THE EFFECT OF ANTEMORTEM STRESS ON POSTMORTEM BEEF AND LAMB CARCASS CHARACTERISTICS

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The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled THE EFFECT OF ANTEMORTEM STRESS ON POSTMORTEM BEEF AND LAMB CARCASS CHARACTERISTICS

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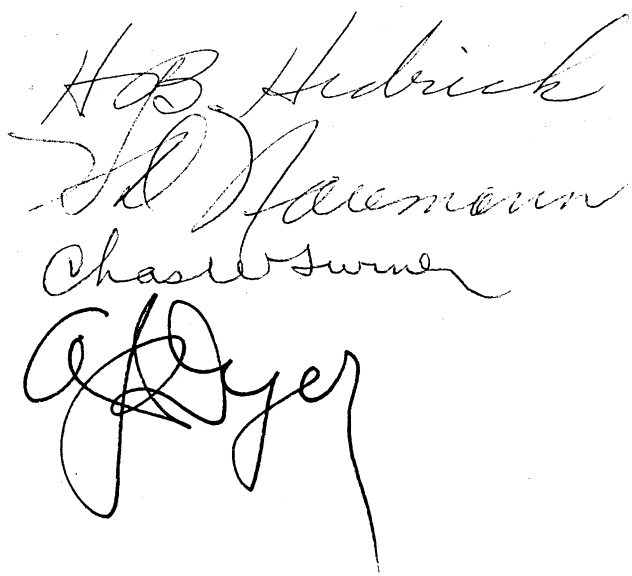

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CHAPTER I

INTRODUCTION

The color of beef and lamb muscle is an important factor in the grading of carcasses and the acceptability of a given retail cut by the consumer. The consumer often associates dark color of muscle with many conditions such as: advanced age of animal, improper handling of animal at the time of slaughter, underfinished animals, a general lack of quality, and the possibility that the product has been cut for an excessive length of time. Dark color of muscle in lamb is associated with carcasses from older animals.

Many theories have been advanced by research workers to explain the variations in the color of beef muscle. Theories which have been advanced as listed by Mackintosh (1935) are: age of cattle at time of slaughter, grass fattening, exercise, delayed bleeding, kind of feed consumed by the animal, amount of blood in the muscle, particular pigmentation and inheritance. Bull (1930) reported age, ration and amount of finish had considerable influence upon the color of beef. Hedrick (1957) reported stress was the cause of dark cutting carcasses. Stress reduces the glycogen content of the muscle, thereby reducing the subsequent lactic acid content of the muscle after the death of the animal. There is a close relationship between the pH value and the color of beef muscle.

Little or no work has been reported on the reason for variation in color of lamb muscle, especially the flank and diaphragm muscles.

Dark cutting beef carcasses and lamb carcasses that are dark in the flank are lowered in grade from one to three grades below carcasses of similar quality, conformation and finish. The main outlet the packer has for dark cutting beef is to hotel and restaurant trade at a reduced price resulting in considerable economic loss.

Numerous compounds are available which are claimed to act as anti-stress agents. Among these compounds are numerous tranquilizers which have been used to control or prevent stress due to anxiety or excitement in several species of animals. Stress caused by excitement has been shown to produce dark cutting beef. It would seem feasible to determine whether the administration of tranquilizers would prevent the carcasses of cattle subjected to excitement from dark cutting.

Hold-over feeding by the processor is a possible means of reducing the incidence of dark cutting beef. The time interval required for cattle to replenish their glycogen stores to normal after being depleted to a level that the subsequent carcass would cut dark is of considerable importance.

The objectives of this investigation were to determine (1) the effect of antemortem stress on the color of

lamb carcasses; (2) the recovery period required to replenish the glycogen stores of cattle following antemortem stress; and (3) the possibility of using insulin and tranquilizers to alleviate dark cutting beef.

CHAPTER II

REVIEW OF LITERATURE

Characteristics of Dark Cutting Beef

Dark cutting beef has definite characteristics which distinguish it from normal bright colored beef. Hall *et al.* (1944) stated that dark beef is in general less acid than bright beef and has a pH value which will be as much as one unit higher than normal. The tissue of dark beef has a low oxidation potential and a sticky gummy texture due to the tissue being less permeable to moisture and oxygen than bright beef. It was not clear whether the deficiency of oxygen was caused by low permeability of the tissue or an excessive demand for oxygen by postmortem metabolic processes. The carbohydrate reserve was nearly nonexistent in the dark beef tissue. Other characteristics recognized for dark cutting muscles were low glucose, practically no glycogen, high inorganic phosphate, and rapid oxygen uptake.

