et al. (1959) reported that glycogen isolated from bovine sternocephalicus muscle after onset of rigor mortis had significantly shorter external chain length than that isolated from pre-rigor muscle. This significant shortening of outer chains was suggested to indicate in vivo heterogeneity with respect to branching characteristics. Glycogen isolated from post-rigor horse muscle did not differ from pre-rigor glycogen. This may have physiological importance since glycogen of high molecular weight appears to be preferentially degraded in vivo and in vitro by muscle phosphorylase (Stetten et al., 1958) and to exchange its glucose units more vigorously with the environment than glycogen of low molecular weight.

The average chain length of glycogen from porcine muscles decreased by approximately 1 glucose residue during post-mortem glycolysis as observed by Sayre et al. (1963d). There was, however, a more severe decrease in both external and internal chain lengths of glycogen from muscles of the Chester White breed which had a slow rate of anaerobic glycolysis while the muscles of Poland Chinas (rapid rate of glycolysis) had only slightly shortened chain lengths during the post-mortem period (24 hr). Ante-mortem sucrose feeding tended to lengthen both the internal and external chain lengths of the muscle glycogen and accelerated the rate of anaerobic glycolysis. These findings suggest that glycogen structure may be altered by nutritional as well as genetic influences and in both instances may influence the rate at which the molecule is degraded (Sayre et al., 1963d).

Kjolberg et al. (1963) determined the average chain length of porcine longissimus dorsi muscle glycogen to be 15 glucose residues. Muscles from Hampshire, Poland China and Chester White pigs having PSE musculature had a lower glycogen content than non-affected animals but the molecular structures of the polysaccharides were identical.

Hexose phosphates. Kastenschmidt et al. (1966) studied the levels of various glycolytic intermediates in porcine skeletal muscles possessing fast and slow glycolytic rates. The accumulation of lactate in muscles with fast glycolysis was nearly complete within 30 min post-mortem, but in muscles with slow glycolysis, lactate continued to rise for 180 min after death. The most striking differences in glycolytic intermediates were in glucose-6-phosphate (G-6-P) and fructose-1,6-diphosphate (F-1,6-diP). Muscles with rapid anaerobic glycolysis contained higher levels of G-6-P especially during the first hr post-mortem, which was interpreted to indicate increased phosphorylation of glycogen. Higher glucose-6-phosphate to fructose-6-phosphate ratios in rapidly glycolyzing muscle than in muscle with slow glycolysis indicated that phosphorylation of glucose was more rapid than its isomerization under these conditions (Kastenschmidt et al., 1966). A large decrease in F-1,6-diP was observed at 15 min post-mortem in muscle with fast glycolysis and this intermediate was virtually absent at 30 min after death. The explanation for this observation was a) an inactivation of phosphofructokinase by decreasing pH and b) an activation of aldolase and subsequent steps in glycolysis.

Myoglobin. Since myoglobin plays an important role in the color of meat, Briskey et al. (1959a) determined the myoglobin content of various classes of ham muscles. Myoglobin values appeared somewhat lower in pale, two-toned muscles than in normal muscles, but the differences were not statistically significant ($P < .05$). As reviewed by Briskey (1964), Hart (1962) reported a slight but significantly lower pigment content in PSE muscles from a large group of animals in which animal age and activity were carefully controlled. Lawrie (1960) found a virtual absence of pigment in the muscles of English breeds of pigs affected by "white muscle" disease. In this investigation, pigment was determined as alkaline hematin. Meyer et al. (1963) noticed a marked difference in myoglobin concentration between PSE and normal longissimus dorsi muscles in loins obtained from a commercial packer. Some of the pigment differences may have been due, however, to drip loss during the period before analyses were conducted. Sayre et al. (1964) found that exudative muscle lost 10% of its weight as drip during an 8-day holding period compared to a 1 to 3% loss for dry, firm muscle. The accumulated drip contained 11 to 14% protein, the protein being sarcoplasmic in origin and thus containing some myoglobin.

Observations by McLoughlin and Goldspink (1963a) implied that the pale color of exudative pork muscle was due to rapid fall of pH after death so that low pH values (5.0 to 5.6) were attained while muscle temperature was still high. A cold water extract of freshly killed muscle was incubated at various temperatures and pH values and the development of turbidity observed. Turbidity increased and acid hematin remaining in

solution decreased under conditions of increasing temperature and decreasing pH. Thus, the combined effects of low pH and high temperature were interpreted to have caused precipitation of previously soluble sarcoplasmic proteins. The color of the muscle myoglobin was apparently masked by the precipitated protein and some myoglobin was also apparently adsorbed onto the precipitated material and removed from free solution.

Vitamins. Niacin, thiamin and riboflavin were determined in fresh and cooked gluteus medius pork muscles with extremely different physical characteristics by Meyer et al. (1963a). Pale, soft and exudative tissue contained about twice as much niacin ($P < .005$) as dark, firm and dry muscle, both fresh and cooked. Fresh, normal muscle had slightly higher riboflavin and thiamin and in the cooked form had higher thiamin. The PSE muscles lost a higher percentage of their vitamin content during cooking, either by heat destruction or drip loss. The authors postulated that the niacin differences reflect a difference in the pyridine nucleotide coenzyme content of the two types of muscle and this may in turn affect post-mortem metabolism of the muscles.

In a later study (Meyer et al. 1963b), only 20 to 25% lower niacin levels were again found in dark colored muscle as compared to lighter colored muscle. There was a tendency for pigs which were physically more active to have lighter colored muscles. The ratio of oxidized to reduced niacin-containing coenzymes was positively correlated ($P < .05$) with level of ante-mortem activity and appeared to be related to muscle color. Additionally, the ratio of oxidized to reduced coenzymes appeared to be related to rate of glycolysis because of its negative correlation with pH values during the initial 2 hr post-mortem.

Minerals. Ludvigsen (1953), in his reports concerning "muscular degeneration" in hogs, found that potassium levels in the blood serum were elevated to 50 to 60 mg/100 ml in animals which had died of the disease. Normal pigs had serum potassium levels of 16 to 20 mg/100 ml. Along with the rise in serum potassium, there was a decrease in sodium. Normally, sodium content ranges between 400 to 550 mg/100 ml, however, values from pigs affected with muscular degeneration were 300 to 360 mg/100 ml. Briskey et al. (1959a), in a study of muscles of various classes, noted no difference between PSE and normal muscles in potassium content. However, PSE muscles contained significantly greater amounts of sodium ($P < .05$) than normal muscles. In a study of 60 market weight pigs, Topel (1965) observed no differences in either muscle or blood plasma levels of sodium or potassium which were related to muscle quality. Severe PSE muscle had a mean sodium and potassium content of 397 and 4217 ppm, respectively, as compared to 387 ppm sodium and 4360 ppm potassium for normal muscle (Topel, 1965).

Cassens et al. (1963) observed marked differences in zinc content between active (dark) and inactive (light) muscles of individual animals; however, there was no variation in zinc content among muscles that exhibited fast or slow glycolysis and consequently became light or dark. The trapezius (dark) muscle contained 182 ppm zinc and the longissimus dorsi (light) muscle 69 ppm.

Histochemical observations. Bodwell et al. (1965) studied the effects of post-mortem heat or cold treatments by histochemical methods. UDPG-glycogen transferase (UDPG = uridine diphosphate glucose), phosphorylase,

and branching enzyme activity were evaluated. No relationship was apparent between enzyme activity and ultimate muscle condition in muscles chilled rapidly immediately after slaughter. There was no detectable UDPG-glycogen transferase activity present at 3 hr post-mortem in muscles held at 37°C. The authors concluded that this enzyme was completely inhibited by conditions of low pH value and high muscle temperature. Heat treatment had a highly variable effect upon phosphorylase and branching enzyme activity.

Hormone Relationship to PSE Muscle

Ludvigsen (1953) cited factors which indicated the significance of hypothyroid function in the pathogenesis of "muscular degeneration" in pigs in Denmark. First, a pronounced seasonal variation in the frequency of the disease was demonstrated with a higher number of cases during the summer, when thyroid function is normally lower, and a lower frequency during the winter, when the activity of the gland is higher. Secondly, the pathological condition was produced experimentally in carcasses by blocking the thyroid gland with methyl-thiouracil. Feeding 1 g of this substance daily for 10 days prior to slaughter resulted in the development of PSE musculature. Experiments also showed that the muscle changes characteristic of "muscular degeneration" could be prevented by feeding iodinated casein containing approximately 3% free Thyroxin at a daily dose level of 2 g for 10 days starting 3 weeks prior to slaughter (Ludvigsen, 1953). The author also found an increased incidence of PSE in Danish pigs subjected to forced exercise and excitement immediately preslaughter and a 75% reduction in the incidence if a 24 hr rest period was allowed before slaughter.

In a later report (Ludvigsen, 1957), a lowered function of the adrenal cortex in the development of PSE muscle was postulated since the corticotrophin content of the anterior lobe of the pituitary was found to be lower than that of normal pigs. Moreover, Ludvigsen (1957) found that in contrast to normal pigs, pigs with "muscle degeneration" showed no increase in lactic acid level in the blood during moderate exercise. He postulated this was due to vasoconstriction in the musculature. Pretreatment with hydrocortisate increased the lactic acid output in the blood to normal levels and no symptomatic reactions occurred during exercise. The author concluded that adrenal cortical hormones play a key role in the complex of reactions governing vasodilation in skeletal muscles and suggested a possible antagonistic reaction between adrenalin and cortisone.

More recently, Briskey (1964) reported higher thyroid to adrenal gland ratios in pigs that ultimately developed PSE muscle. The larger thyroid glands also showed histological evidence of hypo-activity. Topel and Merkel (1966) studied the effects of exogenous goitrogens upon some properties of porcine muscle. Both methyl-thiouracil and Tapazole caused hypertrophy of the thyroid gland to approximately the same degree. Longissimus dorsi muscle pH, protein solubilities and non-protein nitrogen, sodium and potassium levels of the muscle were not affected by the goitrogens. However, some methyl-thiouracil treated hogs developed slightly pale, soft and watery muscles. While goitrogen treatment lowered the adrenal gland weights and plasma 17-hydroxycorticosteroid levels, the differences were not statistically significant. The authors concluded

that goitrogenically-induced hypothyroidism per se apparently had little effect upon the ultimate physical and chemical properties of porcine muscle, in disagreement with Ludvigsen (1953). However, development of PSE musculature may be the indirect result of secondary effects of methylthiouracil upon the adrenal gland (Topel and Merkel, 1966).

Following injection of iodine-131, Judge et al. (1966) found a significantly higher percentage of the plasma iodine-131 present as protein-bound iodine-131 in pigs which ultimately became pale, soft and exudative compared to pigs with normal muscle. Thyroid iodine-131 was slightly lower in the PSE group. The authors could find no differences in urinary excretion of catecholamines, 17-ketosteroids, or 17-hydroxycorticosteroids when animals were grouped according to muscle color and structure and rate of pH fall. However, among the Poland China barrows in the study, in which the muscles varied from PSE to normal, positive correlations were found between 17-ketosteroids and gluteus medius quality score ($r = .74$; $P < .05$) and between 17-hydroxycorticosteroids and the length of delay phase to onset of rigor mortis ($r = .76$; $P < .01$) and the total time to completion of rigor mortis ($r = .64$; $P < .05$). Judge et al. (1966) implied that pigs which rapidly develop the changes associated with rigor mortis in PSE muscle also have elevated blood levels of protein-bound iodine, depressed thyroid iodine uptake, and deficiencies in adrenocortical hormone production.

Histological examination of porcine adrenal glands revealed two types of material which were stained by Sudan black B: 1) fine granules and 2) large masses (Cassens et al., 1965). Of special interest was the frequent appearance of large masses of sudanophilic material localized in the zona

reticularis of adrenal cortices from Poland China animals (rapid glycolysis). The large masses of lipid were seldom seen in the glands from Chester White pigs (slow glycolysis). Although the border between the zona reticularis and zona fasciculata was not clearly defined, the large lipid masses, when present, always extended outward from the clearly defined border of the medulla. Cassens et al. (1965) did not believe this phenomenon was due to pigment accumulation since staining with hematoxylin and eosin revealed no apparent accumulation of granules or pigment. It was suggested that the presence of large lipid masses might represent a degenerative change.

Topel et al. (1967) found that plasma levels of free 17-hydroxycorticosteroids were 3.3 ug/100 ml lower in pigs which were ultimately classified as severe PSE than in pigs classified as normal. This difference approached significance ($P < .08$). A considerable range in plasma 17-hydroxycorticosteroid levels was observed among animals within each group and was attributed to the method of collection of the blood sample and the stress induced during collection. The authors also recognized that plasma levels of 17-hydroxycorticosteroids are not an accurate measure of adrenal secretory activity over a period of time but only reflect plasma levels at the time of sampling. Topel et al. (1967) stated that plasma glucocorticoid levels were associated with the ultimate physical and biochemical properties of post-mortem skeletal muscle, in agreement with Ludvigsen (1957). Topel and Merkel (1967) also reported that feeding or injection of either prednisolone or methylprednisolone in pigs produced adrenal gland atrophy and significantly lowered plasma levels of 17-hydroxycorticosteroids.

However, injection of prednisolone or methylprednisolone did not result in the development of PSE musculature in any of the animals or in fast rates of pH fall post-mortem. Prednisolone treatment caused a slight sodium retention in the muscle while methylprednisolone resulted in sodium dimunition. Neither steroid affected longissimus dorsi muscle potassium content.

Bendall and Lawrie (1962) reported on the effect of ante-mortem injection of various drugs on post-mortem changes in muscle. Injection of adrenalin 4 hours before slaughter significantly depleted muscle glycogen in rabbits and raised ultimate muscle pH. The accepted explanation for this effect is that adrenalin causes activation of phosphorylase so that glycolysis proceeds more rapidly along the anaerobic pathway to lactic acid (Cori and Illingworth, 1956). However, cortisone injection 20 hours before adrenalin administration completely protected the animal against the adrenalin effect without itself altering the normal ultimate pH or rate of chemical changes in the muscles.

