LYSOSOMAL ENZYME ACTIVITY IN NORMAL AND PALE, SOFT AND EXUDATIVE PORCINE MUSCLE

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ABSTRACT

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by Thayne R. Dutson

The primary objectives of this study were to determine any differences in lysosomal enzyme activity between PSE and normal porcine muscle, and to ascertain any similarities between PSE and degenerative muscle. Histochemical measurements of enzyme activity were carried out for 5 different enzymes, including acid phosphatase, esterase, aryl sulphatase, β-glucuronidase and β-galactosidase. The activity of each enzyme was determined at 0 hour, 45 minutes and 3 hours post-mortem on muscle samples from 25 pigs. Of the 25 pigs, 10 were Landrace (5 normal and 5 PSE), 10 were Poland China (5 normal and 5 PSE) and 5 were Chester White (all normal).

There was no significant difference in lysosomal activity between PSE and normal pigs for any of the enzymes studied. However, there was a significant difference in acid phosphatase activity ($P < .05$) between the Chester White pigs, which have a low incidence of the PSE condition, and the other two breeds, that are known to have a high incidence of PSE muscle. There was no significant difference between breeds for the other enzymes studied.

No β-glucuronidase or β-galactosidase activity was found in the muscles of any of the pigs. Similarly, different times post-mortem did not influence the activity of any of the enzymes studied, except esterase. Esterase activity was found to diminish with increasing time post-mortem for all pigs, and practically disappeared between 45 minutes and 3 hours post-mortem.

Results of this study suggest that the PSE condition is not associated with any definite increase in lysosomal enzyme activity. However, significant differences in acid phosphatase activity were found to exist in muscle from stress-susceptible and stress-resistant breeds, which may indicate an indirect involvement in the PSE condition.

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By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

INTRODUCTION

The chemical and physical changes that take place in pork muscle after death are extremely important factors in determining carcass quality. Pork muscle with a fast rate of post-mortem glycolysis develops an abnormal accumulation of lactic acid and a low pH, while body temperature is still high. This gives rise to a condition in which the muscle becomes pale in color, soft in texture and exudative in appearance and is known as pale, soft and exudative or PSE pork. The incidence of PSE pork is quite high, especially in some localities and in some breeds and strains of hogs. The average incidence in the United States has been estimated to be about 18%, however, for some isolated strains has been reported to be as high as 88% (Briskey, 1964). The lower quality, higher shrinkage and undesirable processing qualities of PSE pork carcasses cause direct monetary losses to the processor, retailer and consumer.

There is a marked decrease in creatine phosphate and ATP levels for PSE pork. The reduced ATP level is probably one of the factors responsible for the accelerated rate of glycolysis. Two possible reasons for the reduced levels of ATP and creatine phosphate are: (1) an increase in the activity of the ATPase system; and (2) an uncoupling of oxidative phosphorylation. The uncoupling would reduce the capacity of the tissues to replenish the ATP, which is broken down during the death struggle.

The uncoupling of oxidative phosphorylation and activation of ATPase are the first events that take place upon lysosome disorganization (Tappel, 1966). The PSE condition then might be due to lysosome abnormalities. Also there is a marked increase in the activity of the lysosomal enzymes in white muscle disease (Desai, 1966), which shows some similarities in appearance to PSE muscle.

McClain (1968) stated that further studies on the influence of lysosomes and the naturally occurring collagenases of PSE muscle may be useful in elucidating some mechanisms of PSE muscle formation. Bodwell (1964) suggested that the relationship of acid phosphatase (a lysosomal enzyme) activity to ultimate post-mortem muscle condition warranted further study.

In light of the above information, the present study was undertaken to determine if there are any differences in the lysosomal enzyme activity between normal and PSE muscle. In addition, possible similarities between PSE musculature and white muscle disease were investigated.

REVIEW OF LITERATURE

Properties of Lysosomes

According to Hirsch and Cohn (1964) lysosomes are small cytoplasmic organelles which contain various hydrolytic enzymes enclosed within their own membranes. These authors indicated that disruption of these membranes is required for maximal enzyme activity. They also stated that there are two types of lysosomes: (1) primary, which are hydrolase containing granules manufactured by the cell; and (2) secondary lysosomes, which are formed by the fusion of primary lysosomes with phagocytic, pinocytic or autophagic vacuoles.

Some questions still remain as to how the enzymes obtain entry into the autophagic vacuoles (Swift and Hruban, 1964; de Duve and Wattiaux, 1966). After membrane coalescence, the hydrolytic enzymes may digest the foreign substances brought into the cell by pinocytosis, or in the case of autophagy may directly digest the cellular contents, such as the mitochondria (de Duve and Wattiaux, 1966).

Enzymes

There are many different enzymes contained within the lysosomes. These enzymes, all of which have an acid pH optimum, include a number of esterases, nucleases, proteases, polysaccharidases and glycosidases (de Duve and Wattiaux, 1966). Strauss (1967) has compiled a list of

enzymes that have been found in lysosomes of various tissues. The list includes acid phosphatase, acid ribonuclease, acid deoxyribonuclease β-glucuronidase, cathepsin, arylsulphatase, β-galactosidase, β-N-acetyl-glucosamine hydrolase, α-mannosidase, collagenase, α-glucosidase, hyaluronidase, phosphatidate phosphatase, esterase, $NADH_2$ cytochrome c reductase and other enzymes in lower concentrations. Strauss (1967) also stated that the presence of some of these enzymes in low concentrations might be due to sequestration of other cellular particles in the process of autophagy.

Enzymes Found in Muscle Tissue

Acid phosphatase is the main enzyme used for cytochemical demonstration of lysosomes (Strauss, 1967), and gives an indication of the lysosomal content of various tissues. Relatively few acid phosphatase-positive granules have been found in skeletal muscle cells (Strauss, 1967). Woessner (1965) has reviewed the literature on lysosomal enzymes and has given tables listing all tissues where cytochemical staining has shown the presence of acid phosphatase and β-glucuronidase. No cellular localization of these enzymes in skeletal muscle was reported. There was, however, some lysosomal enzyme activity associated with the fibroblasts and collagen fibers of the muscle fascia and tendons.

Ogata and Mori (1963) found no acid phosphatase or arylsulphatase reactions in mammalian skeletal muscle. However, they found a slight amount of β-glucuronidase and β-galactosidase activity, as well as a

marked staining reaction for esterase. They stated that the esterase activity was more concentrated in the small red fibers than in the large white fibers. The fibers intermediate between the red and white were also intermediate in staining intensity.

Bodwell et al. (1965) have shown variable amounts of positive staining for acid phosphatase in isolated skeletal muscle cells in each of 7 hogs. These authors concluded that the muscle cells showing intense staining were in an active state of degeneration.

Barron et al. (1966) demonstrated the presence of a sarcoplasmic esterase, which by inhibition studies was identified as a β-type esterase. These authors noted the same fiber distribution for this enzyme as reported by Ogata and Mori (1963). Barron et al. (1966) indicated that this esterase was of sarcoplasmic origin but did not state whether or not it was of lysosomal origin.

There has been some quantitative demonstration of lysosomal enzyme activity in muscle tissue. These include acid phosphatase (Pennington, 1963), β-glucuronidase (Tappel et al., 1962; Desai et al., 1964), β-galactosidase (Desai et al., 1964; Maio and Rickenberg, 1960) and aryl-sulphatase (Tappel, 1966; Desai, 1966). There is some evidence, however, that the positive reactions observed might be due to lysosomes originating from macrophages or from other blood and connective tissue cells that were present in the tissues (Tappel, 1966).

Enzyme Effects on Tissues

According to Hirsch and Cohn (1964), lysosomes probably perform both digestive and autolylic functions in cell physiology and pathology. These

authors stated that when foreign substances are engulfed by cellular phagocytosis, the contents of the lysosomes within the phagocyte are discharged directly into the phagocytic pouch to attack and degrade the engulfed material. They also stated that the release of lysosomal enzymes into the cytoplasm by the lytic action of substances, such as bacterial toxins and low pH, causes cell damage and eventually death. If the lysosomal enzymes are released directly into the cytoplasm, it is liquified, which is the immediate cause of death (Hirsch and Cohn, 1964).

Autophagy, which is the process of engulfing or sequestration of internal cellular contents into lysosomes followed by their eventual degradation and digestion, also plays an important role in lysosome function. Swift and Hruban (1964) stated that autophagy, which goes on continually at a low level in normal cells, is greatly increased by certain toxic agents. Substances such as, β-3-thienylalanine and azaserine, which are biosynthesis inhibitors, and triparanol, a cholesterol inhibitor, are examples of toxic agents that cause an increase in autophagic action. These authors also showed that autophagy is increased by certain atrophic stimuli. For example autophagy is increased in the liver by the physiological response to starvation and in the ventral prostate gland by castration.