The National Livestock and Meat Board (1949) reported that dark cutting beef was characterized by lower acidity, lower glycogen and lower reducing sugar content. Further study indicated that there was no reason to believe that dark cutting beef was different from light beef in nutritive value.

Bull *et al.* (1941) stated that the color of dark beef can range from a dark red to a purplish-black color.

Hedrick (1958) stated that normally when a beef carcass is ribbed, the color of the muscle is purplish and upon exposure to the atmosphere the color will brighten to a cherry red color. In dark cutting beef, oxygenation of myoglobin to oxymyoglobin does not occur; or, if so, at a much slower rate. The pH and dark color of muscle are closely related. At pH 5.4 the muscle of beef will be a bright cherry red, at 5.8 the color is shady or dark red, at 6.2 - 6.8 the color is purplish black. The muscle membrane is less permeable to oxygen at low hydrogen ion concentrations and myoglobin is not oxygenated. The membrane is also less permeable to the passage of moisture out of the cell when the hydrogen ion concentration is lower. This accounts for the characteristic sticky, gummy texture of dark muscle. Steaks from dark cutting carcasses were found to be comparable in eating qualities to steaks from bright cutting carcasses of like grade and age. Determinations were made for tenderness, flavor, juiciness and aroma by a taste panel. No significant relationship was found between the carcass pH value and subsequent organoleptic characteristics.

Significance of Dark Cutting Beef

Dark cutting beef is of considerable economic importance to both the producer and processor. Mackintosh (1932) stated that about five per cent of beef carcasses are lowered in grade because of the color of the lean. Beef carcasses may cut dark regardless of grade, sex or age of animal.

Bull et al. (1941) stated that as a result of discrimination by consumers, the grade and wholesale price of dark cutting beef is lowered. Depending on the severity of darkness, the market grade may be lowered from one to three grades lower than cattle of like conformation and finish.

Hall et al. (1944) stated that the sale of dark cutting beef must be induced by a sacrifice in grade and price.

Munns and Burrell (1958) took the pH values of 1800 beef carcasses and found 12.2 per cent to have a pH value above 6.0. The percentages of carcasses with high pH values by sex were: steers 13.9, heifers 11.7, cows 9.5, yearlings 8.6 and baby beeves 9.9. As a continuation of this work, pH values were taken on 6,589 hot graded cattle. This group was made up of 4,914 cows, 882 steers and 793 heifers. Of the cows, 11.2 per cent had high loin pH values, steers 5.8 percent and heifers 5.7 per cent. The incidence for the cows was significantly higher than for

steers and heifers. The incidence of carcasses with high pH values by week ranged from 0.9 per cent to 26.3 per cent. This variation though not explained could possibly be attributed to variation in weather conditions or differences in handling conditions.

Factors Associated With Dark Cutting Beef

Consumers prefer beef with a bright cherry red color and object to beef with a very dark red or purplish black color. The objection to dark beef is probably based on the supposition that the beef is dark because it was produced by a low quality animal, or that the animal was handled improperly at the time of slaughter, or the subsequent carcass was handled improperly. For these reasons, dark cutting beef has been of interest to research workers for many years. During this time, many factors have been investigated as possible causes of variations in color of beef.

Mackintosh (1932) stated that many packers consider pasture as being the cause of dark cutters and therefore discriminate against grass fat cattle. Bull et al. (1930) found the color of partly finished beef to be lighter in color than the carcasses of grass fat cattle. Mackintosh (1935), Longwell (1936), and Bull et al. (1941) stated that grass fat cattle had a brilliance of color equal to that of cattle finished in dry lot. The color of the lean was equally as bright as with cattle fed grain in dry lot or cattle fed grain on pasture. The conclusions were drawn from these experiments that grass as a feed would not produce dark lean in beef and that full feeding cattle while on pasture would not cause dark cutting beef.

Bull et al. (1930), Mackintosh and Hall (1935), and

Longwell (1936) stated that the age of the animal has a direct effect on the color of beef muscle. Older cattle have darker red lean while young cattle have a light bright red color lean. However, with advanced age the dark color was not comparable to that of dark cutting beef. Helser et al. (1930) reported that with increasing age of animals, color of the muscle darkens.