Physiological Observations

Forrest (1965) reported on the effect of ante-mortem heat treatment upon heart rate, respiration rate, partial pressures of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) in the blood, and blood pH of pigs and also the relationship of these factors to post-mortem muscle quality. Heat treatment caused a sharp rise in heart rate in pigs of the Poland China breed, a significant increase in P_{CO_2}, a sharp decrease in P_{O_2} and a fall in blood pH from 7.2 to 6.8. A sharp rise followed by a fast decline similar to respiratory arrest was noted in respiratory rate. In Chester White pigs,

heat treatment resulted in a slight elevation in heart rate, a continuous increase in respiration rate, decreased P_{CO_2} and a slight increase in P_{O_2}. The *longissimus dorsi* muscles of all treated Poland Chinas were extremely PSE at 24 hr post-mortem while all Chester Whites had normal muscles. The author concluded that circulatory and respiratory difficulties leading to increased blood P_{CO_2} and decreased P_{O_2} could be major contributors to the production of PSE muscle, particularly if the animals were subjected to warm temperatures and stress immediately pre-slaughter.

Preslaughter Factors and Methods of Prevention of PSE Pork

Briskey *et al.* (1959b,c) reported that exhaustive exercise immediately before slaughter significantly decreased initial muscle glycogen and increased 24 hr pH values. The muscles from exercised pigs were dark in color, dry and firm in appearance and possessed relatively low expressible water ratios. In contrast to this response, hogs which were placed on high sucrose rations and given no exercise had muscles that were pale, soft in structure and exudative in appearance.

Sayre *et al.* (1961) placed market-weight swine in cold water (0.5°C) for 30 to 40 min to simulate an environmental change. The extreme change from a warm to cold environment decreased the initial muscle glycogen level with a resultant decrease in lactic acid concentration and increase in color intensity of the chilled muscle. However, water binding capacity of the chilled muscle was not consistently affected. The authors concluded that factors other than fresh muscle glycogen content, chilled pH and rate of glycolysis were important in determining the water binding capacity of muscle.

Increased muscle temperature and a rapid rate of post-mortem glycolysis resulted upon subjecting pigs to elevated environmental temperatures immediately before slaughter (Sayre et al., 1963c). Heat treatment produced decreased color intensity of the muscle and a marked acceleration of rigor mortis onset. Short-term excitement and exercise immediately prior to slaughter resulted in muscles with low color and texture scores and inferior water binding properties (Sayre et al., 1963b). Long- and short-term sucrose feeding elevated the muscle glycogen level at slaughter and ultimately resulted in muscle that was slightly soft and pale. Fasting 70 hours prior to slaughter lowered the initial glycogen content of muscle and also slowed pH decline and color change during post-mortem glycolysis.

Kastenschmidt et al. (1964) studied the effects of regulation of antemortem temperature upon porcine muscle quality. Treatments were 1) warm (42-45°C for 30-60 min), 2) cold (1-3°C water for 30 min), 3) warm to cold, and 4) cold followed by warm. The warm treatment was reported to induce extremely pale, soft, exudative tissue with a marked loss of protein solubility. The most desirable muscles were produced by the warm to cold treatment, the muscles being dark, dry and firm. The other two treatments had less marked effects. In a later, more comprehensive study, Kastenschmidt et al. (1965) found that mild warm air followed by cold air treatments, which were not severe enough to influence muscle temperature at slaughter, slowed the glycolytic rate and were most effective in improving muscle quality parameters.

Borchert and Briskey (1964) removed the entire loin or the ham from the carcass immediately after slaughter and immersed them in liquid nitrogen

for varying lengths of time. Liquid nitrogen treatment was quite effective in preventing the development of PSE musculature and in improving water holding capacity and subjective color and structure scores. Equilibration temperatures, either 4 or -18°C, had no effect on the results. Borchert and Briskey (1965) also reported that liquid nitrogen treatment resulted in higher sarcoplasmic and myofibrillar protein extractability and also improved the emulsifying properties of the muscle proteins.

EXPERIMENTAL METHODS

This study was divided into two separate investigations. Part I consisted of a study of the adenylic deaminase activity of porcine muscle and its relationship to rate of post-mortem pH decline and visual properties of the muscle. Fourteen market weight (200 to 220 lb) Poland China pigs, purchased from a local breeder, were included in this study. Enzymatic activity and pH fall were determined in the longissimus dorsi, gluteus medius and rectus femoris muscles since these muscles often vary considerably in quality within the same carcass. In addition, AMP and IMP levels were determined on samples obtained from the longissimus dorsi and gluteus medius muscles at slaughter. AMP and IMP were determined only on the first 8 animals slaughtered.

In addition to the determination of adenylic acid deaminase activity and AMP and IMP levels, part II of the investigation also included determination of phosphorylase activities, glycogen, inorganic phosphate, and ATP levels as related to glycolytic rate and some objective indicators of muscle quality. Twenty-two market weight Poland China pigs were again obtained from a local breeder. The animals, most of which were gilts, were divided randomly among three treatment groups. The first group, including 8 pigs, served as controls. Each of the eight pigs included in group 2 was injected intramuscularly (biceps femoris) with 5 mg of epinephrine (1:1000 solution) per 100 lb body weight 10 min prior to slaughter. The third group of 6 pigs each received a daily intramuscular (biceps femoris) injection of 100 mg of prednisolone (delta-1-hydrocortisone)

for 10 days prior to slaughter. The pigs were slaughtered 24 hr after the last prednisolone injection. Ten min prior to slaughter, the pigs in group 3 also received an intramuscular injection of epinephrine as previously described for group 2.

Slaughter, Cutting and Sampling Procedure

Animals were transported to the MSU Meat Laboratory and allowed to rest 4 to 5 hr prior to slaughter. Water was provided ad libitum but feed was withheld during this period. The pigs were electrically stunned, bled and dressed according to conventional procedures. In part I of the study, muscle samples were obtained at 10 min post-mortem from the lumbar region of the longissimus dorsi and from the gluteus medius and rectus femoris muscles of the ham. Samples were frozen in liquid nitrogen immediately after excision from the carcasses. Muscle pH was determined on samples of the longissimus dorsi at 15 min, 45 min and 1 hr post-mortem and at 30 min intervals thereafter until 3 hr post-mortem. Only the longissimus dorsi muscle was sampled in part II of the study. Muscle samples for analysis and pH determination were obtained and prepared as described in part I. After a 24 hr chill period, all carcasses were separated into wholesale cuts according to procedures outlined by the Pork Carcass Evaluation Committee of the Reciprocal Meat Conference (1952). In part II, a 1 in. thick chop was removed from the 10th rib region of the loin (longissimus dorsi muscle) for determination of color and transmission value and then was frozen for subsequent 24 hr glycogen determination.

All frozen muscle samples in part II were powdered before analysis. Powdering was performed with pre-chilled equipment in a -20°C cold room. The frozen tissue block was chipped into smaller pieces and placed in a Waring blender with an approximately equal weight of dry ice. The blender was operated at full speed until the sample was finely powdered, usually 30 to 60 sec. The dry ice was then allowed to sublime from the powdered tissue after which the powder was stored at -20°C until used for analysis.

Muscle pH

Approximately a 5 g muscle sample was homogenized for 1 min in a small Waring blender containing 25 ml of 0.005M sodium iodoacetate for the pH determinations which were made during the first 3 hr post-mortem. Twenty-five ml of deionized water was blended with a 5 g muscle sample for ultimate pH determinations at 24 hr post-mortem. All pH estimates were performed in duplicate with a Corning Model 12 expanded scale pH meter.

AMP and IMP Determinations

Extraction. Samples were not thawed prior to this extraction. In part I, 10 g of frozen tissue was weighed into a VirTis homogenizer flask (Dannert, 1966). Approximately 25 ml of distilled water was added and the sample was homogenized at maximum speed for 2 min. Following quantitative transfer to a 100 ml volumetric flask, the homogenate was boiled for 5 min. It was then cooled before bringing the volume of the extract to 100 ml with distilled water. The extract was centrifuged for 20 min

at 2000 x G after which it was filtered through S & S No. 588 filter paper. In part II, 10 g frozen powdered muscle was placed in 50 to 60 ml of boiling deionized water. The slurry was boiled for 10 min, quantitatively transferred to a 100 ml volumetric flask and allowed to cool to room temperature before being brought to volume. This extract was centrifuged for 10 min at 14,000 x G and subsequently filtered through S & S No. 588 filter paper. The clear filtrate resulting from the extractions in both parts I and II was adjusted to pH 8.0 with 1N sodium hydroxide. A 10 ml aliquot of the extract was then applied to an anion exchange column for separation of mononucleotides.

Chromatographic separation. The method of Lento et al. (1964) as modified by Macy and Bailey (1966) was used for nucleotide separation. Bio-Rad Laboratories analytical grade anion exchange resin AG 1 x 8, 200-400 mesh, was washed with distilled water to remove fines. It was then packed in a glass column, 12 mm inside diameter, to a height of 160 mm. The resin was converted to the hydroxyl form by washing the column with 3 bed volumes of 1N sodium hydroxide. Excess sodium hydroxide was washed from the column with distilled water and the resin was next converted to the formate form by washing with 3 bed volumes of 6N formic acid. Excess reagent was again washed from the column and the aliquot of muscle extract was placed on the column. The resin was washed free of ultraviolet absorbing materials with distilled water until a transmission of nearly 100% was obtained. The mononucleotides were now ready for elution. The columns were recharged after the nucleotides had been eluted according to the above procedure. Each column was recharged a maximum of 15 times.

The nucleotides were eluted by the three phase gradient system described by Macy and Bailey (1966). Three aspirator bottles were aligned one above another. The lower bottle leading to the column contained 125 ml of deionized water, the middle bottle 250 ml of 0.5N formic acid, and the upper bottle 500 ml of 0.2M sodium formate. Magnetic stirrers assured proper mixing in the lower 2 bottles during elution.

Flow rate was maintained constant at 2.5 ml per min by means of a chromatographic pump. The absorbance of the column effluent was monitored with an Isco, Model UA, ultraviolet flow densitometer at 254 mu and 10 ml fractions were collected with a Rinco fraction collector. Ultraviolet absorbing peaks were combined, their volume measured and the nucleotide concentration determined as described below.

Quantitative evaluation. The method of Lento et al. (1964), which is specific for 5'-ribonucleotides, was employed for this determination. One ml of 0.01N periodic acid was added to a 5 ml aliquot of sample and the mixture heated for exactly 3 min at 70°C in order to oxidize the ribose moiety between the 2 and 3 carbon position. Oxidation was stopped by addition of 1 ml of 0.05N sodium arsenite (Na_2HAsO_3) followed immediately by 1 ml of concentrated hydrochloric acid, and then by 1 ml of a saturated solution of 2,4-dinitrophenylhydrazine (2,4-DNPH) in 2N hydrochloric acid. After mixing, the tubes were placed in boiling water for 10 min to hydrolyze oxidation products and facilitate precipitation of the bis-hydrazone. The precipitate was collected on medium porosity fritted glass filters and washed free of unreacted 2,4-DNPH. The hydrazone was then dissolved

in acetone, diluted to volume and the absorbance of the yellow solution determined at 435 mu in a Beckman DU spectrophotometer. According to Lento et al. (1964), the nucleotide content of each fraction can be determined from a single standard, since the extinction coefficients of each 5'-nucleotide derivative formed are essentially the same. However, a standard curve using AMP was run and used for actual determinations (Figure 1).

5'-Adenylic Acid Deaminase

The enzyme was extracted from frozen muscle tissue as described by Lee (1963). Frozen tissue, which had been stored a maximum of 7 days, was cut into small pieces and homogenized for 1 min in a Waring blender with 3.5 volumes of a cold buffer solution containing 0.3M potassium chloride, 0.09M potassium dihydrogen phosphate, and 0.06M potassium monohydrogen phosphate, pH 6.5. The deaminase was extracted by efficient stirring at 3°C for 1 hr. Assay of enzymatic activity was conducted by the spectrophotometric method of Lee (1963) with modifications as suggested by Smiley et al. (1967). Reagent solutions needed for the assay were 0.15M succinate buffer, pH 6.5, and 3×10^{-3}M 5'-AMP in distilled water. For the determination of enzymatic activity, 0.1 ml of properly diluted (1:6 or 1:8 depending upon protein concentration and enzyme activity) muscle extract was added to 2.9 ml of prewarmed (30°C) reaction mixture containing 0.1 ml of AMP solution, 2.0 ml of succinate buffer and 0.8 ml of distilled water. The reaction is thus carried out at a total volume of 3 ml in the presence of 0.1M succinate and 1×10^{-4}M AMP. The rate of decrease (initial velocity) in absorbance at 265 mu was measured in a Beckman DU spectro-