Lysosome Stability

There have been many studies dealing with the effects of various substances and treatments on the stability of the lysosomal membrane

(de Duve, 1966). Vitamin A, ultraviolet radiation, streptolysins, pyrogenic steroids, endotoxin, lysolecethin, carbon tetrachloride and detergents have all been demonstrated to be labalizers of the lysosomal membrane, while cortisone, cortisol and chloroquine are stabilizers (Weissmann, 1964; de Duve, 1966; Woessner, 1965; and Dingle, 1963). Cortisone and other stabalizers may also have an antogonizing effect on the various labalizers (Weissmann, 1964; and de Duve, 1966). Upon lowering the pH and holding the temperature at 37° C, the effects of the labalizing agents are greatly increased according to Weissman (1964). He also stated that lowering of the pH alone has a labalizing effect on the lysosomes.

Lysosomes in Muscular Dystrophy

Involvement of lysosomal enzymes in muscular dystrophy has been studied by many authors. These studies have included genetic muscular dystrophy of mice (Fennell and West, 1963) and chickens (Tappel, 1966), and nutritional muscular dystrophy in chickens (Bunyan *et al.*, 1967; and Tappel, 1966), rabbits (Tappel, 1966) and lambs (Desai, 1966).

Tappel (1966) using genetically dystrophic chickens stated the earliest hydrolytic degenerative changes observed in the muscle are due to increased lysosomal enzymes in the tissue. He also stated that later degeneration is probably due to invading macrophages.

Fennell and West (1963) reported that the outstanding feature in genetically dystrophic mice is the strong acid phosphatase reaction of

the atrophic muscle fibers. They also suggested that the increased acid phosphatase activity was catabolic in nature.

Desai (1966) has compared the activity of 5 lysosomal enzymes in white muscle disease of lambs - a type of nutritional muscular dystrophy - with that of normal controls. He found that the activity of these enzymes was markedly increased in white muscle disease. The diseased compared to the normal controls showed a 35-fold increase for arylsulphatase, a 5-fold increase for β-glucuronidase, a 3-fold increase for β-galactosidase and cathepsin, and a 2-fold increase for acid phosphatase. The author stated that the increases in activity were similar to that for chicks and rabbits suffering from nutritional muscular dystrophy. Desai (1966) also concluded that the phenomenon of increased lysosomal activity is directly associated with the degradative changes occurring in dystrophic tissues. Tappel (1966) and Bunyan et al. (1967) have reported similar changes as a consequence of nutritional muscular dystrophy in chicks.

Pale, Soft, Exudative Pork

Ludvigsen (1953) found that some muscles of Danish Landrace pigs were abnormally light colored and exudative in appearance. These muscles had an extremely low pH (5.3 to 5.5) at 45 minutes post-mortem, whereas, the pH of normal muscles was much higher (6.8 to 7.0). He called this condition "muscle degeneration disease". The same condition has been described by many authors (Wismer-Pedersen, 1959; Briskey et al., 1959a,

1959b; Briskey and Wismer-Pedersen, 1961a, 1961b; Bendall and Wismer-Pedersen, 1962; McLoughlin and Goldspink, 1963) and has more recently been called PSE (pale, soft and exudative) by Briskey (1964).

Incidence and Importance of PSE Pork

The PSE condition has been encountered in many countries, and its incidence varies widely from country to country (Briskey, 1964). The incidence has been reported to range from a high of 35 to 40% in Denmark (Clausen and Thomsen, 1960) to a relatively low percentage in Ireland (McLoughlin, 1965), whereas, an incidence of 18% has been estimated in the United States (Briskey, 1964).

According to Briskey (1964), many different factors influence the incidence of PSE muscle. Among these factors are geographical area, temperature, season, weight, sex, and the lean-to-fat ratio. Briskey (1964) also indicated that breed is one of the most important factors affecting the incidence of the PSE condition. He reported that Landrace, Hampshire and Poland China pigs had a higher incidence of PSE muscle than Yorkshire, Berkshire and Chester White pigs.

Monetary losses caused by the PSE condition are manifest in all phases of the pork industry from curing and processing to the retail sale of fresh cuts. Briskey (1964) citing unpublished data by Borchert and Briskey showed that the yields from PSE products were lower than those from normal controls by 3% for fully cooked hams, 6% for canned hams, 10% for Canadian bacon, 2% for smoked picnics, and 2% for smoked

butts. Karmas and Thompson (1964) reported that cooked PSE hams retained the original color differences and the gelatinous cookout was 4 to 8% higher than that for normal hams.

Using an average incidence of 18% for PSE pork in the United States, Briskey (1964) calculated that a processing plant, which slaughtered 8000 pigs per day, would have a monetary loss of 1 1/2 to 2 million dollars per year. He also stated that even though the merchandising problems and adverse consumer reactions to fresh PSE pork are difficult to assess monetarily, they are real and must be considered.

Characteristics of PSE Pork Muscle

A rapid rate of post-mortem glycolysis, which results in a low pH at an early time post-mortem, is one of the major attributes of PSE muscle (Ludvigsen, 1953; Wismer-Pedersen, 1959; Briskey and Wismer-Pedersen, 1961a; McLoughlin, 1963; Briskey et al., 1960; Briskey, 1964). Although the relationship between the incidence of PSE muscle and a rapid rate of post-mortem glycolysis is well established, the basic cause for the extremely rapid glycolytic rate is unknown (Briskey, 1964).

The rapid glycolysis of PSE muscle produces an accumulation of lactic acid, which results in a low pH while the tissues are still at a high temperature (Briskey, 1964). The morphological and some of the biochemical changes that take place in PSE muscle have been attributed to the concerted action of low pH and high temperature on the tissues (Briskey and Wismer-Pedersen, 1961a; Bendall and Wismer-Pedersen, 1962; Briskey, 1964; Cassens, 1966). Cassens (1966) stated that a combination

of high temperature and low pH soon after death may be responsible for the visual loss of color and the drastic lowering of water-binding capacity, which has been reported by Wismer-Pedersen and Briskey (1961b) and Bendall and Wismer-Pedersen (1962). In the latter paper it was reported that low pH in combination with high temperatures did not change or denature the fibrillar proteins, but the sarcoplasmic proteins were altered. Bendall and Wismer-Pedersen (1962) suggested that the low water binding capacity of PSE muscle develops as a consequence of denaturation of the sarcoplasmic proteins and their being deposited on the myofibrils. Sayre and Briskey (1963) have also reported a decrease in the sarcoplasmic protein fraction of muscle held at a high temperature and a low pH soon after death of the animal. Scopes and Lawrie (1963) have shown that creatine phosphoryltransferase is particularly susceptible to the low pH-high temperature denaturation.

Briskey and Wismer-Pedersen (1961a) found a rapid decrease in labile phosphate compounds concurrently with the rapid drop in pH of PSE pigs. Bendall *et al.* (1963) reported that the onset of rigor mortis occurred only after the ATP concentration declined to about 30% of the initial level. Briskey *et al.* (1962) and Bendall *et al.* (1963) have shown that muscles which ultimately become PSE exhibit rapid development of rigor mortis.

Histochemistry of PSE Muscle

In histochemical studies involving the effects of temperature upon post-mortem changes in pork, Bodwell *et al.* (1965), concluded that there

was no UDP-glycogen transferase activity in carcasses subjected to high temperatures at a low pH. These authors reported a small amount of acid phosphatase and alkaline phosphatase activity in some muscles and concluded that such muscles were in an active state of degeneration. They also studied the relative proportions of red and white fibers by staining for succinic dehydrogenase activity.

Using histochemical staining procedures, Cooper et al. (1968) have shown that PSE muscle has more intermediate and fewer red fibers than normal muscle. They also stated that PSE muscles are higher in phosphorylase and ATPase activity than normal muscles.

MATERIALS AND METHODS

Experimental Animals

A total of 41 hogs were killed in this study, including 19 Poland China, 16 Landrace and 6 Chester White. The Poland China and Landrace pigs were obtained from breeders that were known to have a high incidence of PSE pork. The Chester White pigs were also obtained from a local breeder. The pigs were transported to the Michigan State University Meat Laboratory and were fasted for 24 hours, but were allowed free access to water before slaughter.

From the original animals slaughtered, two groups were selected as having PSE and normal tissues. Selection was based on muscle pH at 45 minutes post-mortem and a subjective quality score. Subjective quality scores were based on 5 points each for marbling, color and firmness, giving a total possible score of 15 points. Those pigs with the highest pH and highest quality score were classified as normal and those with the lowest pH and lowest quality score were termed PSE. The Landrace and Poland China breeds were represented in both the PSE and normal groups with 5 pigs per group, while the Chester White pigs were all normal. Therefore, only 5 Chester White pigs were used. This gave a total of 25 carcasses that were utilized for histochemical characterization of PSE and normal muscle.

Slaughtering and Sampling Procedures

The pigs were killed without stunning by sticking anterior to the sternum, thus severing the carotid artery and the jugular vein. A 0-

hour post-mortem sample was removed from the longissimus dorsi muscle in the region of the 4th or 5th lumbar vertebra immediately after sticking and before bleeding was complete. The sample was trimmed to about one cm. square and frozen on cork discs with liquid nitrogen. The portion of the sample removed during trimming was frozen in liquid nitrogen and was later used for pH measurements.

Subsequent samples were removed anterior to the location of the first sampling site at 45 minutes and 3 hours post-mortem. They were treated in the same manner as the 0 hour sample. All samples were stored at -78°C in dry ice until removed for further use. The carcasses were skinned, eviscerated, washed and prepared for cooling between the 45 minute and 3 hour sampling periods. However, they were not chilled until after removal of the 3 hour post-mortem sample.