Mackintosh and Hall (1935) stated that in theory exercise may be the cause of dark cutting but later Bull and Rusk (1942) found that there were no indications that either light or heavy exercise had any effect upon the color of the lean as determined by spectrophotometric studies. Proctor and Best (1932) exercised dogs for a period of two to three weeks and found there was a glycogen buildup in the muscle for a short period of time. However, if the training was continued for a longer period of time there was no significant rise over the basal two to three weeks.

Hall et al. (1944) found that dark color is not caused by the presence of any abnormal pigments. An aqueous extract of dark beef was found to be normal bright red in color with normal spectrophotometric characteristics. If adequate oxygen was introduced into dark meat it would brighten and become a normal bright color. This indicated that the dark color might be due to incomplete oxygenation of muscle myoglobin. It seemed evident that a deficiency

of oxygen existed in dark beef. However, it was not clear whether the deficiency was caused by low permeability of the tissue or excessive demand for oxygen by postmortem metabolic processes. These workers also found a relation between the pH of the meat and the color. The characteristic high pH values in dark beef indicate inhibition of acid forming enzyme systems in the tissue.

The National Livestock and Meat Board (1949) reported on their studies using 4-H club calves exhibited at the 1938, 1939, 1940 and 1941 International Livestock Expositions. Their data was substantiated by a study of a large number of commercial cattle. Significant findings resulting from these studies were that dark cutting beef is characterized by lower acidity, lower glycogen level and lower reducing sugar content. Dark cutters were produced experimentally through the lowering of muscle sugar content by administering massive doses of insulin.

The following factors were found responsible for, or attributing to dark cutting beef: (1) the calves cutting lightest in color had received a higher ratio of grain to protein supplement than those which were off color; and, (2) when the calves were chilled and feed was withheld for a certain period, five per cent were dark cutters, whereas no dark cutters were found among similar calves which were well fed but chilled. This would seem to indicate that combined chilling and a lack of feed increased the

incidence of dark cutters.

Hall et al. (1944) reported on calves that were subjected to rigorous exposure and were not fed for two days prior to slaughter. With no other cause for excitement, twenty eight per cent of a lot of fifty three cattle were dark cutters. These animals had been accustomed to ample provisions and comfortable quarters and were left in the cold and a rain without any shelter before slaughter. This would indicate that dark cutters are caused by inadequate carbohydrate reserves and inadequate conditioning of the animals to cultivate resistance against shock from drastic changes.

Hedrick (1957) stated that dark cutting beef is produced as the result of cattle being subjected to prolonged periods of stress shortly prior to slaughter. These studies suggest that approximately twenty four hours of stress were required to sufficiently deplete the muscle glycogen of normal animals to cause subsequent dark cutting carcasses. Shorter periods of stress had no marked effects on subsequent carcass characteristics. From these studies it was concluded that dark cutting beef was the result of decreased glycogen in the animal muscle, thereby causing a low lactic acid content. When stress such as excitement or the administration of adrenaline was prolonged, the homeostatic mechanism for maintaining normal glycogen stores was exhausted and dark cutting carcasses

subsequently resulted. In this study a total of sixty three yearling Shorthorn cattle were stressed by the following methods; (1) periodic prodding with an electric hot-shot; and (2) intramuscular or subcutaneous injections of adrenaline.

The administration of adrenaline produced hyperglycemia and glycosuria. This induced physiological stress simulates those reactions which occur when an animal is subjected to an environmental stress. Either subcutaneous or intramuscular injections of three milligrams of adrenaline per one hundred pounds body weight consistently produced dark cutting carcasses. As the level of adrenaline was increased, the color of the subsequent muscle darkened, the muscle pH became higher, and the texture of the muscle became stickier and gummier.

Stress arouses the sympathetic nervous system causing the adrenals to secrete adrenaline. Adrenaline causes the activation of phosphorylase in muscle and liver which causes hyperglycemia and glycosuria. Such factors as excitement, trauma, fatigue, and exposure to adverse weather would arouse sympathetic-adrenal mechanism and cause the depletion of the glycogen reserves. The susceptibility of the individual cattle to stress and the intensity and duration of such conditions will determine the prevalence of dark cutting carcasses.