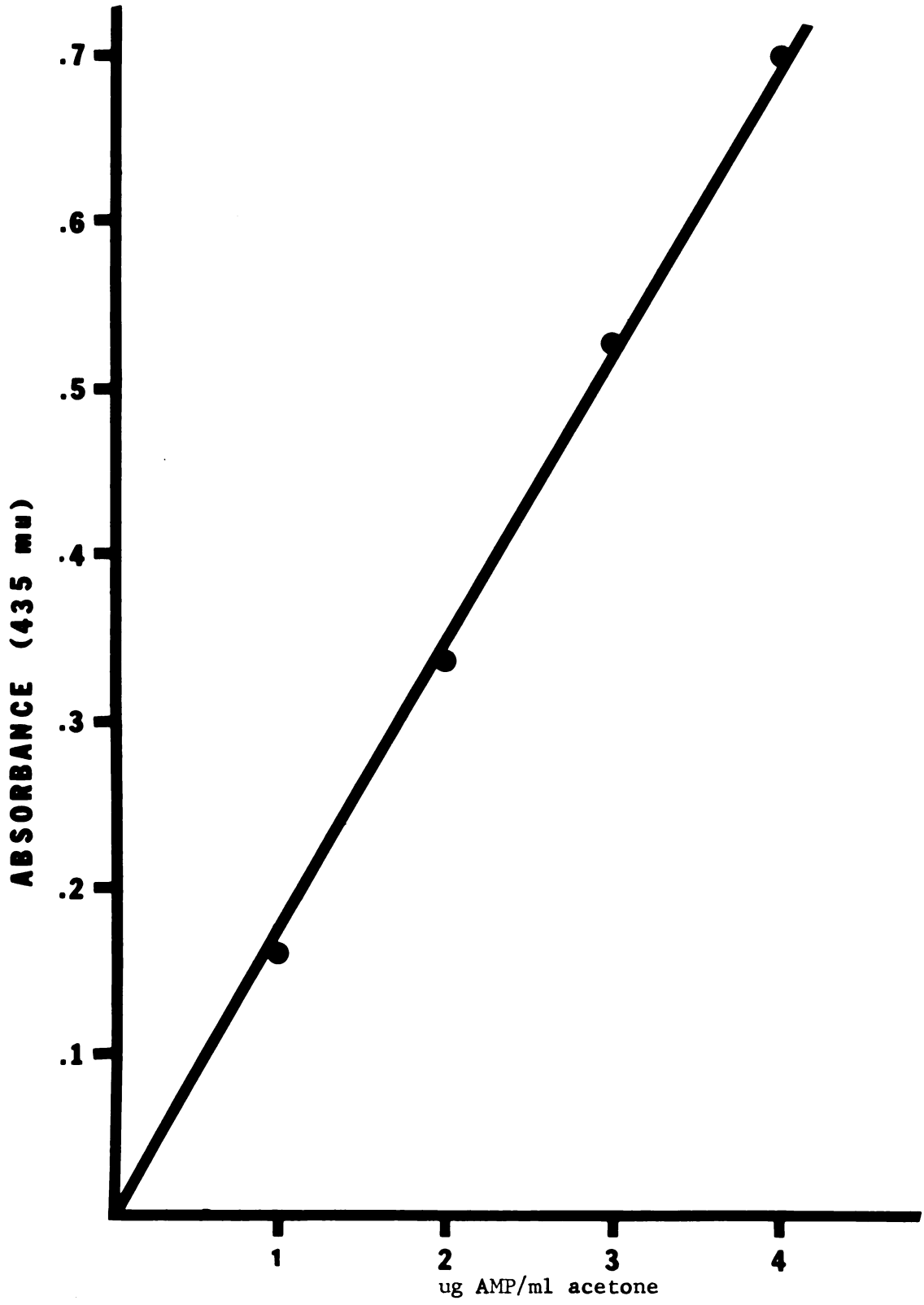

Figure 1. 5'-Adenosine monophosphate standard curve.

photometer equipped with a Gilford, Model 220, absorbance indicator. Protein concentration was determined by 280/260 mu absorbance ratio. Specific activity of the enzyme (umoles AMP deaminated/min/mg protein) was calculated according to the following formula (Smiley et al., 1967):

$$\text{Specific activity} = \frac{\text{Absorbance units decrease/min}}{(8.86)(\text{protein content in mg per assay})}$$

Phosphorylase Assay

Frozen muscle powder was extracted for phosphorylase within 12 hr post-mortem by the method of Posner et al. (1965). Powdered muscle (approximately 500 mg) was extracted at -20°C with 2 ml of a 60% glycerol solution containing 0.02M sodium fluoride, 0.005M ethylenediaminetetra-acetic acid (EDTA), 0.03M cysteine hydrochloride and 0.04M sodium-β-glycerophosphate adjusted to pH 6.8 with sodium hydroxide. The material was allowed to extract for 20 min and was then diluted to 10 ml with a cold aqueous solution of the above salts. The suspension was stirred for 10 min (2 to 4°C), centrifuged at 2000 x G for 30 min and decanted. The supernatant was immediately assayed for glycogen phosphorylase.

Phosphorylase was assayed as described by Illingworth and Cori (1953). Solutions required included: 4% glycogen, 0.064M glucose-1-phosphate (dipotassium salt), pH 6.8, and 0.064M glucose-1-phosphate containing 0.004M AMP, also pH 6.8. Two-tenths ml of 4% glycogen was added to 0.4 ml of enzyme extract (in duplicate) and the solution incubated at 30°C for 20 min. The reaction was started by adding 0.2 ml of either the glucose-1-phosphate (for phosphorylase a activity) or glucose-1-phosphate

plus AMP (for total phosphorylase activity) solution to the two samples. Final concentrations in the reaction mixture were: 0.01M sodium fluoride, 0.0025M EDTA, 0.015M cysteine, 0.02M sodium-β-glycerophosphate, 1% glycogen, 0.016M glucose-1-phosphate and 0.001M AMP (when present). At appropriate times after starting the reaction (1 min intervals with AMP, 4 min intervals without AMP), 0.2 ml aliquots were pipetted from the reaction mixture into 7 ml of dilute (approximately 0.07N) sulfuric acid. The sulfuric acid stopped the reaction but was too dilute to allow much additional hydrolysis of glucose-1-phosphate at room temperature in 60 min (Illingworth and Cori, 1953).

The amount of inorganic phosphate released was determined by a modified Fiske-Subbarow procedure (Clark, 1964) and the amount of inorganic phosphate present in the enzyme was corrected for by running blanks on the muscle extract. Protein concentration of the muscle extract was determined by 280/260 mu absorbance ratio. Results were expressed as u moles of inorganic phosphate released/min/mg protein either with AMP (total phosphorylase) or without AMP (phosphorylase a). Phosphorylase a activity was also expressed as a percentage of total phosphorylase activity.

Percent Reflectance and Munsell Value

Lightness or darkness of the longissimus dorsi muscle at 24 hr post-mortem was objectively determined using a Photovolt Photoelectric Reflection Meter, Model 610. The instrument was standardized against a magnesium oxide block using the green filter. A chop removed from the longissimus dorsi muscle was allowed to oxygenate for 30 min prior to

measuring percentage reflectance at 3 positions in the cross-section of the muscle: in the medial one-third of the muscle, in the center one-third, and in the lateral one-third. The reflectance readings from the 3 positions were averaged and converted to the equivalent Munsell value according to the conversion factors of Nickerson (1946). A Munsell value of 0 indicates pure white and a value of 10 pure black; intermediate values indicate various shades of gray.

Transmission Value

The transmission value method described by Hart (1962b) was used in this investigation as an objective measurement of muscle quality. The method is based upon the fact that PSE muscle has lower sarcoplasmic protein solubility than normal muscle. Transmission value is defined as the percentage of light transmitted through a water extract of muscle in which the proteins have been precipitated by a citric acid-phosphate buffer, pH 4.6. Since fewer water-soluble proteins are extracted from PSE muscle, a smaller quantity of proteins are in turn precipitated, the turbidity of the resulting solution is lower and the transmission value higher.

Reagents for the test included 0.2M dibasic sodium phosphate and 0.1M citric acid. The reagents were combined in the ratio of 9.35 ml sodium phosphate and 10.65 ml of citric acid solution to form a pH 4.6 buffer. Ten g of finely ground meat was placed in a centrifuge tube previously calibrated at 40 ml. Cold distilled water was added until the total volume was 40 ml and the mixture was then thoroughly stirred.

The mixture was held at 2 to 4°C for 20 hr after which it was stirred again and then centrifuged at 2000 x G for 20 min. The supernatant was filtered through S & S No. 588 filter paper. One ml of the clear filtrate was mixed with 5 ml of pH 4.6 buffer solution, temperature 20°C, in a colorimeter tube. This mixture was held at exactly 20°C for 30 min. After this incubation, the solution was mixed again by inversion of the tube and read at 600 mu in a Bausch and Lomb Spectronic 20 colorimeter against a blank containing 1 ml of muscle extract and 5 ml of distilled water. Percent transmission was recorded as the transmission value.

Glycogen Determination

Glycogen was extracted from the frozen muscle powder as described by Hansen et al. (1952). A 200 to 500 mg sample, accurately weighed, was hydrolyzed for 20 to 30 min in 3 ml of 30% potassium hydroxide in a boiling water bath. After cooling, 4 ml of 95% ethanol was added and gently mixed with the contents of the tube. The tubes were heated in boiling water until bubbles appeared in the mixture and then allowed to stand at 2 to 4°C for 3 hr to allow complete precipitation of glycogen. The tubes were next centrifuged for 15 min at 2000 x G, the supernatant decanted and the precipitated glycogen dissolved in 3 ml of distilled water. Glycogen was reprecipitated with 95% ethanol, centrifuged and drained as before. The resulting glycogen precipitate was dissolved in 3 ml distilled water in the case of determinations on 24 hr muscle or 10 to 20 ml of distilled water for determinations at 15 min post-mortem.

Glycogen was assayed by the method of Dubois et al. (1956). Two ml of glycogen solution (containing 10 to 60 ug of glycogen) was placed in a test tube and 0.5 ml of 8% phenol solution added. Five ml of concentrated sulfuric acid was then rapidly added and the contents of the tube were well mixed. After standing for 10 min, the mixture was incubated at 25 to 30°C for 20 min and then read at 490 mu in a Bausch and Lomb Spectronic 20 colorimeter. The amount of glycogen was determined from a linear standard curve constructed for pure rabbit liver glycogen (Sigma Chemical Co.) between 10 and 80 ug of glycogen.

Inorganic Phosphate

The method of Lowry and Lopez (1946) was used for inorganic phosphate estimation. The powdered muscle sample was deproteinized with cold 5% trichloroacetic acid, brought to pH 4.0 to 4.2 by addition of 4 volumes of 0.1N sodium acetate, filtered, and the filtrate diluted with pH 4 acetate buffer. To each volume of diluted extract was added 0.1 volume of 1% ascorbic acid and 0.1 volume of 1% ammonium molybdate in 0.05N sulfuric acid. Readings of color development were taken at 5 min and again at 10 min after molybdate addition at a wavelength of 700 mu. Simultaneous readings were made on a standard and a blank. If a difference was observed in the readings of the unknown at 5 and 10 min as compared to the standard, the values were extrapolated to zero time.

Adenosine Triphosphate

Muscle ATP was extracted from frozen powdered muscle with boiling distilled water. A 0.4 to 0.5 g sample was accurately weighed into the hot water and boiled for about 1 min then cooled and filtered. The filtrate was assayed for ATP by the bioluminescence method described by Strehler and Trotter (1952) and Strehler (1953). In order to prepare the enzyme system needed for the assay, 100 mg of vacuum dried firefly lanterns (Sigma Chemical Co.) were extracted with 0°C 0.1M sodium arsenate. The filtered solution from this extraction was diluted with 15 ml of deionized water and then 48 mg of magnesium sulfate were dissolved in the solution. This enzyme solution was maintained constantly at 0°C. Before assaying for ATP, 0.5 ml of enzyme solution was placed in 2 ml of distilled water and warmed to room temperature. The reaction was started by addition of 0.5 ml of unknown sample or standard to the diluted enzyme solution. Fluorescence was measured at 550 mu 30 sec after starting the reaction using a Aminco-Bowman Spectrophotofluorometer.

Statistical Analyses

The statistical methods employed in this study were outlined by Snedecor (1956). Analyses of variance, standard deviations, and standard errors were calculated for the various treatment groups. Duncan's Multiple Range test as given by Steel and Torrie (1960) was used to test for differences among means. Simple correlation coefficients were determined between various factors both within and between treatments.

RESULTS AND DISCUSSION

Part I. Adenylic Acid Deaminase in Porcine Muscle

Typical PSE porcine muscle is very pale, almost white, in color, soft in texture and extremely exudative in visual appearance. Many processing difficulties occur with PSE muscle, especially excessive loss of fluid and lower yield of processed product. PSE muscle yields a lower quality processed meat product. The development of PSE muscle is largely a direct result of extremely rapid post-mortem glycolysis and pH fall, with pH values of 5.5 and below being attained while muscle temperature is high (Briskey, 1964). This low pH is usually attained during the first hr post-mortem (Briskey and Wismer-Pedersen, 1961a). Thus, pH measurements must be taken during the first 60 min post-mortem in order to assess rate of pH fall. The combination of high temperature and low pH causes precipitation and insolubilization of the muscle proteins. The lower water holding capacity and pale color of PSE muscle are believed to be a direct result of the low pH - high temperature conditions.

The objective in part I of this study was to determine the relationship of 5-adenylic acid (AMP) deaminase activity of porcine muscle to the development of PSE musculature. AMP deaminase is responsible for the degradation of AMP to IMP. One mole of ammonia is released for each mole of AMP degraded. It is recognized that AMP influences the activity of certain glycolytic enzymes; notably phosphorylase b is able to degrade glycogen to glucose-1-phosphate in the presence of sufficient quantities

of AMP (Cori and Green, 1943). However, it has generally been accepted that AMP concentration is much too low in resting muscle to activate phosphorylase b (White et al., 1964). Secondly phosphofructokinase activity is increased by AMP as reported by Passonneau and Lowry (1962). It would be possible, therefore, that adenylic acid deaminase activity of porcine muscle might indirectly affect the rate of glycolysis and thus have some bearing upon ultimate muscle properties. In this study, deaminase activity was determined in three muscles, specifically chosen because of their varying susceptibility to the PSE condition. The gluteus medius muscle of the ham is readily subject to development of PSE muscle morphology, the longissimus dorsi is somewhat intermediate in susceptibility, while the rectus femoris of the ham is quite resistant. The levels of AMP and IMP present in the longissimus dorsi and gluteus medius muscles immediately after slaughter were also determined in addition to deaminase activity.