Measurement of pH

The frozen sample was powdered by placing it in a Waring Blender at -20°C. Two grams of the powdered muscle were added to 25 ml of a 0.005 M sodium iodoacetate solution and the pH was read on a Corning, model 12, pH meter.

Histochemical Procedures

Sectioning

The cork discs containing the frozen blocks of tissue were frozen to the microtome specimen holder (S.L.E.E., 1964) using a few drops of

distilled water and then immersing the specimen holder in a dry ice-acetone bath. The specimens were then placed in the S.L.E.E. "Pearse" Cold Microtome (cryostat) and sections were cut 12 μ in thickness. The temperature of the cryostat was set at -18°C. The sections were mounted on coverslips and subjected to the procedures outlined below.

Acid Phosphatase

Acid phosphatase activity was determined by the method of Pearse (1960). Sections were incubated for 60 min in a mixture of 10-20 mg of sodium α-napthyl phosphate, 20 ml of 0.1 M acetate buffer at pH 5.0, 1.5 g polyvinyl pyrrolidone and 20 mg of the stable diazotate of o-amino azotoluene (Fast Garnet GBC salt). The sections were then washed in running water for 2-3 minutes and mounted in glycerine jelly.

Arylsulphatase

The method for determining arylsulphatase was according to the procedure outlined by Pearse (1960). Sections were pretreated for 3 minutes each in successive solutions of 0.85, 1.0 and 2.0% sodium chloride. The sections were then incubated for 8 hours in a substrate solution, which was made up by dissolving 25 mg of potassium 6-benzoyl -2-napthyl sulphate in 80 ml of hot 0.85% sodium chloride solution, then adding 20 ml 0.5 M acetate buffer (pH 6.1), and making the solution hypertonic by adding 2.6 g of solid sodium chloride. The sections were taken from the substrate solution, washed twice in cold saline and placed in an ice cold freshly prepared solution of Fast Blue B salt

(1 mg/ml) in 0.05 M phosphate buffer (pH 7.6) for five minutes. The sections were washed three times in cold 0.85% saline, once in water and then were mounted in glycerine jelly.

Beta-Glucuronidase

Beta-glucuronidase was determined by the method of Pearse (1960). Sections were incubated for 8 hours in a substrate solution, which contained 30 mg of 6-bromo-2-napthyl-β-D-glucuronide, 5 ml of absolute ethanol, 20 ml phosphate-citrate buffer (pH 4.95) and 75 ml of distilled water. The sections were then rinsed in tap water, immersed in a 1 mg/ml solution of Fast Blue B salt in cold 0.02 M phosphate buffer (pH 7.5) for 2 minutes, rinsed twice in cold distilled water and once in a 0.1% acetic acid solution before mounting in glycerine jelly.

Beta-Galactosidase

The procedure for estimating β-galactosidase was according to the method of Pearse (1960). The sections were placed in an incubating medium for 8 hours, containing 100 mg of 6-bromo-2-napthyl-β-D-galactopyranoside, 15 ml methanol, 300 ml of distilled water and 85 ml phosphate-citrate buffer (pH 4.95). The sections were removed and washed in 3 changes of distilled water for 3 minutes each. They were then transferred to a freshly prepared 1 mg/ml solution of Fast Blue B salt in cold 0.02 M phosphate buffer (pH 7.5) for 3 minutes, washed 3 times in cold distilled water and mounted in glycerine jelly.

Esterase

The staining procedure for esterase activity was that described by Pearse (1960). Sections were incubated for 8 hours in a freshly prepared solution of 1.3 mg 5-bromindoxylacetate, 0.1 ml of 95% ethanol, 2.0 ml of 0.1 M tris HCl buffer (pH 7.6), 1.0 ml of 0.05 M potassium ferricyanide, 1.0 ml of 0.05 M potassium ferrocyanide, 1.0 ml of 0.1 M calcium chloride and 3.6 ml distilled water. The nuclei were counter-stained by placing the sections in Mayer's Carmalum for 10 minutes. They were then removed, rinsed in water and mounted in glycerine jelly.

Succinic Dehydrogenase

Succinic dehydrogenase activity was determined by the method of Pearse (1960). Sections were incubated for 20 minutes in a medium which contained equal volumes of a Nitro-BT solution (1 mg/ml) and a stock succinate solution. The stock succinate solution was made up by combining equal volumes of 0.2 M phosphate buffer at pH 7.6 and 0.2 M sodium succinate. After incubation the sections were washed in saline and fixed in 10% formol-saline for 10 minutes. They were then rinsed in 15% alcohol for 5 minutes and mounted in glycerine jelly.

Ratings for Intensity of Staining

The intensity of the reactions that resulted from application of the different staining procedures was scored individually and rated as follows: 1 = none, 2 = trace, 3 = weak, 4 = moderate, 5 = moderately strong, and 6 = strong. A Leitz Dialux research microscope was used for rating all slides.

Photomicrographs were made of representative sections using a Leitz Dialux microscope equipped with a Kodak Colorsnap 35 mm camera and Photo-Multiplier and Automatic Integrating Timer Unit (Vickers Instruments, Ltd.). Kodachrome II (KPA 135-36) film was used and black and white prints were made.

Statistical Treatment

Regression analysis and analysis of variance were carried out to investigate the effects of breed and of pH change on the activity of acid phosphatase and esterase using the procedures described by Snedecor (1956).

RESULTS AND DISCUSSION

Post-Mortem pH

The 0 hour and 45 minute pH values obtained for the PSE and normal pigs of the Landrace breed are shown in table 1. For the Poland China pigs the same information is shown in table 2 and for the Chester Whites in table 3. The live weight, sex, muscle pH (at 0 hour, 15 minutes, 45 minutes and 3 hours post-mortem), quality score and muscle condition (PSE or normal) for all Landrace pigs are shown in Appendix 1. The same information for all Poland China pigs is presented in Appendix 2 and for all Chester White pigs in Appendix 3. Only those pigs classified as characteristic of PSE and normal were used for subsequent studies.

Table 1. Values for pH in normal and PSE Landrace pigs.

PSE Group			Normal Group		
	Muscle pH			Muscle pH	
Animal No.	0 hr	45 min	Animal No.	0 hr	45 min
1	6.30	5.48	2	6.48	6.26
3	6.22	5.70	8	6.36	6.26
5	6.17	5.97	10	6.36	6.18
6	6.20	5.20	12	6.36	6.27
11	6.34	5.98	13	6.30	6.20
Mean	6.24	5.66		6.37	6.23

Table 2. Values for pH in normal and PSE Poland China pigs.

PSE Group			Normal Group		
	Muscle pH			Muscle pH	
Animal No.	0 hr	45 min	Animal No.	0 hr	45 min
14	6.14	5.61	3	6.44	6.55
15	6.19	5.42	8	6.28	6.10
16	6.20	5.27	10	6.26	6.05
17	6.02	5.33	11	6.36	6.05
19	6.33	5.80	13	6.21	6.22
Mean	6.18	5.49		6.31	6.19

Table 3. Values for pH in Chester White pigs.

Normal Animals[1]		
	Muscle pH	
Animal No.	0 hr	45 min
1	6.14	6.07
2	6.34	6.45
4	6.31	6.26
5	6.28	6.38
6	6.31	6.13
Mean	6.27	6.25

[1]No PSE animals were obtained from the Chester White breed.

The mean 45 minute post-mortem pH values for the PSE and normal pigs were 5.66 and 6.23, respectively, for the Landrace pigs and 5.49 and 6.19 for the Poland China pigs (Tables 1 and 2). All Chester White pigs were classified as normal and had a mean 45 minute post-mortem pH value of 6.25 (Table 3). The differences in pH between the PSE and normal groups would be expected since pH values served as the basis for selecting the two groups. However, selection on this basis is justified by the results of McLoughlin and Goldspink (1963), who stated that a rapid post-mortem fall in pH (below 6.0 at 45 minutes) is associated with pale-exudative porcine muscle.

Individual pigs did not always show the relationship between pH and pale-exudative muscle. Pigs PC18 (Appendix 2) and LR-5 (Appendix 1) have a 45 minute post-mortem pH values of less than 6.0, but their quality scores were high. Since the PSE pigs were selected on the basis of a low 45 minute pH, LR-5 was placed in the PSE group, even though the color was normal. This pig had a quality score of 11, which is fairly high. The high rating is a consequence of subjective score being based on a combination of marbling, color and firmness, with the first two factors counteracting the low score for firmness. If some other measurement, such as turbidity, had been used along with pH, this discrepancy may have been eliminated.

Results also show that there are different gradations of the PSE condition. This can be seen by the wide range of 45 minute post-mortem pH values presented in Tables 1, 2 and 3. These results agree with those of other workers (Bodwell et al., 1966; Briskey, 1964), who have

also observed considerable variability in the intensity of the PSE condition.