Howard and Lawrie (1957) reported that steers which

were exercised immediately after removal from a train and then slaughtered had a statistically significant decrease in glycogen in the liver and muscles when compared with steers which were fed, watered and rested for fourteen days after removal from the train before being slaughtered. The ultimate pH of the exercised steers was higher than the controls. Steers which received massive doses of insulin to the point of severe convulsions from insulin tetany had greatly depleted muscle glycogen stores as compared to normal animals. Pyrophosphate injections in steers caused accelerated glycogenolysis to occur in the liver and caused considerable aerobic glycolysis to occur in the musculature, resulting in high pH. The glucose level was much lower for injected steers than for the controls. Injections of ephedrine had no effect in lowering muscle glycogen and none on the ultimate pH.

Howard and Lawrie (1956) reported that a steer given large injections of magnesium sulfate had higher initial glycogen, ATP, and initial pH values than did a control. Steers which received large quantities of glucose had a high muscle glycogen content and therefore reached a normal ultimate pH. In an experiment using 200 units of insulin per kilogram body weight over a twenty hour period, the ultimate pH of the longissimus dorsi was 7.15. This was much higher than occurred in a control steer. In another study involving thirty steers, the steers showing

indications of extreme excitability had a higher ultimate pH and a lower glycogen level than did more docile animals.

It was found that when steers were fasted, then forcibly exercised for one and one half hours, the ultimate pH value would be significantly higher than control animals and the glycogen level would be lower than that of the control animals. However, neither fasting nor forced exercise alone would result in a high pH or in low glycogen levels.

Brisky et al. (1957) stated that the effects of forced exercise of hogs were darker color, higher pH value than normal and a sticky, gummy surface.

Rongey (1958) reported that a more uniform color of the lean ham area resulted from antemortem injections of adrenaline. The same results could be achieved by exciting the hogs with an electric hot-shot for fifteen minutes. The adrenaline injections and the forced exercise were administered fourteen hours antemortem. The muscle area of the hams from hogs injected with adrenaline was darker than the muscle area of the hams from the controls receiving no adrenaline.

The Physiological Effect of Stress on Farm Animals

Dukes (1955) stated that when an animal is submitted to conditions causing a state of stress, the adrenal medulla releases adrenaline. As adrenaline is released there is an inhibitory effect on intestinal movements, acceleration of the heart rate, increase in blood pressure and mobilization of glucose in the blood. This is accompanied by a break down of lymphocytes in the blood and transformation of non-sugars into carbohydrates. These conditions take place regardless of the cause of the stress conditions. Stress can be caused by physical alarm, exposure to excessive cold or by the use of anesthetics and bacterial invasion.

Selye(1950) reported that many factors will produce stress in animals, these being; trauma, nervous stimuli, muscular exercise, hormones, diet, temperature, hemorrhage, electric injury, anoxia, asphyxia and drugs. Strong emotions, such as rage and fear, can produce shock; and if they are prolonged for sufficient lengths of time, they can produce all three stages of what is termed the general-adaptation-syndrome. The three successive stages of the general-adaptation-syndrome are the "alarm reaction", then the "stage of resistance", and finally the "stage of exhaustion".

In times of stress, glucose is the most important

source of available energy. The proper function of both the central nervous system and the muscles is almost entirely dependent upon glucose. The neuro-muscular activity plays an important role during the "fight or flight" reactions of animals under stress.

The sympathetic nerve centers in the hypothalamus are stimulated when an animal is stressed, causing increased blood sugar formation from the hepatic glycogen reserves. This mechanism immediately yields a large amount of sugar; however, it is of limited duration.

Bailey (1958) stated that animals which are subjected to stress will develop the general-adaptation-syndrome. This syndrome involves three successive stages: the alarm reaction, the stage of resistance, and the stage of exhaustion. The alarm reaction affects large portions of the body and may be divided into two phases, the shock phase and the phase of counter-shock. The shock phase may be characterized by such changes as a decrease in temperature, vascular tension and muscle tone. Other changes associated with shock are lowered blood concentration, deranged capillary and cell membrane permeability and a fall in blood sugar, with acute gastrointestinal erosion also present. Shock is a condition of suddenly developing systemic damage. The phase of counter-shock is characterized by an enlargement of the adrenal cortex with increased secretory activity. There is a rise in blood

pressure and blood volume, increase in diuresis and hyperglycemia. These changes are largely dependent upon the discharge of corticoids into the blood stream. During the resistance stage, the body develops an increased resistance to the particular stress or agent to which it has been exposed and, at the same time, a decreased resistance to other stimuli. The stage of exhaustion is that stage when no resistance is possible.