An example of the elution pattern obtained upon separation of nucleotides on the anion-exchange column is shown in Figure 2. Although the method of separation is well documented in the literature, the eluted peaks were identified by applying known compounds to the column. Cytidine monophosphate was the first peak eluted, followed by AMP and IMP. Uridine monophosphate, when present, was eluted from the column between AMP and IMP. Recovery of AMP and IMP from the column was checked by chromatographing known amounts of the two nucleotides. Recovery values ranged between 95 and 105%.

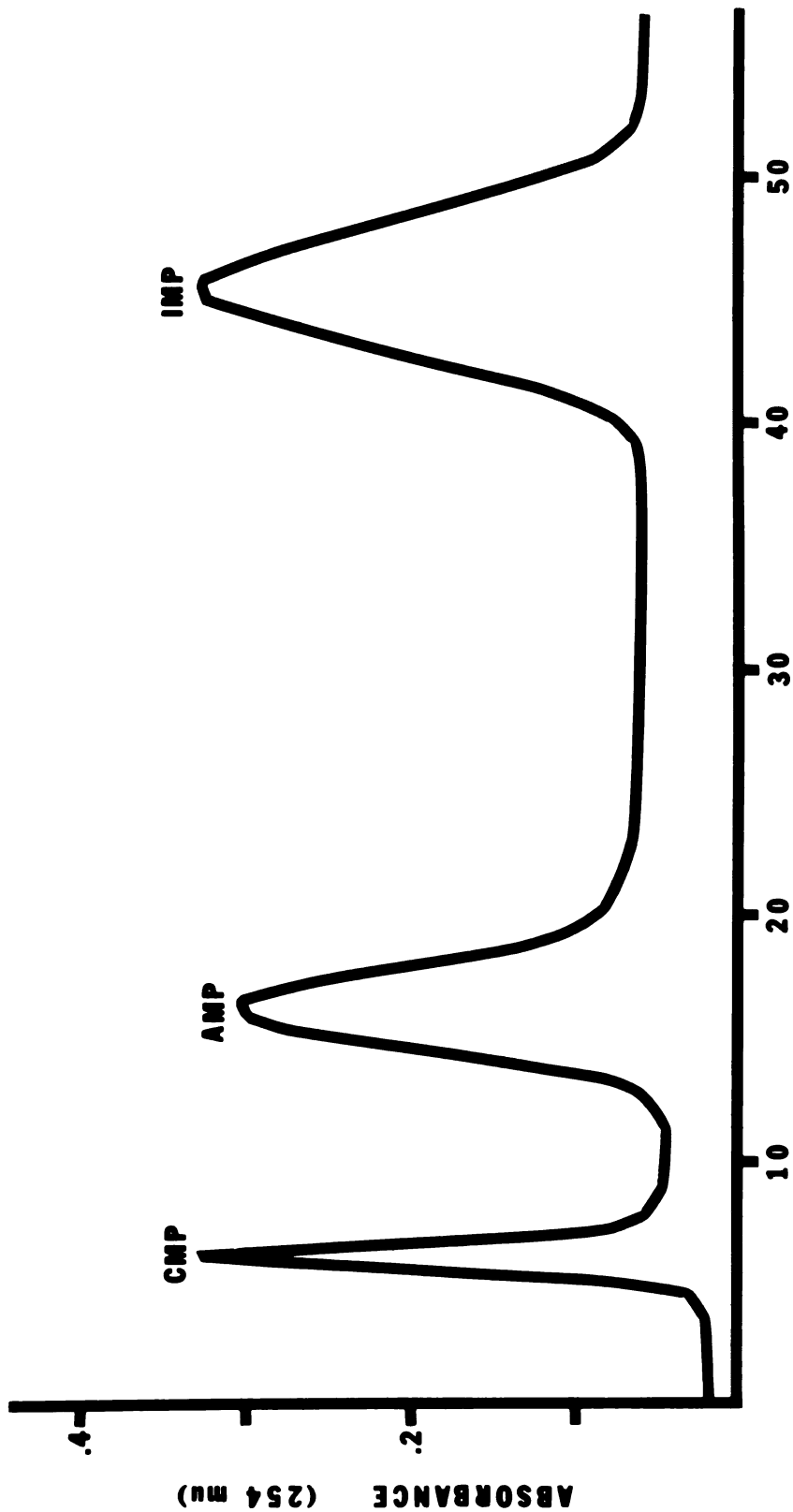

Figure 2. Chromatogram of nucleotides extracted from muscle and separated by anion-exchange chromatography.

As shown in Table 1, there were no significant differences in adenylic acid deaminase activity among the three porcine muscles studied. It is interesting to note, however, that the mean activity of the rectus femoris muscle is slightly lower than that observed in the longissimus dorsi or gluteus medius.

Table 1. Means and standard error of the means of adenylic acid deaminase activity, AMP, and IMP in three porcine muscles.

Muscle	Adenylic acid deaminase[1]		AMP[2]		IMP[2]	
	Mean	Std. error	Mean	Std. error	Mean	Std. error
longissimus dorsi	.031	.001	.60	.11	6.15	.42
gluteus medius	.031	.002	.50	.06	6.03	.14
rectus femoris	.027	.002	-	-	--	-

[1]Adenylic acid deaminase activity is expressed as umoles AMP deaminated/min/mg protein.
[2]umoles/g fresh muscle.

The levels of AMP and IMP at 15 min post-mortem (Table 1) were not statistically different between longissimus dorsi and gluteus medius muscles. Few data concerning porcine muscle nucleotide levels are available in the literature and those values which have been reported were for muscle which had been held for varying lengths of time post-mortem. The nucleotide levels of such muscle would undoubtedly vary greatly due to enzyme degradation of nucleotides. For example, at 48 hr post-mortem, Dannert (1966) found IMP concentrations of 3.12, 3.23, and 2.98 umoles/g in the biceps femoris, longissimus dorsi and semimembranosus porcine muscles, respectively, and AMP levels of 0.90, 0.33, and 0.33 umoles/g.

The IMP levels found by Dannert (1966) are substantially lower than those found at 15 min post-mortem in the present investigation.

It has been shown by several researchers (Briskey and Wismer-Pedersen, 1961a; Bendall and Wismer-Pedersen, 1962; Briskey et al., 1962; McLoughlin, 1963) that an abnormally rapid post-mortem glycolytic rate is among the causative factors in development of PSE musculature. As a result of rapid glycolysis, pH drop is also very rapid during the initial post-mortem period (1/2 to 1 hr). In this study, glycolytic rate was assessed by following rate of pH decrease in the musculature. In order to evaluate the relationship between PSE muscle development and adenylic acid deaminase activity, simple correlation coefficients were calculated between enzyme activity and muscle pH at 15 and 45 min post-mortem (Table 2). Positive correlations were obtained between adenylic acid deaminase activity and muscle pH in the three muscles studied. However, only the correlation coefficients between deaminase activity and pH at 45 min ($r = .71$) in the gluteus medius and enzyme activity and pH at 15 min ($r = .56$) in the rectus femoris muscle were statistically significant ($P < .05$).

Simple correlation coefficients between muscle pH and AMP levels revealed no apparent relationship since all of the correlation coefficients were low. This would seem to be in conflict with the relationship observed between deaminase activity and pH decline. The reason for this difference is not evident from these data. IMP concentration was negatively correlated with pH in all but one case in which a low positive correlation was obtained (IMP and 15 min pH in the longissimus dorsi muscle). This negative relationship was most pronounced in the gluteus medius muscle with the correlation between IMP and 45 min pH being significant ($r = -.84$, $P < .05$).

Table 2. Simple correlation coefficients between pH, adenylic acid deaminase activity, and AMP and IMP levels in three porcine muscles.

	pH 15 min	pH 45 min
Longissimus dorsi muscle		
Adenylic acid deaminase	.29	.41
AMP	.23	.00
IMP	.11	-.44
Gluteus medius muscle		
Adenylic acid deaminase	.48	.71*
AMP	.17	.12
IMP	-.65	-.84*
Rectus femoris muscle		
Adenylic acid deaminase	.56*	.43

*Significant ($P < .05$)

Deficiencies in adenylic acid deaminase activity in skeletal muscle have been reported in certain pathological conditions. Pennington (1961) reported that 5'-adenylic acid deaminase activity was nearly 3 times lower in skeletal muscles of dystrophic mice than in those from normal mice. Engel *et al*. (1964) found a markedly lower activity of this enzyme in skeletal muscles of human patients suffering from periodic hypokalemic paralysis. Activity differences of this magnitude were not observed in

the present investigation. None of the animals used in this part of the study yielded carcasses with severe PSE musculature. However, a wide range of muscles qualities and rates of pH fall was present among the pigs in this study. Much greater animal variation in adenylic acid deaminase activity should have been noted if the enzyme were involved in development of PSE muscle. The absence of high positive relationships between rate of pH fall and both deaminase activity and AMP concentration in the muscle supports the conclusion that adenylic acid deaminase activity is not directly involved in the development of PSE porcine musculature.

Part II. Observations on Control, Epinephrine and Prednisolone Injected Pigs

The objective in the second part of this study was to determine the effects of epinephrine injection immediately pre-slaughter upon some physical and biochemical properties of porcine muscle. Ante-mortem stress and excitement have been cited as causative factors in the development of PSE muscle (Briskey, 1964) and it was hoped to simulate stress conditions by epinephrine injection. Epinephrine was also shown to activate phosphorylase by initiation of the b to a conversion (Cori and Illingworth, 1956; Danforth et al., 1962). It was thought that epinephrine injection might thus accelerate the rate of post-mortem glycolysis in porcine muscle and possibly increase the incidence of PSE development.

The effects of epinephrine injection were also studied in pigs which had been injected with large doses of prednisolone in an attempt to suppress adrenal cortical secretion. It was of interest to study the

effects of epinephrine upon porcine muscle properties by supressing adrenocortical secretion since the latter has been implicated in the etiology of PSE musculature as well as stress conditions (Ludvigsen, 1957; Judge et al., 1966; Cassens et al., 1965; Topel et al., 1967). The effect of prednisolone injection upon adrenal gland weight and plasma glucocorticoid level was not evaluated in this study; however, Topel (1965) reported that prednisolone injected pigs, at the dosage used in the present study, had a 25 to 29% decrease in adrenal gland weight and an 8.0 ug/100 ml decrease in plasma 17-hydroxycorticosteroids. Normal porcine plasma concentration of 17-hydroxycorticosteroids was reported to be 21.8 ± 6.4 ug/100 ml. Topel (1965) has shown that muscle from prednisolone injected pigs has a different post-mortem pH pattern than muscle from non-injected pigs. The results for control, epinephrine and prednisolone plus epinephrine injected pigs are shown in Tables 3 through 8 and in Figure 3.

Muscle pH. Ultimate muscle pH (24 hr post-mortem) was not significantly affected by either epinephrine or prednisolone plus epinephrine treatment (Table 3). Hedrick et al. (1957) reported that injection of epinephrine at 24 hr and 12 hr prior to slaughter significantly elevated the ultimate pH of bovine muscle. Epinephrine injection 4 hr pre-slaughter significantly raised the ultimate pH of rabbit muscle (Bendall and Lawrie, 1962). The explanation for the elevated ultimate pH was that muscle glycogen was depleted ante-mortem by epinephrine injection thus decreasing the quantity of lactic acid produced during anaerobic, post-mortem glycolysis. Since ultimate pH was not affected by epinephrine treatment in the present study, it indicates that muscle glycogen was not significantly depleted by the 10 min ante-mortem injection.

Rate of post-mortem pH decline was not affected by epinephrine injection. Muscle pH values did not differ significantly between control and treated pigs either at 15 min or at 45 min after exsanguination. However, Bendall and Lawrie (1962) found faster rates of pH fall in rabbits injected with epinephrine 4 hr ante-mortem, even though ultimate pH was higher in the muscles of treated rabbits.

The rate of pH fall was unaffected by exogenous epinephrine in the pigs injected with 100 mg prednisolone for 10 days prior to slaughter. Hypoadrenocorticoidism is characteristic of an animal after prolonged prednisolone administration as shown by Topel (1965), with resultant adrenal atrophy and lower plasma 17-hydroxycorticosteroids. Glucocorticoids play an important role in the ability of an animal to withstand stress (Guyton, 1961). Bendall and Lawrie (1962) showed that pre-injection of cortisone in rabbits (24 hr ante-mortem) protected against the glycogen wasting effect of exogenous epinephrine (4 hr ante-mortem) and restored a normal rate of post-mortem pH fall and normal ultimate pH. It was expected that simulated stress in the present experiment (epinephrine injection) might accelerate the post-mortem glycolytic rate in prednisolone injected animals. It was noted that after several days of prednisolone injection, the pigs were less active physically and were easily exhausted by a small amount of exercise.

Munsell value and transmission value. Munsell value did not differ significantly between control, epinephrine, or prednisolone plus epinephrine treated pigs (Table 3). Higher values indicate that the muscle

was paler in color. However, it is of interest that the mean value for epinephrine injected pigs was slightly higher than controls while the value for prednisolone treated pigs was lower than controls. It is not surprising that significant differences were not observed for Munsell value since wide variation existed within groups. Hedrick et al. (1957) reported that darker colored bovine muscles resulted from 12 or 24 hr ante-mortem epinephrine injection and significantly darker colored porcine muscles were also observed (Hedrick et al., 1964) following 24 hr ante-mortem administration of epinephrine.

Simple correlation coefficients were calculated between Munsell value and pH at various times post-mortem (Table 4). Munsell value was negatively correlated with pH at 15 min post-mortem ($r = -.69$, $P < .01$) and at 45 min post-mortem ($r = -.70$, $P < .01$). This indicates that color intensity of porcine muscle was related to the rate of post-mortem pH decline in agreement with other workers (Wismer-Pedersen, 1959; Briskey and Wismer-Pedersen, 1961a; Sayre et al., 1963c). Muscle color was positively correlated with muscle protein extractability as shown by the correlation ($r = .78$, $P < .01$) between Munsell value and transmission value.