Histochemical Observations

Acid Phosphatase

Tissues from both PSE and normal pigs of each breed showed some degree of positive reaction for acid phosphatase. Alternate serial sections were stained for acid phosphatase and succinic dehydrogenase activity in order to determine the type of fibers which contained the greatest amount of acid phosphatase activity. Results show that acid phosphatase activity was limited mostly to the red fibers (those staining positive for succinic dehydrogenase) with a very small amount of activity in the intermediate fibers (those with a very small amount of positive staining for succinic dehydrogenase).

Figure 1 shows a photomicrograph of pork muscle stained for succinic dehydrogenase, while Figure 2 shows a serial section taken from the same area and stained for acid phosphatase activity. By comparing these two photomicrographs, one can see that the fibers giving a more positive reaction for succinic dehydrogenase also showed a stronger reaction for acid phosphatase.

In order to ascertain whether the acid phosphatase positive granules were due to the action of the enzyme on the substrate or to an artifact, serial sections of muscle were taken. One was incubated in the normal staining solution for acid phosphatase, while the other was

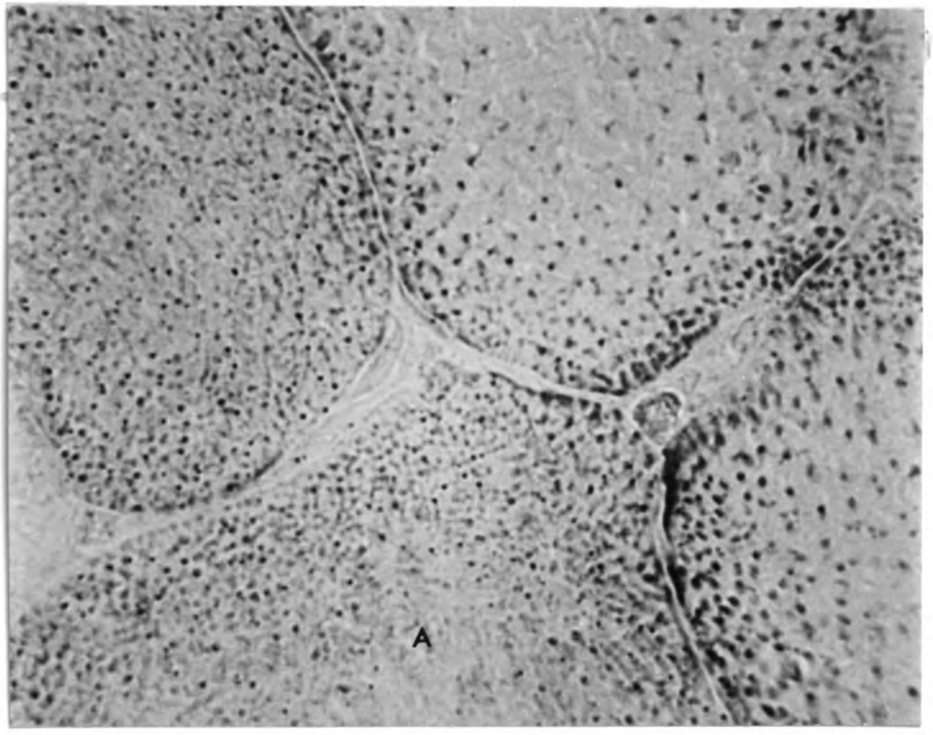

Figure 1. A photomicrograph of pig muscle stained for succinic dehydrogenase activity. The large fiber (A) is the same as in Figure 2. The small granules indicate enzyme activity.

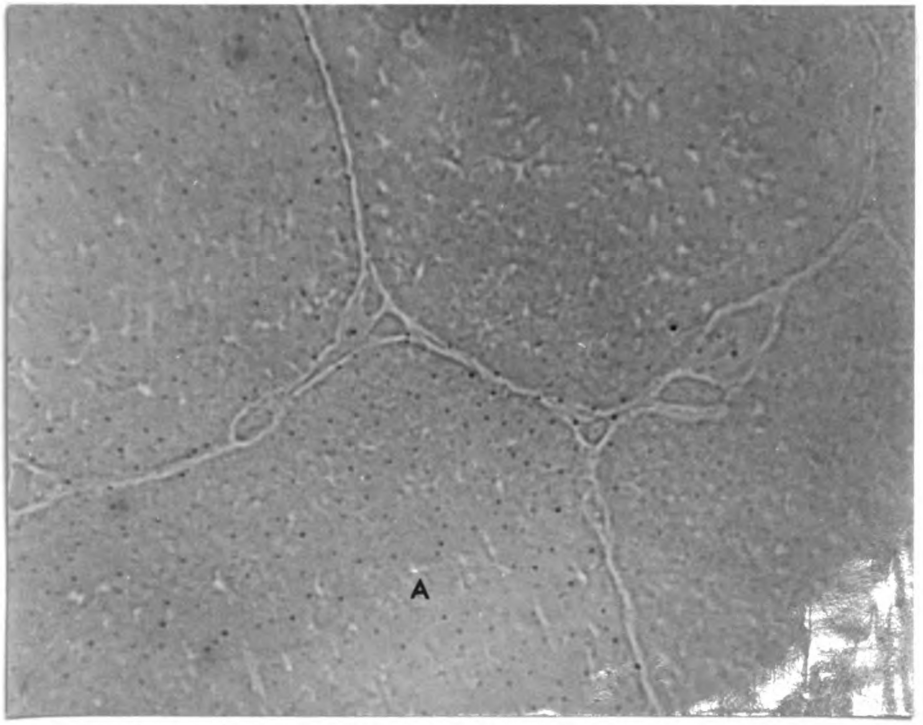

Figure 2. A photomicrograph of pig muscle serial to Figure 1 stained for acid phosphatase activity. The large fiber (A) is the same as in Figure 1. The small granules indicate enzyme activity.

incubated in the same staining solution without added enzyme substrate. Other than the enzyme substrate being absent in the one case, the two samples were treated identically. Results are shown in Figures 3 and 4. Figure 3 represents a typical photomicrograph of the section incubated in the acid phosphatase staining solution with substrate, while Figure 4 is a photomicrograph of the section incubated in the same solution without substrate. Figure 3 shows approximately the same amount of staining as does Figure 2, whereas, Figure 4 has no acid phosphatase positive granules. This indicates that the positive staining reaction is due to the effect of acid phosphatase on the substrate and suggests that the reaction is not an artifact.

Results of the present study suggest that there are acid phosphatase positive granules within skeletal muscle cells per se, which is in disagreement with other research. Some authors have stated that there are relatively few acid phosphatase positive granules present in skeletal muscle fibers (Straus, 1967; Bodwell et al., 1965), while others have stated that there are no acid phosphatase positive granules present in skeletal muscle cells (Ogata and Mori, 1963; Woessner, 1965). Further work should be done to determine the nature of these granules and to ascertain if they are of lysosomal origin.

Results show that there was no significant difference in the acid phosphatase activity of muscle from PSE and normal pigs. These results are summarized in Table 4 which presents mean values for the acid phosphatase activity of PSE and normal pigs for each breed. The values for

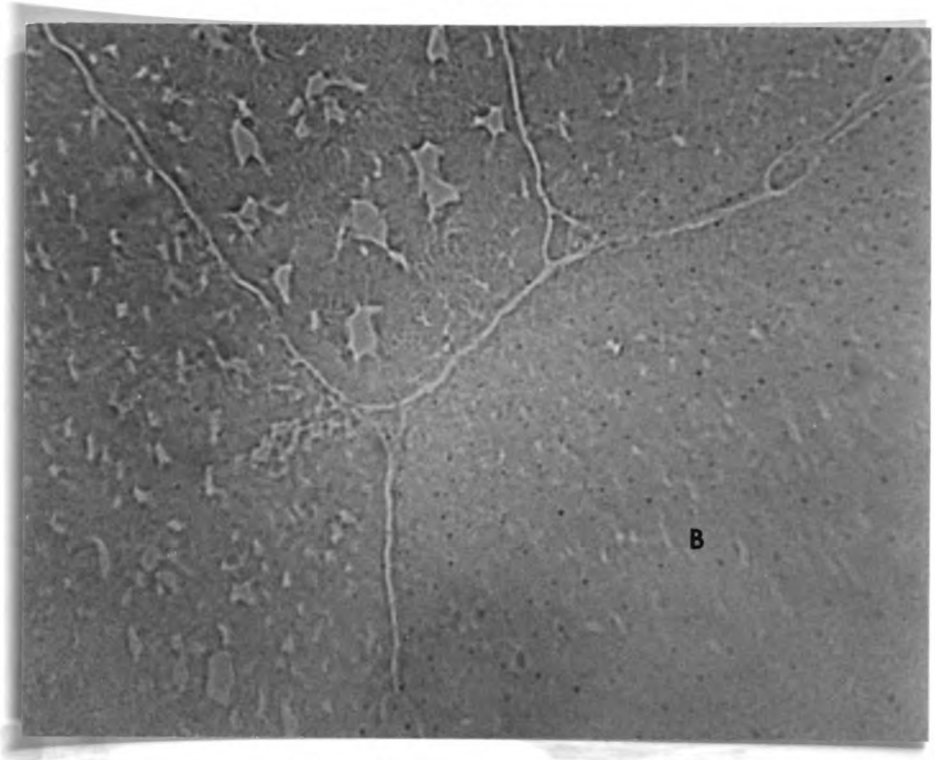

Figure 3. A photomicrograph of pig muscle stained for acid phosphatase activity. Fiber B is the same as in Figure 4. The small granules indicate enzyme activity.