As shown in Table 3, transmission value was not significantly affected by epinephrine or prednisolone plus epinephrine injection (high transmission value indicates muscle with low protein extractability). Although transmission value for prednisolone plus epinephrine treated pigs was substantially lower than for the other treatments, the difference was not statistically significant. As expected, transmission value was highly

negatively related to rate of pH decline. Correlation coefficients between transmission value and pH at 15 and 45 min post-mortem were -.76 and -.88, respectively (Table 4). This indicates the important relationship between rate of pH fall and porcine muscle quality. Hart (1962b) demonstrated that transmission value was very high in PSE (low quality) muscle and quite low in normal muscle.

Table 3. Means and standard error of the means for pH, Munsell value, and transmission value in control, epinephrine, and prednisolone plus epinephrine treated pigs.

	Control	Epinephrine	Prednisolone + epinephrine	Standard error
pH - 15 min	6.35	6.25	6.29	.09
pH - 45 min	5.93	5.85	5.98	.17
pH - 24 hr	5.45	5.48	5.42	.02
Munsell value	5.58	5.70	5.43	.14
Transmission value	50.8	53.5	41.4	8.7

Table 4. Simple correlation coefficients between Munsell value, transmission value and pH of porcine muscle.

	pH 15 min	pH 45 min	Munsell value
Munsell value	-.69**	-.70**	
Transmission value	-.76**	-.88**	.78**

*Significant ($P < .05$)
**Significant ($P < .01$)

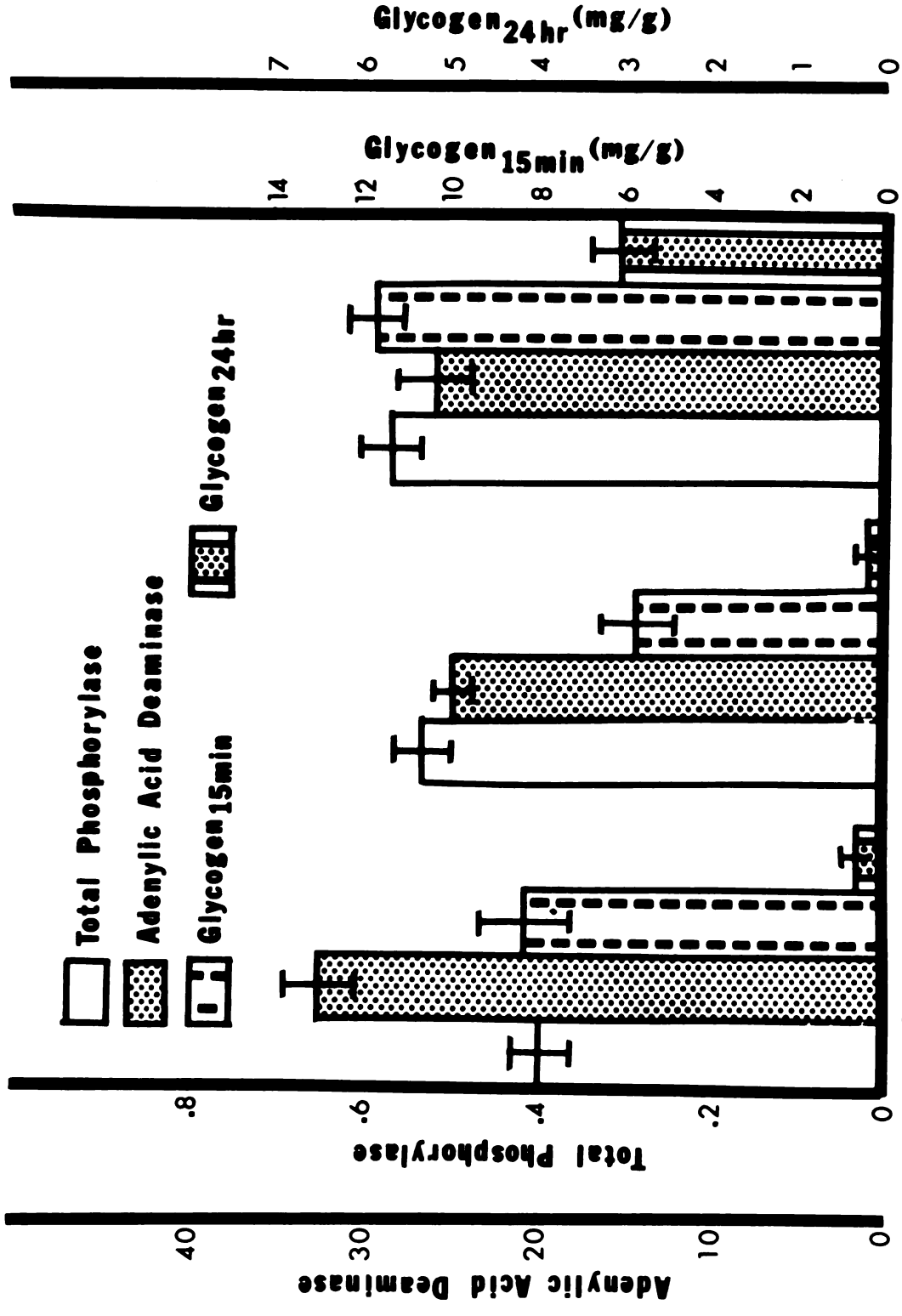

Figure 3. Total phosphorylase, adenylic acid deaminase and 15 min and 24 hr glycogen in control, epinephrine, and prednisolone plus epinephrine injected pigs.
Phosphorylase activity = umoles Pi released/min/mg protein
Adenylic acid deaminase activity = umoles AMP deaminated/min/mg protein

Phosphorylase. Total phosphorylase activity was significantly increased by epinephrine injection (Table 5 and Figure 3). The activity of this enzyme in the muscle of control pigs was .40 umoles Pi released /min/mg protein compared with .53 for epinephrine injected pigs and .57 for prednisolone plus epinephrine treated pigs. However, the increased total phosphorylase activity in epinephrine treated pigs could not be entirely accounted for by higher phosphorylase a activity. This may have been due to the fact that phosphorylase a is more active in the presence of AMP (used for total phosphorylase assay) than in the absence of AMP as explained by Cori (1956), thus causing total phosphorylase to appear greater. Also, since approximately 10 min elapsed between bleeding of the animal and excision of the muscle sample, considerable breakdown of phosphorylase a by phosphorylase phosphatase could have occurred. Posner et al. (1965) reported 2 to 4 fold increases in the ratio of phosphorylase activity without AMP to activity with AMP in frog and rat muscles 12 min after epinephrine injection. In the present study, phosphorylase a activity was slightly higher in epinephrine treated pigs. The difference between controls and prednisolone plus epinephrine treated pigs approached significance ($P < .10$). Phosphorylase a activity, expressed as a percentage of total phosphorylase activity did not differ significantly between treatments (Table 5).

Simple correlation coefficients between phosphorylase activity and pH values, Munsell value and transmission value are presented in Table 6. Low, nonsignificant correlations were obtained between total phosphorylase activity and pH at either 15 ($r = -.05$) or 45 min ($r = .05$) post-mortem

Table 5. Means and standard error of the means for total phosphorylase, phosphorylase *a* and % phosphorylase *a* in muscle of control, epinephrine, and prednisolone plus epinephrine treated pigs.[1]

	Control	Epinephrine	Prednisolone + epinephrine	Standard error
Total phosphorylase activity[2]	.40[a]	.53[b]	.57[b]	.03
Phosphorylase *a* acitivity[2]	.03	.05	.07	.01
% phosphorylase *a*	7.7	10.2	12.0	2.4

[1]Means with different superscripts are significant ($P < .05$).
[2]Phosphorylase activity = umoles Pi released/min/mg protein.

Table 6. Simple correlation coefficients between phosphorylase activity and pH, transmission value and Munsell value of porcine muscle.

	Total phosphorylase	Phosphorylase *a*	% phosphorylase *a*
pH - 15 min	-.05	.20	.22
pH - 45 min	.05	.28	.30
Munsell value	-.05	-.22	-.22
Transmission value	-.05	-.31	-.33

(rate of pH fall). This is in disagreement with Wismer-Pedersen (1959) but agrees with the results of Sayre *et al.* (1963b) who found no relationship between total phosphorylase and rate of pH decline. Even though phosphorylase *a* activity and % phosphorylase *a* were slightly associated pH at 15 and 45 min post-mortem, the correlations did not approach significance ($P < .05$). In addition, low relationships were observed between Munsell value and transmission value and the various measures of phos-

phorylase activity. These results tend to negate any postulation that some abnormality in the phosphorylase system is responsible for development of PSE muscle.

Adenylic acid deaminase, AMP and IMP. Specific activity of 5'-adenylic acid deaminase (expressed as umoles AMP deaminated/min/mg protein) was significantly lower ($P < .05$) in the muscle of epinephrine (.025) and prednisolone plus epinephrine (.026) treated pigs than in control animals (.032) as shown in Table 7. However, differences between treatment groups in AMP and IMP concentrations were not statistically significant. Moreover, correlation analysis revealed that neither AMP nor IMP level was highly related to 5'-adenylic acid deaminase activity. The reason for lower adenylic acid deaminase activity in muscle from epinephrine treated pigs is not known. Low positive correlation coefficients were found between adenylic acid deaminase activity and the rate of post-mortem pH fall in agreement with results of part I of this study. The values were $r = .26$ and $r = .09$ between enzyme activity and pH at 15 and 45 min post-mortem, respectively (Table 8).

As in part I of this study, AMP level was not highly related to muscle pH at either 15 min or 45 min post-mortem, $r = .24$ and $r = .13$, respectively. In contrast, IMP level was very highly but negatively correlated with rate of pH fall, the values being $r = -.94$ at 15 min post-mortem and $r = -.93$ at 45 min. This almost linear, inverse relationship indicates that in porcine muscle with very rapid post-mortem glycolysis, there would also be a very rapid liberation of ammonia, 1 mole of ammonia

being released for each mole of IMP formed. Briskey and Wismer-Pedersen (1961a) reported that liberation of ammonia from PSE muscle was complete at 2 hr post-mortem while normal muscle required 8 to 10 hr.

Table 7. Means and standard error of the means for adenylic acid deaminase, AMP, IMP, and glycogen in control, epinephrine, and prednisolone plus epinephrine treated pigs.[1]

	Control	Epinephrine	Prednisolone + epinephrine	Standard error
Adenylic acid deaminase activity[2]	.032[a]	.025[b]	.026[b]	.002
AMP[3]	1.82	1.72	2.10	.16
IMP[3]	2.53	2.74	2.54	.46
Glycogen - 15 min[4]	8.27[a]	5.85[a]	11.66[b]	.87
Glycogen - 24 hr[4]	.34[a]	.21[a]	3.14[b]	.18

[1]Means with different superscripts differ significantly ($P < .05$).
[2]Adenylic acid deaminase activity = umole AMP deaminated/min/mg protein.
[3]umole/g fresh muscle.
[4]mg/g fresh muscle.

Table 8. Simple correlation coefficients between pH, adenylic acid deaminase activity, and AMP and IMP concentrations of porcine muscle.

	pH 15 min	pH 45 min
Adenylic acid deaminase	.26	.09
AMP	.24	.13
IMP	-.94**	-.93**

**Significant ($P < .01$)

The AMP and IMP levels found in this part of the study differ substantially from those found in part I. In part II, AMP levels were 2 to 3 times higher and IMP concentrations approximately one-half of that reported in part I. These differences were due to the slightly different extraction procedure used in part II, in which approximately 5 volumes of boiling water was added directly to the frogen muscle powder. This resulted in almost instantaneous coagulation of the muscle proteins and much more rapid inactivation of the enzyme systems responsible for degradation of AMP and IMP. The extraction method of Dannert (1966) used in part I involved homogenization of frozen tissue with water and allowed 2 to 3 min before heat coagulation of protein, during which time considerable change in nucleotide levels could conceivably take place due to enzyme degradation. Therefore, the AMP and IMP concentrations in part II more nearly reflect actual muscle concentrations.

The mean AMP concentrations observed in this study were 1.82 umole/g of muscle in controls, 1.72 umole/g in epinephrine treated, and 2.10 umole/g in prednisolone plus epinephrine treated pigs. Cori et al. (1943) and Cori (1956) have reported that rabbit muscle phosphorylase b is activated 1/2 maximally in the presence of 1 x 10^{-5}M AMP. The concentrations reported above for porcine muscle are substantially greater than the amount of AMP needed for 1/2 maximal activation of phosphorylase b.

Morgan and Parmeggiani (1963) observed a 20 fold increase in rate of glycogen breakdown by rat heart muscle phosphorylase b when the reaction was carried out in .6 mM AMP, 4 mM ATP and 10 mM inorganic phosphate (anaerobic levels) as opposed to conducting the reaction in .15 mM AMP,

8 mM ATP, and 1 mM inorganic phosphate (aerobic levels). They stated that phosphorylase b activation would appear to be an important factor in the rapid rate of glycogenolysis under anaerobic conditions.

Based upon the AMP levels found in porcine muscle, it would therefore be possible for phosphorylase b to be active during post-mortem glycolysis. However, the AMP requirements of porcine muscle phosphorylase b may possibly be quite different from those reported for rabbit or rat muscle phosphorylase.

Glycogen. Prednisolone plus epinephrine treated pigs had significantly ($P < .05$) greater amounts of muscle glycogen at 15 min post-mortem and at 24 hr post-mortem (Table 7 and Figure 3), than either control or epinephrine treated pigs. The average 15 min muscle glycogen level in epinephrine treated pigs was lower than that for controls, however, the difference was not statistically significant ($P < .05$). Lower muscle glycogen at 15 min post-mortem in the epinephrine treated group was no doubt a reflection of the higher total phosphorylase activity found in this treatment as compared to the control pigs. Yet, rate of pH fall was not different between control and epinephrine treated pigs.

The fact that 15 min and 24 hr glycogen was higher in prednisolone treated animals and that initial rate of pH decline was not altered by this treatment indicates that the muscle glycogen reserves were higher in prednisolone injected pigs. The reason for this action of prednisolone is not known. As stated previously, prednisolone injected pigs were less active and more easily exhausted after the 5th or 6th injection.

Limited information is available concerning the effects of adrenal steroids upon post-mortem muscle properties. Faludi et al. (1964) reported loss of weight and muscle atrophy in dogs injected with large doses of prednisolone and methyl prednisolone for prolonged periods. The dogs were also lethargic and quite weak after several days of injection. Other authors have reported similar effects in rabbits following large dosages of cortisone (Ellis, 1956; Germuth et al., 1951).

One important function of the glucocorticoids is to enhance gluconeogenesis (Guyton, 1961; Zarrow et al., 1964). Along with increased gluconeogenesis, there is greater storage of glycogen in the liver but not in muscle. This illustrates that there is a basic difference in the effects of glucocorticoids upon liver glycogen and cellular glycogen elsewhere in the body. As stated by Zarrow et al. (1964), glucocorticoids are believed to interfere with glucose utilization at the cellular level by inhibition of the hexokinase reaction.

These data indicate that the incidence of PSE muscle was no greater in either epinephrine injected or prednisolone plus epinephrine injected pigs than in controls. Although total phosphorylase activity was increased by epinephrine administration, rate of post-mortem pH fall and frequency of PSE muscle were essentially unaffected.

Observations on Porcine Muscle With Fast and Slow Rates of Post-Mortem Glycolysis

Since variation in post-mortem pH fall existed among the control pigs in this study, they were divided into two groups on the basis of

rate of pH decline. Those with 45 min pH < 5.65 were designated as fast glycolysis while those with 45 min pH > 5.85 were designated slow glycolysis. Only 1 of the pigs in the slow group had 45 min pH below 6.0, the others being greater than 6.1. The data obtained on the slow and fast groups were subjected to analysis of variance, in order to observe any differences between the two groups.

The striking difference in rate of pH fall between the two muscle types is shown in Figure 4. At 3 hr post-mortem, pH of the slow group had fallen to 5.80 while the fast group had reached pH 5.46 at the end of 1 hr. The difference in pH between the two groups was already evident at 15 min post-mortem, when the first determination was made and became more pronounced at 45 min post-mortem (Table 9). However, the ultimate pH (24 hr) did not differ significantly between muscles with rapid or slow pH fall.

Table 9. Means and standard error of the means for pH, Munsell value and transmission value in porcine muscle with fast and slow rates of post-mortem glycolysis.[1]

	Fast	Slow	Standard error
pH - 15 min	6.18[a]	6.52[b]	.09
pH - 45 min	5.52[a]	6.33[b]	.14
pH - 24 hr	5.46	5.44	.02
Munsell value	5.78[a]	5.37[b]	.10
Transmission value	68.9[a]	32.8[b]	6.8

[1]Means with different superscripts differ significantly ($P < .05$).

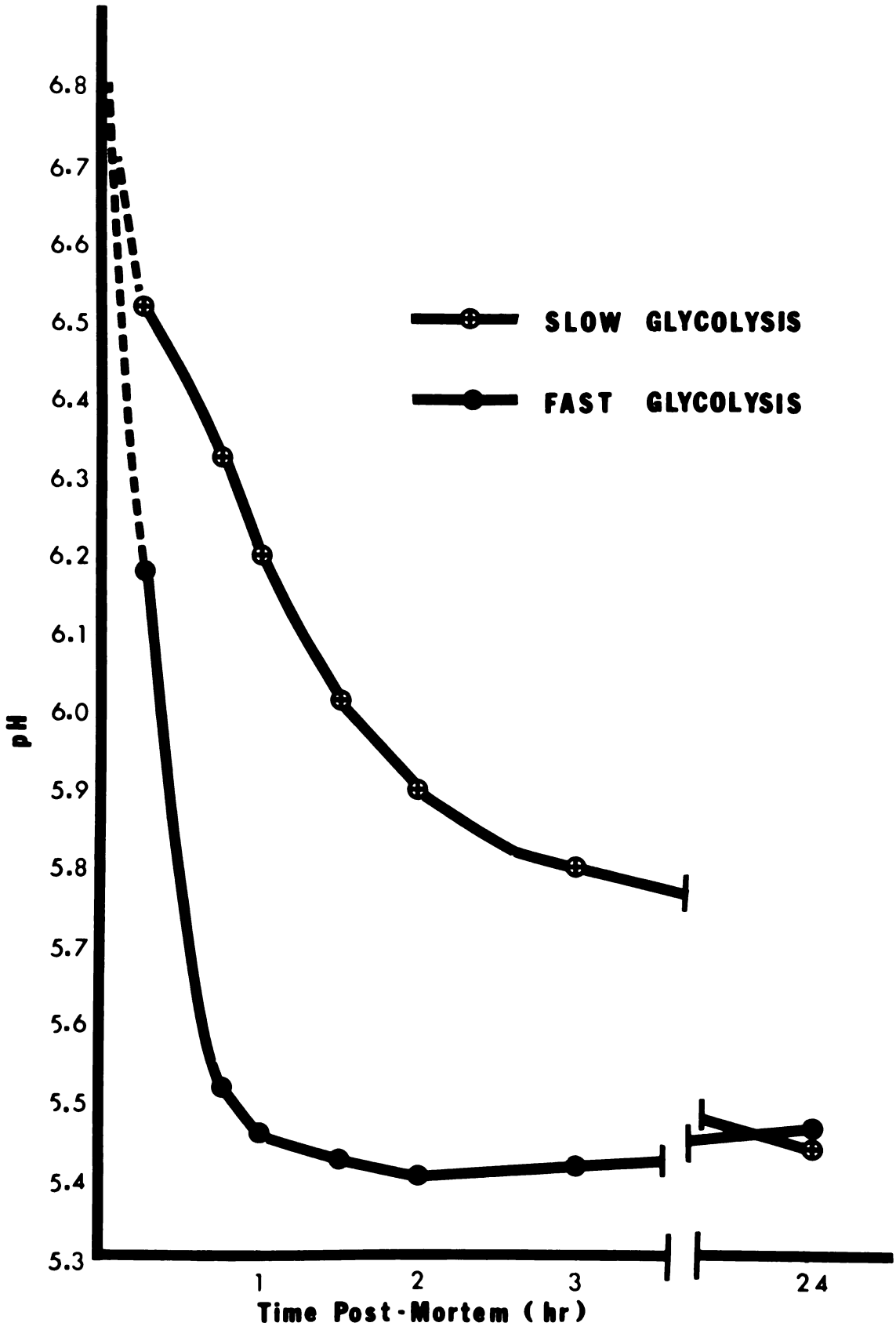

Figure 4. Rate of pH fall in muscles with slow and fast glycolysis.

Briskey (1963) described 6 different types of post-mortem pH pattern in porcine muscle. Two very gradual types of pH fall were found, one of which resulted in ultimate pH of 6.0 to 6.5 and the other an ultimate pH of 5.7 to 6.0. At the other extreme, a rapid decrease to a pH of 5.1 to 5.4 at 1/2 to 1 1/2 hr and the retention of this low pH or a slight subsequent elevation to an ultimate pH 5.3 to 5.4 was also reported (Briskey, 1963). This latter pattern corresponds to the fast group observed in the present study; however, the very gradual types of pH decline were not observed.

Munsell value and Transmission value. Average Munsell value and transmission value for muscles with slow and fast rates of pH fall are shown in Figure 5. Muscles with fast glycolysis ultimately became significantly lighter colored as indicated by the higher Munsell value ($P < .03$). This group of muscles also possessed a significantly higher transmission value indicative of some PSE muscle development. Transmission value of the slow group was 32.8 versus 68.9 for the fast pH decline group (Table 9). Hart (1962b) stated that normal high quality muscle has a transmission value of 0 to 20 while severe PSE muscle averages 80 to 100.

In agreement with results reported earlier, Munsell value and transmission value were negatively related to rate of pH fall (Table 10). Although only one of the correlation coefficients was statistically significant, but they were all approaching significance at the 5% level. Munsell value and transmission value were again positively related but not quite as highly as observed previously. Muscle color and morphology

were not subjectively scored as described by Topel (1965), however a close relationship was apparent between the transmission value and the visual appearance of the muscle. Muscle which was visually classified as PSE always had a high transmission value.

Table 10. Simple correlation coefficients between pH, Munsell value, and transmission value in porcine muscle with slow and fast rates of post-mortem glycolysis.[1]

	pH 15 min	pH 45 min	Munsell value	Transmission value
pH - 15 min	1.0			
pH - 45 min	.93	1.0		
Munsell value	-.69	-.63	1.0	
Transmission value	-.65	-.83	.58	1.0

[1] $r > .71$ are significant ($P < .05$)

These results further substantiate the postulation that rapid rates of glycolysis and the concomitant accelerated pH fall are closely related to the ultimate quality of porcine muscle (Wismer-Pedersen, 1959; Briskey and Wismer-Pedersen, 1961a,b; Bendall and Wismer-Pedersen, 1962).

Phosphorylase and adenylic acid deaminase. Total phosphorylase and phosphorylase a activities of muscle with slow and fast rates of pH decline are shown in Table 11 and Figure 5. Muscles in the slow group had a slightly lower average value for total phosphorylase, however, there was rather large variation between animals and thus the differences were not statistically significant. Phosphorylase a activity was higher in the slow group but a large standard error was also present and the differences

were not significant. It is concluded from these results that phosphorylase activity, as assayed in this study, is not related to the rapid glycolytic rate often observed in porcine muscle or to the development of PSE muscle.

There was no significant difference in adenylic acid deaminase activity between the muscle groups possessing fast or slow pH decline.

Table 11. Means and standard error of the means for total phosphorylase, phosphorylase *a*, % phosphorylase *a*, and adenylic acid deaminase in porcine muscle with fast and slow rates of post-mortem glycolysis.[1]

	Fast	Slow	Standard error
Total phosphorylase activity[2]	.41	.38	.04
Phosphorylase *a* activity[2]	.02	.04	.02
% phosphorylase *a*	5.85	9.52	2.65
Adenylic acid deaminase activity[3]	.033	.031	.004

[1]Means with different superscripts are different significantly ($P < .05$).
[2]Phosphorylase activity = u mole P_i released/min/mg protein.
[3]Adenylic acid deaminase activity = u mole AMP deaminated/min/mg protein.

Glycogen. Glycogen content of the longissimus dorsi muscle at 15 min and 24 hr is shown in Table 12 and Figure 6. Fifteen min glycogen was significantly lower ($P < .05$) in muscles with rapid rates of glycolysis (6.27 mg/g) compared to muscles with slow glycolysis (10.28 mg/g). Muscle pH at 15 min post-mortem was 6.18 in the slow group versus 6.52 in the fast group. Thus, the difference in glycogen is probably largely due to more rapid degradation as reflected in the lower initial pH rather than to differences in glycogen reserves at the time of death. There was no

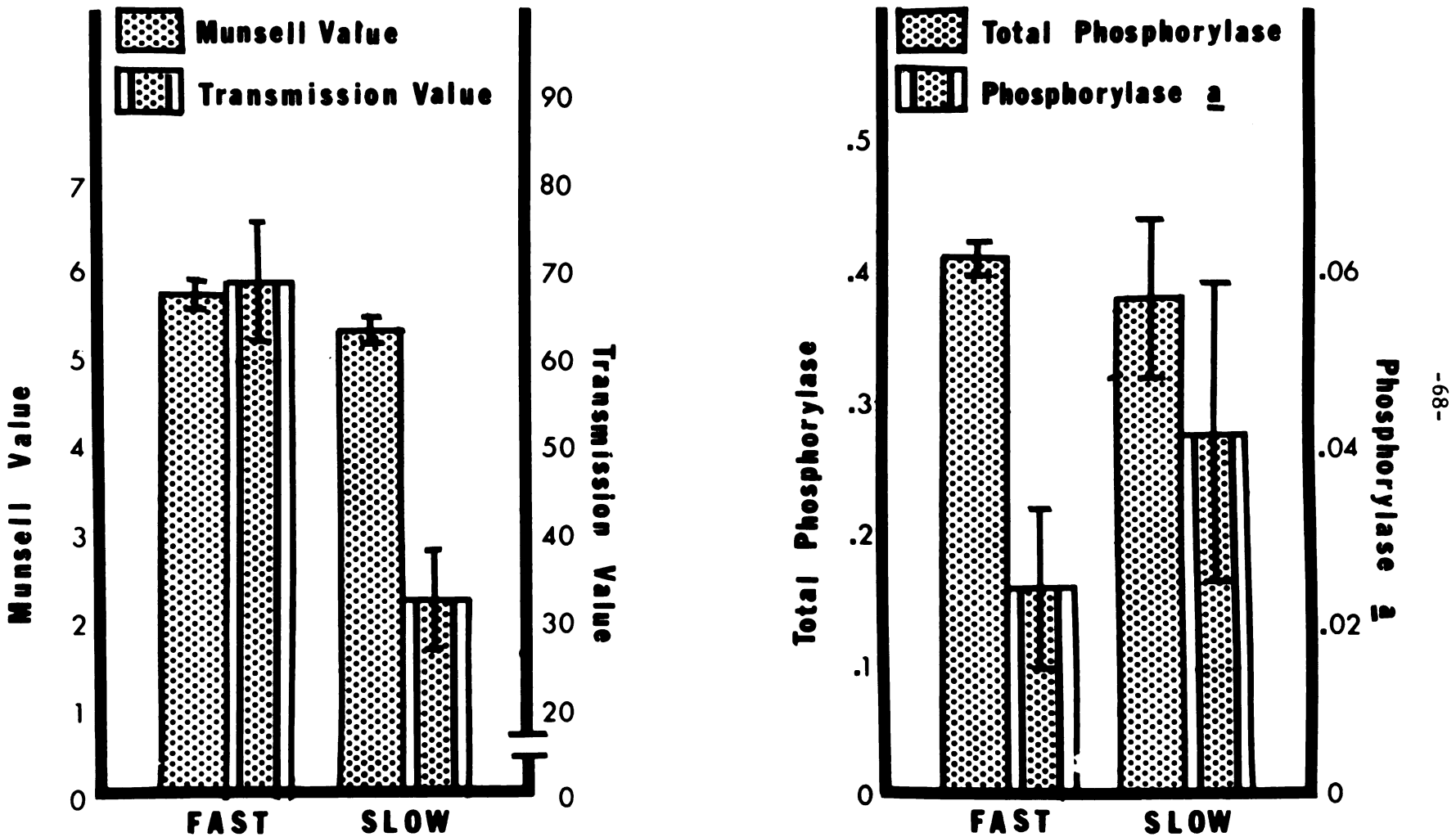

Figure 5. Munsell value, transmission value, total phosphorylase and phosphorylase a in porcine muscle with slow and fast rates of post-mortem glycolysis. (phosphorylase activity = u mole P_i released/min/mg protein).

difference between groups in 24 hr glycogen concentration. It should also be noted that all pigs used in this study were of one breed (Poland China).