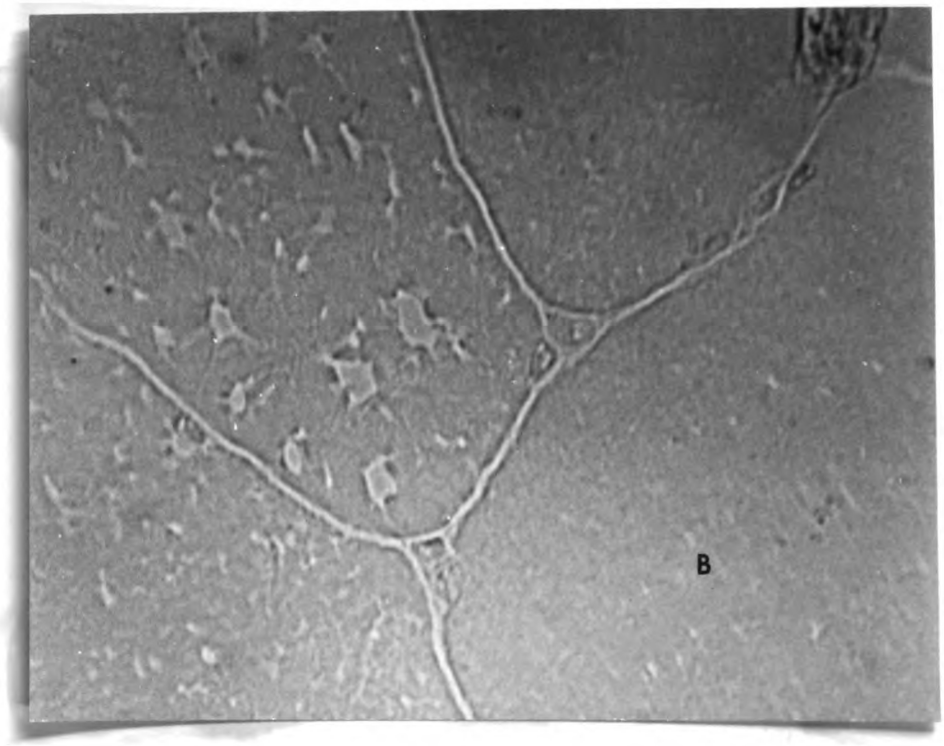

Figure 4. A photomicrograph serial to Figure 3 and incubated in the acid phosphatase staining solution without substrate. Fiber B is the same as in Figure 3.

Table 4. Mean values for acid phosphatase activity in Landrace, Poland China and Chester White pigs.[1]

Breeds	Time post-mortem		
	0 hr	45 min	3 hr
Landrace PSE	4.0	4.1	3.5
Landrace Normal	4.6	4.6	4.5
Poland China PSE	4.2	4.1	3.2
Poland China Normal	4.2	3.8	3.8
Chester White Normal[2]	2.9	2.6	2.1

[1]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

[2]All Chester White pigs were classified as normal.

acid phosphatase activity for all pigs of each breed are shown in Appendix Tables 7, 8 and 9.

Although there was no significant difference in acid phosphatase activity between PSE and normal pigs, there was a significant difference ($P < .05$) between the acid phosphatase activity of the Chester White pigs and the other two breeds. This shows that although different breeds may vary in their susceptability to the PSE condition (Judge et al., 1966), breed alone is not a good criterion for PSE muscle. Thus, selection for the PSE condition should be based upon a combination of post-mortem pH, quality score and turbidity measurements.

The fact that the PSE animals did not have a greater amount of acid phosphatase activity suggests that the muscles fibers were not degenerative and could not be classified as dystrophic. On the other

hand, high acid phosphatase activity has been shown to be indicative of muscle degeneration or dystrophy in the rat (Fennell and West, 1963), the lamb (Desai, 1966) and the rabbit (Tappel, 1966; and Bunyan et al., 1967). It is possible, however, that the methods used in the present study were not adequate to distinguish muscle degeneration, especially if the increased acid phosphatase activity was in the connective tissues.

There was no significant post-mortem change in acid phosphatase activity for any of the pigs studied.

Esterase

Some degree of positive reaction was shown for esterase by both PSE and normal pigs of each breed. Alternate serial sections were stained for esterase and succinic dehydrogenase activity in order to determine the type of fibers, which contained the greatest amount of esterase activity. The results show that esterase activity is limited mostly to the red fibers with a small amount of activity in the intermediate fibers.

Figure 5 shows a photomicrograph of pork muscle, which has been stained for esterase activity, while Figure 6 depicts a serial section stained for succinic dehydrogenase activity. By comparing Figure 5 and Figure 6, one can see that the fibers showing the strongest reaction for esterase activity also show the strongest reaction for succinic dehydrogenase activity.

These results are in agreement with those of Barron et al. (1966) and Ogata and Mori (1963), who stated that the esterase activity of muscle tissue is confined mainly to the red type fibers.

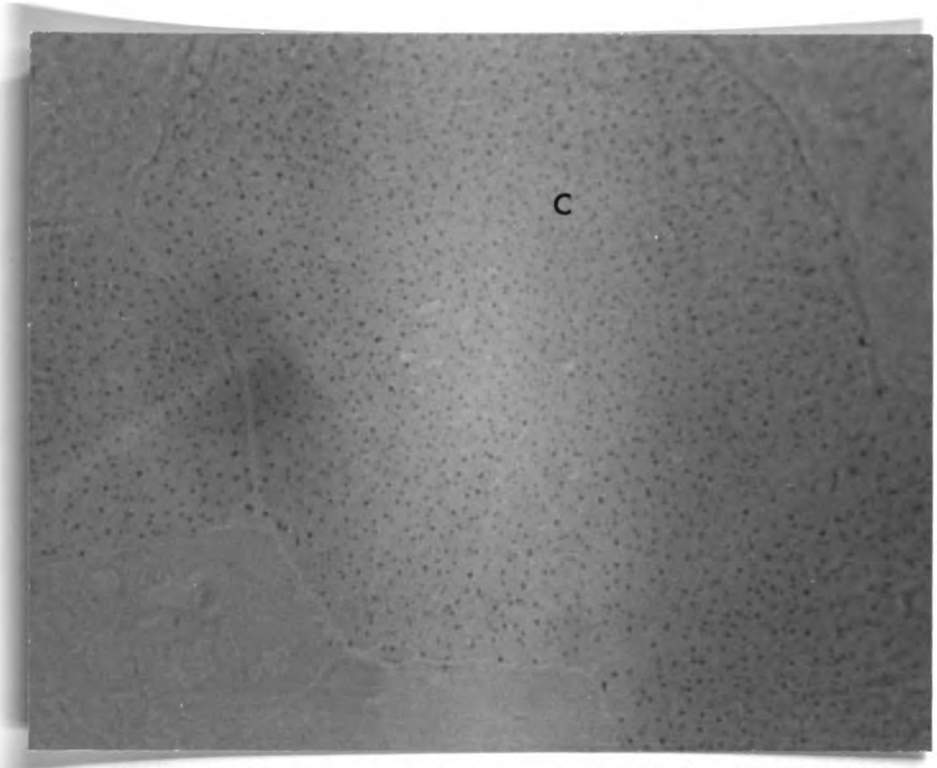

Figure 5. A photomicrograph of pig muscle stained for esterase activity. The small granules indicate enzyme activity. Fiber C is the same as in Figure 6.

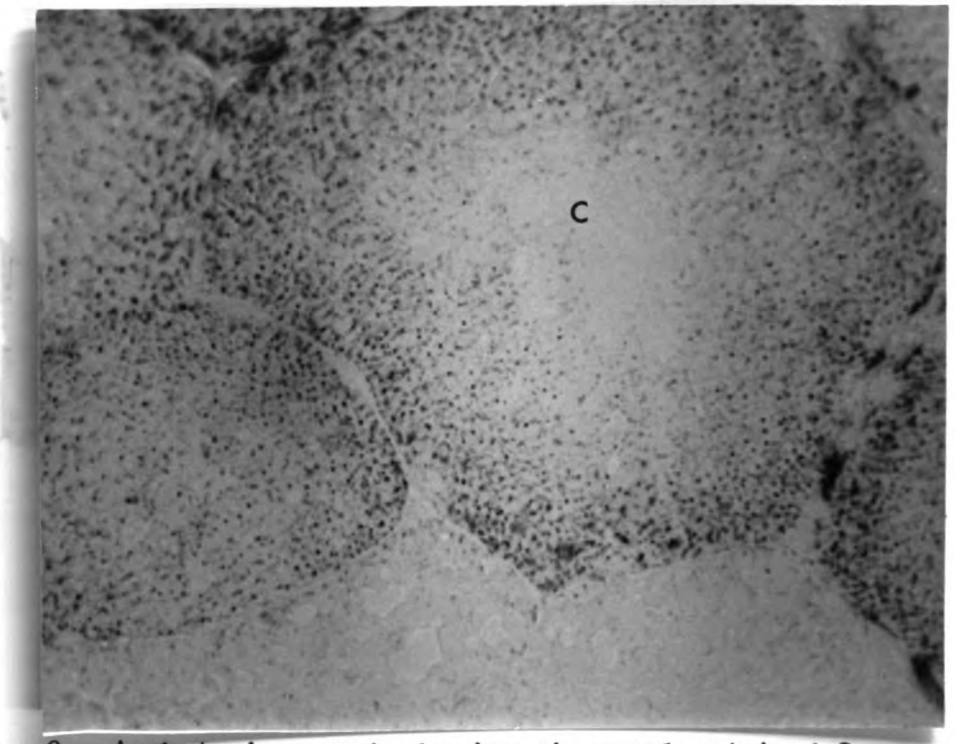

Figure 6. A photomicrograph showing pig muscle stained for succinic dehydrogenase activity (serial to Figure 5). The small granules indicate enzyme activity. Fiber C is the same as in Figure 5.