Sayre et al. (1963a) reported that muscles from the Hampshire breed had 2 to 3 times higher initial glycogen than Poland China muscles. Chester White longissimus dorsi muscle also contained significantly more initial glycogen than Poland Chinas. Poland China muscle consistently had lower initial pH than muscle from the other two breeds. However, the authors did not specify the time lapse from exsanguination of the animal to sampling of the muscle. Kastenschmidt et al. (1966) found slightly lower initial glycogen levels (2 min post-mortem) in porcine muscle which exhibited rapid glycolysis as compared to slowly glycolysing muscle.

AMP, IMP, ATP, and inorganic phosphate. The results of AMP, IMP, ATP and inorganic phosphate determinations are presented in table 12 and illustrated graphically in Figure 7. AMP concentration at 15 min post-mortem was slightly higher in the muscles possessing fast pH decline, however, this difference was not significant between the two groups as greater variation occurred between individuals than between groups. There was a highly significant difference ($P < .01$) in 15 min IMP level between muscle with rapid and slow glycolysis (pH decline). The fast group contained 3.51 u mole IMP/g while the slow group had 1.54 u mole/g of muscle. Inorganic phosphate was also significantly higher ($P < .02$) in the rapidly glycolysing muscle, 25.6 u mole/g fresh muscle versus 17.4 u mole/g for slow glycolysis. However, ATP concentration was higher in the slow group of muscles (2.49 u moles/g versus 1.24 u moles/g of muscle). This difference was statistically significant ($P < .07$).

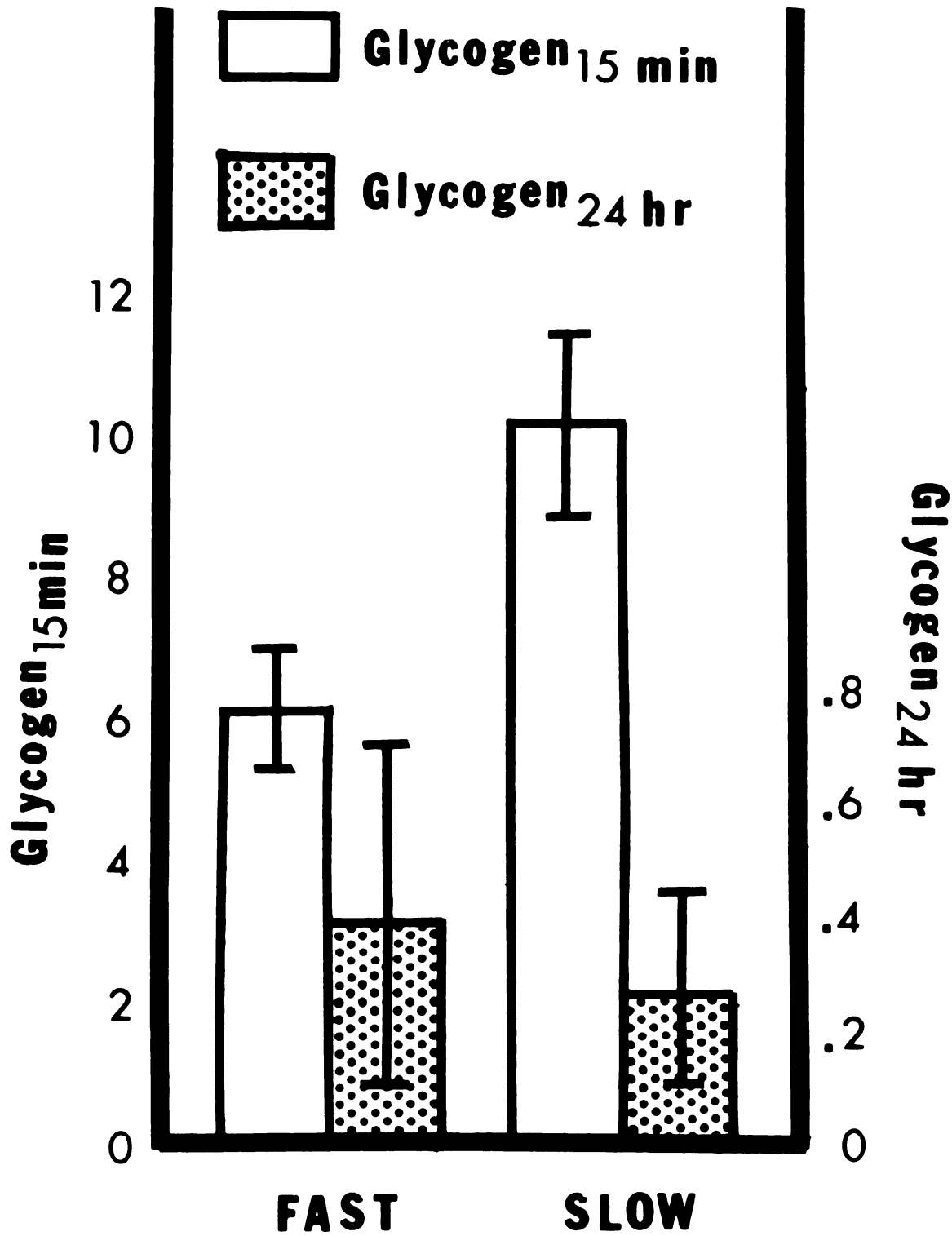

Figure 6. Glycogen at 15 min and 24 hr post-mortem in porcine muscles with fast and slow glycolysis. (glycogen = mg/g fresh muscle)

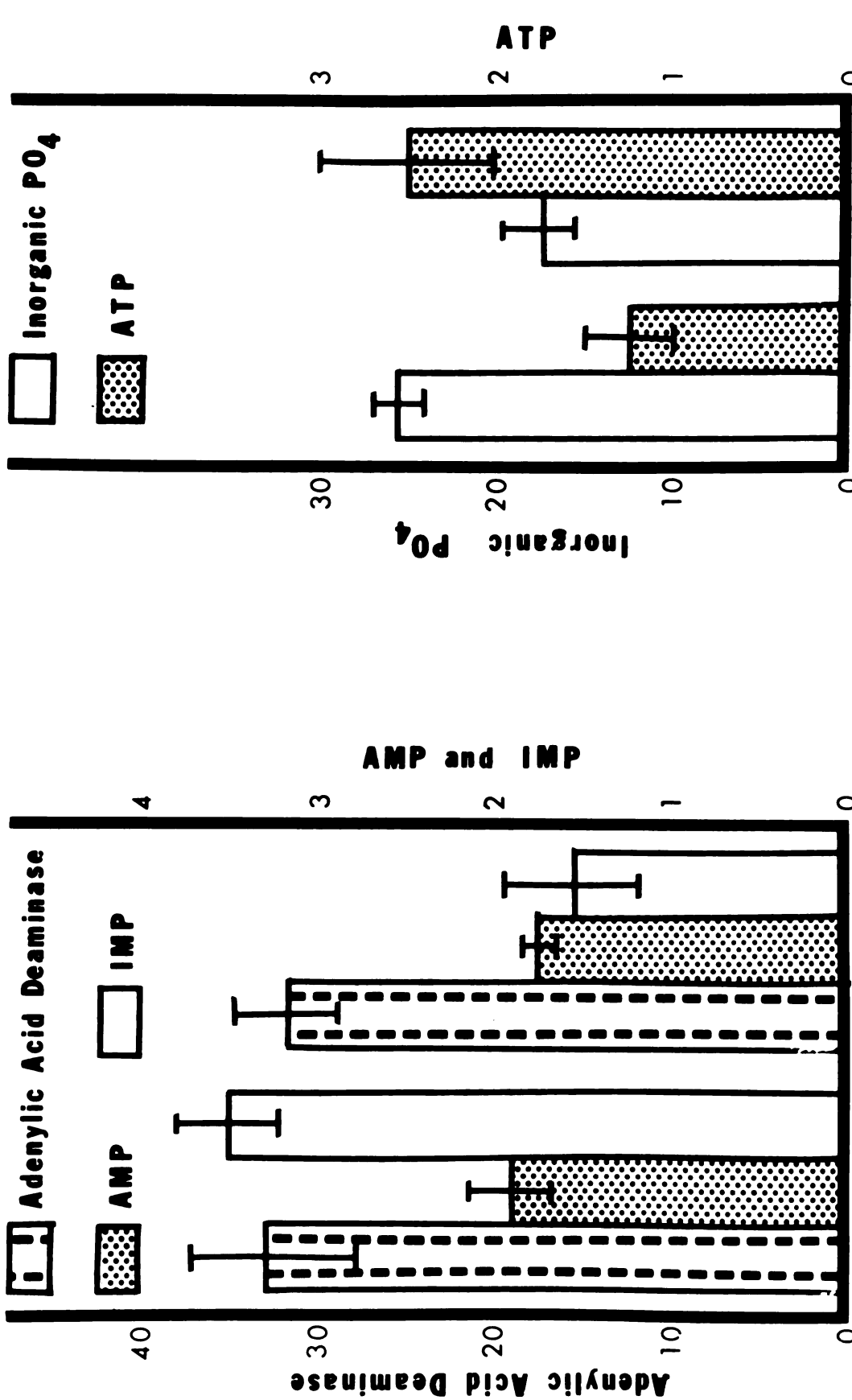

Figure 7. 5'-Adenylic acid deaminase, AMP, IMP, ATP, and inorganic phosphate in porcine muscles with fast and slow glycolysis.
(Adenylic acid deaminase activity = u moles AMP deaminated/min/mg protein; AMP, IMP, ATP, and inorganic phosphate expressed as u mole/g fresh muscle).

Table 12. Means and standard error of the means for glycogen, AMP, IMP, ATP and inorganic phosphate in porcine muscles with fast and slow rates of post-mortem glycolysis.[1]

	Fast	Slow	Standard error
Glycogen - 15 min[2]	6.27[a]	10.28[b]	2.26
Glycogen - 24 hr[2]	.40	.27	.24
AMP[3]	1.88	1.74	.20
IMP[3]	3.51[a]	1.54[b]	.35
ATP[3]	1.24	2.49	.40
Inorganic phosphate[3]	25.6[a]	17.4[b]	1.8

[1]Means with different superscripts differ significantly ($P < .05$).
[2]mg/g fresh muscle
[3]u moles/g fresh muscle

Simple correlation coefficients between pH and AMP, IMP, ATP, and inorganic phosphate are presented in Table 13. It is evident from the low correlation coefficients between pH at 15 and 45 min and AMP levels that no relationship existed between rate of pH fall and AMP concentration. However, IMP, ATP, and inorganic phosphate levels were closely associated with rate of pH decline. All of the correlation coefficients between these factors and pH at either 15 or 45 min post-mortem were highly significant ($P < .01$).

From these results, it appears that a more rapid degradation of ATP occurred during the initial 15 min post-mortem period in muscle with rapid pH fall, as indicated by the lower ATP level and higher inorganic phosphate concentrations. This is in agreement with Bendall *et al*.

Table 13. Simple correlation coefficients between pH and AMP, IMP, ATP, and inorganic phosphate in porcine muscles with fast and slow rates of post-mortem glycolysis.

	pH 15 min	pH 45 min
AMP	-.04	-.26
IMP	-.95	-.95
ATP	.93	.87
Inorganic phosphate	-.88	-.95
$r > .71$ is significant ($P < .05$)	$r > .83$ is significant ($P < .01$)	

(1963). The major pathways for resynthesis of ATP during the post-mortem period are either from creatine phosphate or by anaerobic glycolysis as pointed out by Bendall (1963). The fact that ATP resynthesis is not keeping pace with ATP breakdown is also indicated by the higher IMP and inorganic phosphate levels in muscles with rapid pH fall. Therefore, the question which remains unanswered is what mechanisms are responsible for the rapid ATP degradation.

Kastenschmidt et al. (1966) reported that ATP levels were lower and IMP levels slightly higher at 2 min post-mortem in porcine muscle which elicited a very rapid pH decline (pH < 5.5 at 60 min post-mortem). Whether in vivo levels of ATP are lower in these muscles has not been determined.

Phosphofructokinase, the glycolytic enzyme which catalyzes conversion of fructose-1-phosphate to fructose-1,6-diphosphate, has been shown to be a rate limiting step in glycolysis (Passonneau and Lowry, 1962; Mansour, 1963; Ozand and Narakara, 1964). ATP in high concentrations has been shown to be a potent inhibitor of phosphofructokinase activity

in muscle (Lardy and Parks, 1956). Passonneau and Lowry (1962) demonstrated that ATP inhibition could be overcome by small increases in AMP, ADP and inorganic phosphate. Ammonium ion (NH_4^+) was shown to be a potent stimulator of phosphofructokinase activity (Muntz and Hurwitz, 1951).