Results show that there was no significant difference in the esterase activity of PSE and normal porcine muscle. The amount of esterase activity did not differ significantly between breeds. The amount of esterase activity, however, did decrease markedly between 45 minutes and 3 hours post-mortem for all PSE and normal pigs studied. This reduction in activity is possibly due to the cooling of the samples between 45 minutes and 3 hours post-mortem. The results are summarized in Table 5, which gives the mean values for esterase activity of PSE and normal pigs of each breed. The individual values of esterase activity for all pigs of each breed are shown in Appendix Tables 10, 11 and 12.

Table 5. Mean values for esterase activity in Landrace, Poland China and Chester White pigs.[1]

Breeds	Time post-mortem		
	0 hr	45 min	3 hr
Landrace PSE	4.5	3.8	1.0
Landrace Normal	4.8	4.0	1.0
Poland China PSE	3.6	2.6	1.4
Poland China Normal	3.2	3.2	2.5
Chester White Normal[2]	4.7	4.2	1.0

[1]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

[2]All Chester White pigs were classified as normal.

Aryl Sulphatase

There was no positive reaction for aryl sulphatase activity in the muscle tissues of either the Landrace or the Chester White pigs. However, the muscle tissues of the Poland China pigs showed a very small amount of positive staining for aryl sulphatase activity. The positive reaction was so slight and sporadic that one is unable to draw any definite conclusions concerning its significance. There was no significant post-mortem change in aryl sulphatase activity for any of the pigs studied.

Figure 7 shows a representative photomicrograph of porcine muscle containing aryl sulphatase positive granules. Figure 8 shows a photomicrograph which does not show any aryl sulphatase activity. The mean values of aryl sulphatase activity for each breed are shown in Table 6. The individual values of aryl sulphatase activity for all pigs of each breed are shown in Appendix Tables 4, 5 and 6.

Table 6. Mean values for aryl sulphatase activity in Landrace, Poland China and Chester White pigs.[1]

Breeds	Time post-mortem		
	0 hr	45 min	3 hr
Landrace PSE	1.0	1.0	1.0
Landrace Normal	1.0	1.0	1.0
Poland China PSE	1.3	1.6	1.7
Poland China Normal	1.2	1.6	1.8
Chester White Normal[2]	1.0	1.0	1.0

[1]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

[2]All Chester White pigs were classified as normal.

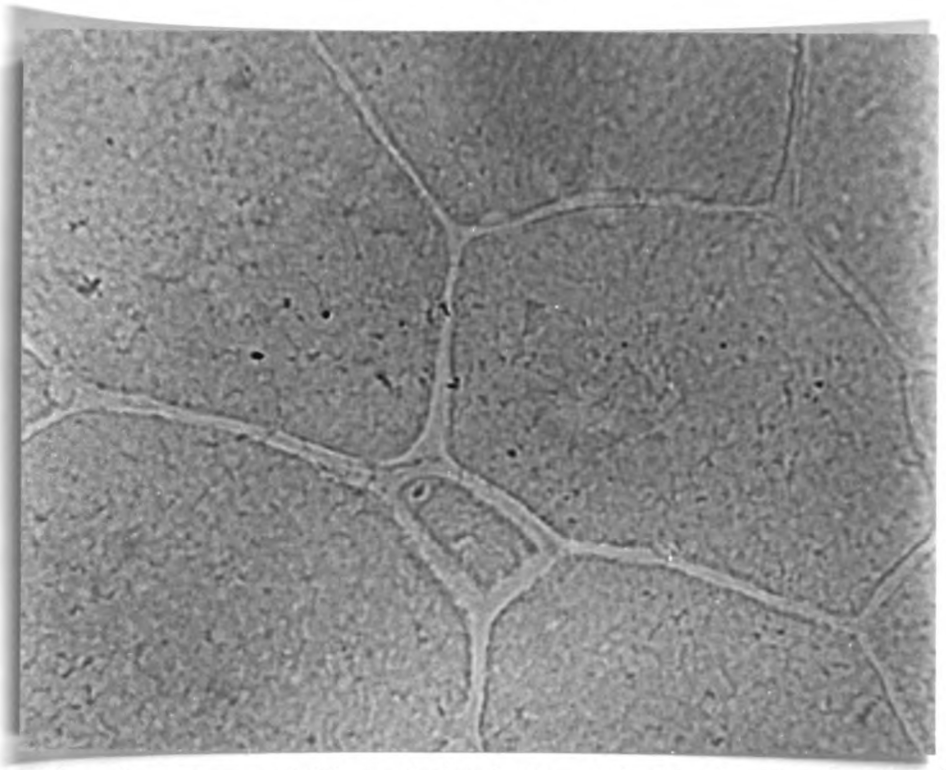

Figure 7. A representative photomicrograph of pig muscle showing a slight amount of aryl sulphatase activity. The small granules indicate enzyme activity.

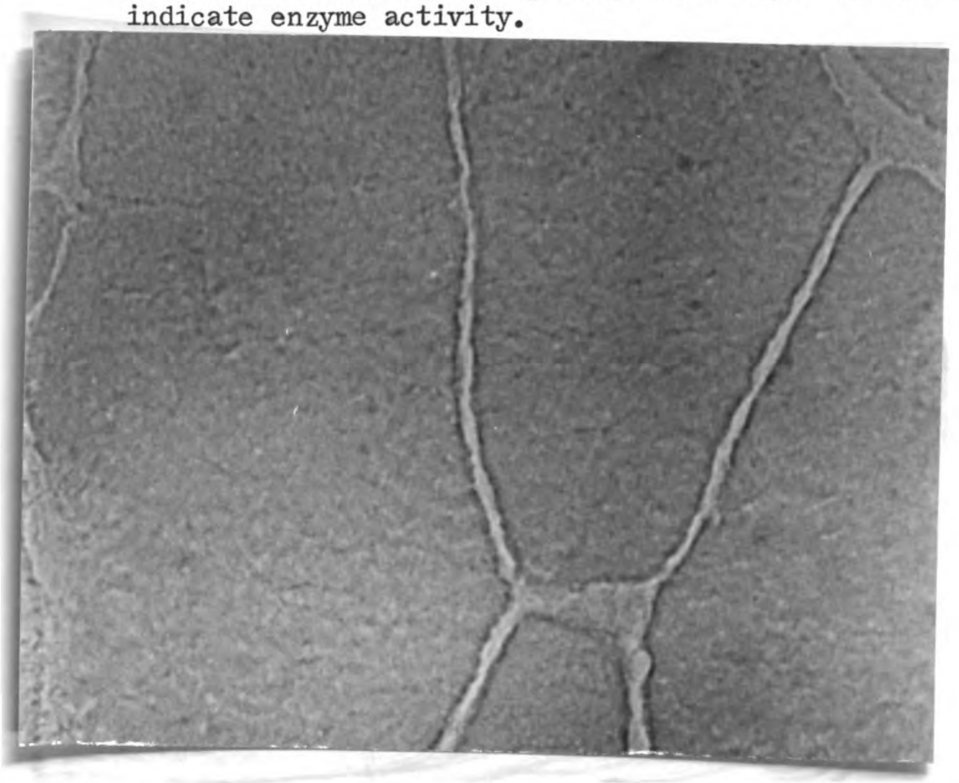

Figure 8. A representative photomicrograph of pig muscle showing no aryl sulphatase positive granules.

The fact that slight aryl sulphatase activity occurred in some of the Poland China pigs and not in the other breeds is interesting, since Poland China pigs tend to have a greater incidence of PSE muscle (Judge et al., 1967). Even though some pigs of the Poland China breed showed positive aryl sulphatase activity there was no difference in the amount of activity between Poland China pigs with PSE and normal muscle. This is verified by the data in Table 6. However, it is still possible that some degree of association between aryl sulphatase activity and PSE muscle may exist but the small numbers used in the present study may have limited significance.

Beta Glucuronidase

There was no positive reaction for β-glucuronidase activity in the muscle tissue of any of the pigs that were used in this study.

A test was made to determine if the staining procedure was in error. This was done by subjecting liver tissue, which has been shown to give a positive reaction for β-glucuronidase (Pearse, 1960), to the same staining solution used for the muscle tissue. The liver tissue gave a strong positive reaction for β-glucuronidase, which is shown in Figure 9. This can be compared to Figure 10, which shows no activity in muscle tissue.

Although no positive reaction was evident for β-glucuronidase in the muscle tissue it is still possible that more sensitive methods for determining activity may have detected slight amounts of this enzyme.

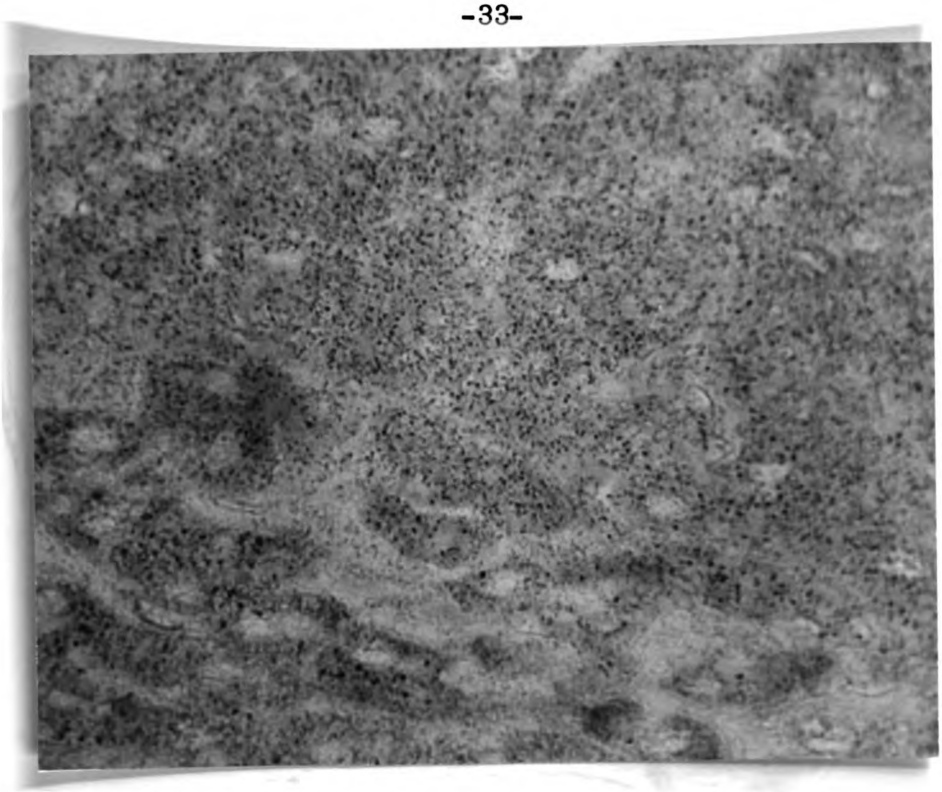

Figure 9. A photomicrograph of liver tissue stained for β-glucuronidase activity. The small granules indicate enzyme activity.

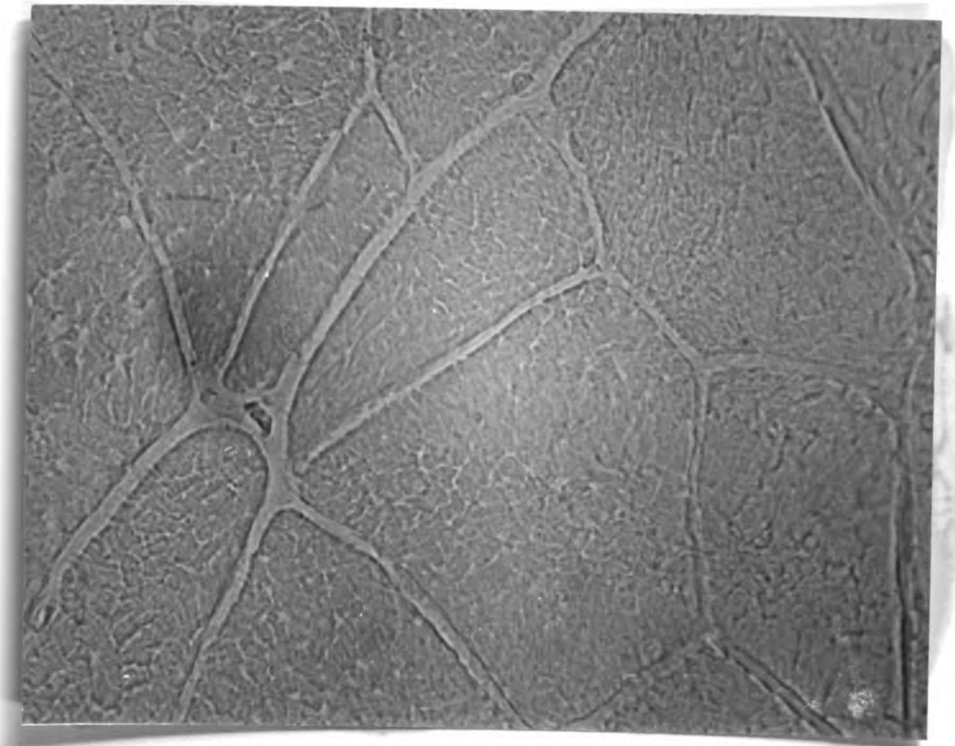

Figure 10. A photomicrograph of pig muscle showing no β-glucuronidase activity.

Beta-Galactosidase

All tests for β-galactosidase were negative in the muscle tissue of all pigs utilized in this investigation.

Figure 11 shows a photomicrograph of liver tissue which was stained for β-galactosidase. The test for activity in muscle tissue is shown in Figure 12. The positive reaction obtained in the liver tissue indicates that the staining procedure was not in error.

Although no positive reaction was found in the muscle tissue for β-galactosidase, it is still possible that more precise methods may have revealed activity for this enzyme.

Figure 11. A photomicrograph of liver tissue stained for β-galactosidase activity. The small granules indicate enzyme activity.

Figure 12. A photomicrograph of pig muscle showing no β-galactosidase activity.

SUMMARY

The activity of 5 lysosomal enzymes, including acid phosphatase, esterase, aryl sulphatase, β-glucuronidase and β-galactosidase, was measured by histochemical methods on each of 25 pigs. The pigs studied consisted of 10 Landrace (5 normal and 5 PSE), 10 Poland Chinas (5 normal and 5 PSE) and 5 Chester Whites (all normal). Enzyme activity was measured at 0 hour, 45 minutes and 3 hours post-mortem.

There was no significant difference in enzyme activity between PSE and normal pigs for any of the enzymes studied. However, there was a significant difference ($P < .05$) in acid phosphatase activity between Chester White pigs, which have a low incidence of the PSE condition, and the other two breeds, which are known to have a high incidence of PSE muscle.

None of the pigs studied gave a positive reaction for β-glucuronidase or β-galactosidase. There was no change in enzyme activity over the different times post-mortem, except for esterase. Practically all esterase activity disappeared between 45 minutes and 3 hours post-mortem.

Results of this study suggest that the PSE condition is not caused by increased lysosomal enzyme activity. However, breed differences were found in acid phosphatase activity between so-called stress-susceptible and stress-resistant pigs, which may indicate an indirect involvement in the PSE condition.

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APPENDIX

Appendix 1. Weight, sex, quality score and pH at different times post-mortem in Landrace pigs.

Hog No.[1]	Live wt	Sex[2]	L.D.[3] post-mortem pH				Quality score[4]	PSE or normal[5]
			0 hr	15 min	45 min	3 hr		
LR-1	216	G	6.30	5.98	5.48	5.14	6^{-}	PSE
LR-2	211	G	6.48	6.43	6.26	5.20	9^{+}	N
LR-3	220	G	6.22	6.05	5.70	5.32	6^{++}	PSE
LR-4	226	G	6.40	6.33	6.08	5.14	6^{+}	
LR-5	226	G	6.17	6.12	5.97	5.23	11^{-}	PSE
LR-6	240	G	6.20	5.84	5.20	5.16	4^{++}	PSE
LR-7	208	B	6.34	6.30	6.16	5.22	5	
LR-8	230	B	6.36	6.31	6.26	5.21	10	N
LR-9	183	G	6.22	6.18	6.18	5.27	10^{-}	
LR-10	196	G	6.36	6.28	6.18	5.30	11^{++}	N
LR-11	196	B	6.34	6.27	5.97	5.22	4^{++}	PSE
LR-12	191	B	6.36	6.29	6.27	5.33	12^{++}	N
LR-13	190	G	6.30	6.32	6.20	5.36	13^{--}	N
LR-14	192	B	6.28	6.24	6.02	5.24	8^{+}	
LR-15	201	G	6.36	6.26	6.12	5.28	9^{-}	
LR-16	192	G	6.32	6.26	6.10	5.28	7	

[1]LR = Landrace
[2]Sex condition is indicated by G = gilt and B = barrow.
[3]L.D. = Longissimus dorsi muscle.
[4]Quality score was determined by a 15 point scale, 5 points each was assigned to marbling, color and firmness.
[5]PSE = Pale, soft and exudative, N = normal, a blank space indicates the pig was not utilized for following experiments and was not classified as to muscle condition.