It is possible, therefore, that increases in phosphofructokinase activity could account for the rapid rate of pH fall observed in porcine muscle. Lower ATP levels and higher inorganic phosphate concentrations were observed in muscles with rapid pH decline in the present study. It must also be assumed that NH_4^+ concentration would also be greater in this type of muscle, since greater amounts of IMP were found. One mole of ammonia (NH_3) is released for every mole of IMP formed so NH_4^+ and IMP should be present in nearly equal molar quantities.

SUMMARY

The results of this study were obtained in two separate investigations with 36 porcine animals. In part I, the adenylic acid deaminase activity and AMP and IMP levels of longissimus dorsi, gluteus medius, and rectus femoris muscles were determined. The relationship of these parameters to post-mortem glycolysis, rate of pH fall, and to development of PSE porcine muscle was evaluated. In part II, epinephrine injection immediately prior to slaughter and the effects of prednisolone administration prior to epinephrine treatment were studied for possible effects upon the development of PSE musculature. Of special interest were the rate of post-mortem glycolysis, phosphorylase activity and adenylic acid deaminase activity of the longissimus dorsi muscle as well as glycogen, AMP, IMP, ATP, and inorganic phosphate levels in the muscle. Phosphorylase and adenylic acid deaminase activities and AMP, IMP, ATP, and inorganic phosphate levels were contrasted in porcine muscles with fast and slow rates of post-mortem glycolysis.

There were no significant differences in adenylic acid deaminase activity, AMP, or IMP between the longissimus dorsi, gluteus medius and rectus femoris muscles. Simple correlation coefficients between muscle pH and adenylic acid deaminase activity indicated a low but positive relationship between enzyme activity and rate of post-mortem pH fall.

Epinephrine or prednisolone plus epinephrine injection did not significantly alter the rate of post-mortem pH decline, ultimate muscle pH,

muscle color (Munsell value) or muscle quality (transmission value). Total phosphorylase activity was significantly increased in muscle from epinephrine injected pigs and phosphorylase *a* activity and % phosphorylase *a* were slightly increased. However, neither total phosphorylase nor phosphorylase *a* activity was related to rate of post-mortem pH fall or incidence of PSE musculature development. Adenylic acid deaminase activity was significantly lower in the epinephrine treated muscles.

AMP and IMP concentrations were not affected by either treatment, however, IMP level was highly related to the rate of pH fall. Epinephrine injection 10 min ante-mortem did not significantly deplete muscle glycogen. Muscle from prednisolone injected pigs contained higher glycogen levels at both 15 min and 24 hr post-mortem.

Pigs in the control group were divided into slow and fast glycolysis groups on the basis of post-mortem pH fall. Muscle with slow glycolysis had lower Munsell values and transmission values. Phosphorylase activity and adenylic acid deaminase activity were not significantly different between muscles with slow and fast glycolysis. Muscle which elicited a rapid pH fall had higher IMP and inorganic phosphate and lower ATP and glycogen levels than did muscle with slow pH fall.

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APPENDIX

Appendix I. Adenylic acid deaminase study.

Animal number	Muscle pH 15 min	Muscle pH 45 min	Muscle pH 24 hr	Longissimus dorsi Adenylic acid deaminase[1]	Longissimus dorsi AMP u mole/g	Longissimus dorsi IMP u mole/g	Gluteus medius Adenylic acid deaminase[1]	Gluteus medius AMP u mole/g	Gluteus medius IMP u mole/g	Rectus femoris Adenylic acid deaminase[1]
5	6.43	5.62	5.51	.021	.53	7.37	.022	.29	6.13	.017
6	6.13	5.51	5.35	.028	.94	6.40	.021	.55	6.62	.029
7	6.70	5.91	5.39	.036	.40	7.91	.030	.77	6.13	.042
9	6.49	6.36	5.39	.029	.92	4.86	.034	.63	5.49	.037
10	6.57	6.59	5.37	.032	.93	4.74	.035	.55	5.84	.029
11	5.93	5.20	5.37	.024	.40	5.79	.022	.53	6.53	.010
13	6.42	6.40	5.53	.034	.42	6.99	.036	.26	6.07	.031
14	6.49	6.53	5.50	.037	.18	5.14	.037	.38	5.43	.021
15	6.21	5.48	5.53	.032			.038			.026
16	6.35	5.96	5.47	.035			.036			.025
17	6.40	6.29	5.45	.034			.033			.033
18	5.85	5.40	5.57	.036			.024			.021
19	5.90	5.49	5.43	.028			.029			.031
20	6.24	6.26	5.45	.027			.036			.025

[1]Adenylic acid deaminase activity = u moles AMP deaminated/min/mg protein.

Appendix II. Control, epinephrine and prednisolone plus epinephrine injected pigs.

Animal number	Muscle pH 15 min	45 min	24 hr	Munsell value	Transmission value	Total phosphorylase activity[1]	Phosphorylase *a* activity[1]	% Phosphorylase *a*
Control								
21	6.26	5.85	5.52	5.34	50.0	.28	.019	6.8
22	6.31	5.50	5.53	5.73	74.0	.38	.014	3.7
23	6.75	6.71	5.51	5.32	28.0	.47	.080	17.0
24	6.63	6.58	5.44	5.51	21.0	.51	.060	11.8
25	6.33	5.65	5.43	5.48	79.0	.44	.020	4.5
26	6.07	5.55	5.43	6.14	75.0	.41	.013	3.2
27	6.02	5.40	5.44	5.77	47.5	.42	.050	12.0
28	6.45	6.18	5.27	5.31	32.0	.27	.007	2.5
Epinephrine								
29	6.10	5.49	5.53	5.87	64.0	.68	.075	11.0
32	5.94	5.38	5.42	6.39	88.0	.56	.075	13.0
33	6.47	6.27	5.49	5.34	33.0	.45	.110	24.4
34	6.40	6.30	5.53	5.80	38.0	.47	.050	10.6
35	6.20	5.44	5.44	6.16	75.0	.47	.020	4.2
36	6.35	6.13	5.48	5.21	21.0	.49	.024	4.9
37	6.02	5.45	5.45	5.76	73.0	.57	.052	9.1
38	6.50	6.32	5.52	5.06	36.0	.58	.028	4.8
Prednisolone + epinephrine								
39	6.00	5.38	5.42	6.11	82.0	.54	.027	5.0
40	6.48	6.42	5.52	5.49	21.0	.48	.018	3.8
41	6.22	5.80	5.39	5.46	42.0	.50	.110	22.0
42	6.06	5.71	5.36	5.36	48.0	.55	.049	8.9
43	6.42	6.16	5.40	5.04	33.0	.60	.065	10.8
44	6.54	6.39	5.42	5.13	22.5	.74	.160	21.6

[1]Phosphorylase activity = u mole P_i released/min/mg protein.
[2]Adenylic acid deaminase activity = u mole AMP deaminated/min/mg protein.

Appendix II (continued). Cnntrol, epinephrine and prednisolone plus epinephrine injected pigs.

Animal number	Adenylic acid deaminase[2]	AMP u mole/g	IMP u mole/g	Glycogen 15 min mg/g	Glycogen 24 hr mg/g	ATP u mole/g	Inorganic phosphate u mole/g
Control							
21	.032	2.08	2.53	6.85	.15	1.88	21.7
22	.044	1.86	3.40	4.40	.08	1.69	28.6
23	.030	1.61	.66	12.30	.18	3.86	13.0
24	.025	1.63	1.45	12.55	.12	2.53	15.0
25	.028	2.61	2.69	6.58	.08	1.76	21.6
26	.033	1.41	3.73	5.62	.09	.76	26.6
27	.027	1.66	4.22	8.47	1.37	.77	25.7
28	.040	1.66	1.53	9.40	.64	1.68	19.8
Epinephrine							
29	.025	1.53	3.15	3.50	.06		
32	.025	1.38	4.60	3.95	.14		
33	.028	2.13	1.24	8.08	.09		
34	.027	1.64	2.14	5.97	.05		
35	.023	1.98	3.18	6.78	.25		
36	.029	1.87	1.46	9.10	.94		
37	.023	1.73	4.71	3.50	.06		
38	.021	1.52	1.47	5.95	.12		
Prednisolone + epinephrine							
39	.019	1.49	3.93	10.90	2.61		
40	.024	2.39	1.68	13.12	4.13		
41	.028	3.11	2.88	9.63	2.23		
42	.022	1.32	3.29	10.78	3.52		
43	.034	2.32	1.75	13.52	3.62		
44	.029	1.99	1.72	12.10	2.76		

[1]Phosphorylase activity = u mole P_i released/min/mg protein.
[2]Adenylic acid deaminase activity = u mole AMP deaminated/min/mg protein.

Appendix III. Simple correlations for control, epinephrine and prednisolone plus epinephrine injected pigs.

	pH 15 min	pH 45 min	Munsell value	Transmission value	Total phosphorylase	Phosphorylase *a*
pH - 15 min	1.00					
pH - 45 min	.93	1.00				
Munsell value	-.69	-.70	1.00			
Transmission value	-.76	-.88	.78	1.00		
Total phosphorylase	-.05	.05	-.05	-.05	1.00	
Phosphorylase *a*	.20	.28	-.22	-.31	.62	1.00
% phosphorylase *a*	.22	.30	-.22	-.33	.39	.94
Adenylic acid deaminase	.26	.09	-.22	-.09	-.52	-.15
AMP	.24	.13	-.35	-.19	-.09	.20
IMP	-.94	-.93	.75	.83	.05	-.17
Glycogen - 15 min	.53	.61	-.52	-.67	.11	.24
Glycogen - 24 hr	.01	.13	-.30	-.32	.32	.18

Appendix III (continued) Simple correlations for control, epinephrine and prednisolone plus epinephrine injected pigs.

	% Phosphorylase a	Adenylic acid deaminase	AMP	IMP	Glycogen 15 min	Glycogen 24 hr
pH - 15 min						
pH - 45 min						
Munsell value						
Transmission value						
Total phosphorylase						
Phosphorylase a						
% phosphorylase a	1.00					
Adenylic acid deaminase	-.11	1.00				
AMP	.27	.16	1.00			
IMP	-.21	-.20	-.23	1.00		
Glycogen - 15 min	.23	-.04	.25	-.56	1.00	
Glycogen - 24 hr	.08	-.17	.28	-.07	.71	1.00

Appendix IV. Simple correlation coefficients for control pigs.

	pH 15 min	pH 45 min	Munsell value	Transmission value	Total phosphorylase	Phosphorylase *a*	% Phosphorylase *a*
pH - 15 min	1.00						
pH - 45 min	.93	1.00					
Munsell value	-.69	-.63	1.00				
Transmission value	-.65	-.83	.58	1.00			
Total phosphorylase	.31	.29	.23	-.11	1.00		
Phosphorylase *a*	.55	.62	-.24	-.63	.71	1.00	
% phosphorylase *a*	.48	.57	-.29	-.64	.60	.98	1.00
Adenylic acid deaminase	-.09	-.25	.05	.29	-.62	-.63	-.62
AMP	-.03	-.26	-.37	.45	-.10	-.28	-.26
IMP	-.95	-.95	.77	.71	-.12	-.45	.41
Glycogen - 15 min	.74	.88	-.52	-.92	.43	.80	.76
Glycogen - 24 hr	-.41	-.27	.07	-.25	-.14	.14	.22
Inorganic phosphate	-.88	-.95	.70	.79	-.34	-.68	-.64
ATP	.94	.87	-.68	-.58	.34	.67	.64

Appendix IV (continued). Simple correlation coefficients for control pigs.

	Adenylic acid deaminase	AMP	IMP	Glycogen 15 min	Glycogen 24 hr	Inorganic phosphate	ATP
pH - 15 min							
pH - 45 min							
Munsell value							
Transmission value							
Total phosphorylase							
Phosphorylase a							
% phosphorylase a							
Adenylic acid deaminase	1.00						
AMP	-.10	1.00					
IMP	.10	.05	1.00				
Glycogen - 15 min	-.53	-.34	-.76	1.00			
Glycogen - 24 hr	-.15	-.25	.36	.14	1.00		
Inorganic phosphate	.47	.06	.92	-.91	.19	1.00	
ATP	-.16	.01	-.88	.68	-.41	-.85	1.00

Appendix VII. Sources of chemicals.

Chemical	Source
Ammonium molybdate	Baker
AMP (sodium salt)	Sigma
Anion exchange resin AG 1 x 8	Bio-Rad
Arsenic thioxide	Baker
Ascorbic acid	Eastman
ATP (disodium salt)	Sigma
Citric acid	Baker
Cysteine - HCl	Eastman
2,4-Dinitrophenylhydrazine	Eastman
Elon (p-methylaminophenol)	Sargent
Ethylenediaminetetra acetic acid (disodium salt)	Baker
Firefly lanterns (vacuum dried)	Sigma
Glucose-1-phosphate (dipotassium salt)	Sigma
Glycogen (rabbit liver)	Sigma
IMP (sodium salt)	Sigma
Periodic acid	Smith
Phenol	National Aniline
Sodium-β-glycerophosphate	Sigma
Sodium iodoacetate	Eastman
Succinic acid	Baker

Baker = J. T. Baker Chemical Co., Phillipsburg, N.J.
Bio-Rad = Bio-Rad Laboratories, 32nd & Griffin, Richmond, Cal.
Eastman = Eastman Organic Chemicals, Rochester 3, N.Y.
National Aniline = National Aniline Division, Allied Chemical & Dye Corp., 40 Rector St., New York 6, N.Y.
Sargent = E. H. Sargent & Co., 8560 W. Chicago, Detroit, Mich.
Sigma = Sigma Chemical Co., 3500 DeKalb St., St. Louis, Mo.
Smith = G. Frederick Smith Chemical Co., Columbus, Ohio