Appendix 2. Weight, sex, quality score and pH at different times post-mortem in Poland China pigs.

Hog No.[1]	Live wt	Sex[2]	L.D.[3] post-mortem pH 0 hr	15 min	45 min	3 hr	Quality score[4]	PSE or normal[5]
PC-1	195	B	6.44	6.18	6.06	5.55	13	
PC-2	180	G	6.18	5.92	5.80	5.64	7	
PC-3	190	G	6.44	6.54	6.55	5.92	10^{+}	N
PC-4	194	G						
PC-5	172	G	6.02	5.52	5.72	5.26	5^{+}	
PC-6	180	G	6.10	6.12	6.06	5.29	8	
PC-7	148	G	6.15	5.94	5.67	5.48	9	
PC-8	161	G	6.28	6.18	6.10	5.55	11	N
PC-9	168	G	6.19	5.88	5.63	5.54	6^{+}	
PC-10	163	G	6.26	6.14	6.05	5.40	7^{-}	N
PC-11	163	G	6.36	6.11	6.05	5.31	10^{-}	N
PC-12	210	G	6.06	5.76	5.65	5.28	5^{+}	
PC-13	209	G	6.21	6.15	6.22	5.37	11^{+}	N
PC-14	191	G	6.14	5.80	5.61	5.34	4^{++}	PSE
PC-15	204	G	6.19	5.58	5.42	5.40	5	PSE
PC-16	165	G	6.20	5.68	5.27	5.34	8^{--}	PSE
PC-17	198	G	6.02	5.66	5.33	5.26	5	PSE
PC-18[6]	205	G	6.35	5.90	5.50	5.40	11^{+}	
PC-19	201	G	6.33	6.14	5.80	5.30	5^{+++}	PSE

[1]PC = Poland China
[2]Sex condition is indicated by G = gilt and B = barrow.
[3]L.D. = Longissimus dorsi muscle.
[4]Quality score was determined by a 15 point scale, 5 points each was assigned to marbling, color and firmness.
[5]PSE = Pale, soft and exudative, N = normal, a blank space indicates the pig was not utilized for following experiments and was not classified as to muscle condition.
[6]PC-18 was soft and exudative but was dark in color.

Appendix 3. Weight, sex, quality score and pH at different times post-mortem in Chester White pigs.

Hog No.[1]	Live wt	Sex[2]	L.D.[3] post-mortem pH				Quality score[4]	PSE or normal[5]
			0 hr	15 min	45 min	3 hr		
CW-1	204	G	6.14	6.09	6.07	5.22	12^{+}	N
CW-2	225	B	6.34	6.36	6.45	5.28	13	N
CW-3	213	B	6.14	6.22	6.16	5.24	7^{-}	
CW-4	198	G	6.31	6.30	6.26	5.32	14^{+}	N
CW-5	192	G	6.28	6.32	6.38	5.58	13^{-}	N
CW-6	210	G	6.31	6.24	6.13	5.22	12^{+}	N

[1] CW = Chester White

[2] Sex condition is indicated by G = gilt and B = barrow.

[3] L.D. = Longissimus dorsi muscle.

[4] Quality score was determined by a 15 point scale, 5 points each was assigned to marbling, color and firmness.

[5] PSE = Pale, soft and exudative, N = normal, a blank space indicates the pig was not utilized for following experiments and was not classified as to muscle condition.

Appendix 4. Aryl sulphatase activity in Landrace pigs.[1]

PSE group				Normal group			
	Time post-mortem				Time post-mortem		
Hog No.	0 hr	45 min	3 hr	Hog No.	0 hr	45 min	3 hr
LR-1	1	1	1	LR-2	1	1	1
LR-3	1	1	1	LR-8	1	1	1
LR-5	1	1	1	LR-10	1	1	1
LR-6	1	1	1	LR-12	1	1	1
LR-11	1	1	1	LR-13	1	1	1
Mean	1	1	1		1	1	1

[1]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

Appendix 5. Aryl sulphatase activity in Poland China pigs.[1]

PSE group				Normal group			
	Time post-mortem				Time post-mortem		
Hog No.	0 hr	45 min	3 hr	Hog No.	0 hr	45 min	3 hr
PC-14	1.0	2.0	3.0	PC-3	1.0	1.5	2.5
PC-15	1.0	1.5	2.5	PC-8	1.0	1.5	1.0
PC-16	2.0	1.5	1.0	PC-10	1.0	1.0	1.5
PC-17	2.5	2.0	1.0	PC-11	1.5	1.5	1.5
PC-19	2.0	1.0	1.0	PC-13	1.5	2.5	2.5
Mean	1.3	1.6	1.7		1.2	1.6	1.8

[1]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

Appendix 6. Aryl sulphatase activity in Chester White pigs.[1,2]

Hog No.	Time post-mortem		
	0 hr	45 min	3 hr
CW-1	1.0	1.0	1.0
CW-2	1.0	1.0	1.0
CW-4	1.0	1.0	1.0
CW-5	1.0	1.0	1.0
CW-6	1.0	1.0	1.0
Mean	1.0	1.0	1.0

[1]All Chester White pigs were classified as normal.

[2]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

Appendix 7. Acid phosphatase activity in Landrace pigs.[1]

PSE group				Normal group			
	Time post-mortem				Time post-mortem		
Hog No.	0 hr	45 min	3 hr	Hog No.	0 hr	45 min	3 hr
LR-1	3.0	3.0	3.0	LR-2	3.0	3.0	3.0
LR-3	4.5	4.5	4.5	LR-8	4.0	5.0	5.5
LR-5	5.0	4.0	4.0	LR-10	5.0	5.0	4.0
LR-6	3.5	5.0	2.0	LR-12	5.0	5.0	5.0
LR-11	4.0	4.0	4.0	LR-13	6.0	5.0	5.0
Mean	4.0	4.1	3.5		4.6	4.6	4.5

[1]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

Appendix 8. Acid phosphatase activity in Poland China pigs.[1]

PSE group				Normal group			
	Time post-mortem				Time post-mortem		
Hog No.	0 hr	45 min	3 hr	Hog No.	0 hr	45 min	3 hr
PC-14	4.5	3.5	4.0	PC-3	4.5	4.0	4.0
PC-15	4.5	3.5	3.5	PC-8	3.5	4.0	4.0
PC-16	4.0	4.0	4.0	PC-10	5.5	3.5	4.0
PC-17	4.5	4.0	3.5	PC-11	4.0	4.0	3.5
PC-19	3.5	5.5	1.0	PC-13	3.5	3.5	3.5
Mean	4.2	4.1	3.2		4.2	3.8	3.8

[1]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

Appendix 9. Acid phosphatase activity in Chester White pigs.[1,2]

	Time post-mortem		
Hog No.	0 hr	45 min	3 hr
CW-1	3.5	2.0	2.0
CW-2	3.5	2.5	3.0
CW-4	2.0	2.5	2.0
CW-5	2.0	2.0	1.0
CW-6	3.5	4.0	2.5
Mean	2.9	2.6	2.1

[1]All Chester White pigs were classified as normal.

[2]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

Appendix 10. Esterase activity in Landrace pigs.[1]

PSE Group				Normal Group			
	Time post-mortem				Time post-mortem		
Hog No.	0 hr	45 min	3 hr	Hog No.	0 hr	45 min	3 hr
LR-1	5.0	4.0	1.0	LR-2	5.0	4.0	1.0
LR-3	4.0	3.5	1.0	LR-8	4.0	4.0	1.0
LR-5	5.5	5.5	1.0	LR-10	5.0	5.0	1.0
LR-6	4.0	2.0	1.0	LR-12	5.0	3.5	1.0
LR-11	4.0	4.0	1.0	LR-13	5.0	3.5	1.0
Mean	4.5	3.8	1.0		4.8	4.0	1.0

[1]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

Appendix 11. Esterase activity in Poland China pigs.[1]

PSE group				Normal group			
	Time post-mortem				Time post-mortem		
Hog No.	0 hr	45 min	3 hr	Hog No.	0 hr	45 min	3 hr
PC-14	4.5	4.5	1.0	PC-3	3.0	5.0	5.0
PC-15	3.5	2.0	1.0	PC-8	3.5	5.0	4.5
PC-16	4.0	3.5	2.0	PC-10	5.0	4.0	1.0
PC-17	5.0	1.0	2.0	PC-11	1.0	1.0	1.0
PC-19	1.0	2.0	1.0	PC-13	3.5	1.0	1.0
Mean	3.6	2.6	1.4		3.2	3.2	2.5

[1]The rating system used for enzyme activity was: 1 = none, 2 = traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.

Appendix 12. Esterase activity in Chester White pigs.[1,2]

	Time post-mortem		
Hog No.	0 hr	45 min	3 hr
CW-1	4.5	4.5	1.0
CW-2	5.0	5.0	1.0
CW-4	5.0	4.5	1.0
CW-5	5.0	4.0	1.0
CW-6	4.0	3.0	1.0
Mean	4.7	4.2	1.0

[1]All Chester White pigs were classified as normal.

[2]The rating system used for enzyme activity was: 1 = none, 2 - traces, 3 = weak, 4 = moderate, 5 = moderately strong and 6 = strong.