Bulato and Cannon (1925) stated that when the splanchnic nerves are stimulated, sugar is liberated into the blood from hepatic stores. The increase in blood sugar is largely, if not wholly, the result of the stimulation of the glycogenolytic process in the liver. The glycogenolytic process in the liver is normally subjected to two influences. It may be stimulated by both nerve impulse and adrenaline in the circulatory blood.

Braid (1928) stated that emotional excitement is accompanied by widespread and intense sympathetic discharge. The diencephalon is responsible for activation of the sympathetic system under conditions of stress.

Howard and Lawrie (1957) reported that the hypothalamus is the center responsible for the control of factors influencing the muscular and general bodily activity and as such may significantly condition the response of steers to stress. Impaired activity of this center causes a rise in body temperature and over-activity leads to drowsiness.

Ruminants utilize acetate, which may automatically inhibit glycogen breakdown as emphasis is shifted to the oxidation of fats. This occurs a few days after the beginning of a fasting period. This is supported by the depletion of glycogen without exercise, when oxidation of fatty metabolites are inhibited by neopyrithiamin. The frequent occurence of a high ultimate pH and low glycogen reserve in the musculature of dairy cows in poor condition would further support this.

Postmortem glycolysis converts all the glycogen initially in the muscle to lactic acid, provided there is no inhibition of the enzyme system involved. In the presence of sufficient glycogen, the production of lactic acid will proceed until the enzyme system is inhibited by the attainment of fixed pH and one component is inactivated. The pH at which glycolysis ceases is not identical for all beef muscles.

Dukes (1955) stated that intravenous injections of adrenaline provoke profound physiological changes, one of which is increased conversion of liver glycogen to lactic acid. Other effects of the adrenaline injections are: vasoconstriction and cardiac acceleration, dilation of the bronchi; relaxation of the stomach and intestines and contractions of their sphincters; stimulation of the glands secreting saliva, tears and sweat; contractions of the pilomotor muscles; and dialation of pupils and bulging of

the eyeballs. Most of these effects can be explained on the assumption that the hormone stimulates the nerves; however, the place of action of the hormones is believed to be a substance that exists between the nerve endings and the tissues supplied, rather than the nerve endings themselves.

Drury and Wick (1958) found that adrenaline promoted the breakdown of muscle glycogen to lactic acid and that this lactic acid is largely reconverted to glycogen by the liver. The adrenaline sets free lactate which serves as an important emergency fuel that can quickly be burned by the organism. The lactate was very quickly oxidized by CO_2 and very little is converted to glucose or glycogen. The lactate is more quickly burned than is the glucose.

Dickman (1958) found that blood glucose concentration was higher in adrenaline treated dogs, than in those which were given no treatment. The results of investigations with dogs demonstrated a dual effect of adrenaline; decrease in glucose uptake by tissue and an increase in the lactate entry into the blood. Dragstedt and Hoffman (1928) reported that adrenaline injected intravenously in dogs would cause increased blood pressure.

Hedrick (1957) found that short periods of excitement with an electric hot-shot caused a two-fold increase in blood sugar in beef cattle. After adrenaline injections, the blood sugar level increased as much as five-fold over

the original level. This rise in blood sugar was due to the glycolytic action induced by the adrenaline. Wide ranges in the peak of blood sugar levels were found to exist between animals which had been injected with the same levels of adrenaline on a body weight basis. These variations could have been caused by: (1) differences in the initial level of glycogen in the liver and muscle; (2) differences in the amount of finish which the animals were carrying; and (3) differences in the renal threshold of various animals. It was observed that more highly finished animals were more adversely affected by given levels of adrenaline than animals which were carrying less finish. Adrenaline affects the glycogen stores of the muscle and liver and has no effect on the fat deposits, therefore, when adrenaline was given on a body weight basis, animals with more finish received more adrenaline per unit of muscle.

A high rise in the urine sugar was noted after subcutaneous injections of adrenaline. A high level of sugar in the urine occurred when the blood sugar level exceeded the renal threshold. This elimination accounts for a major portion of the glycogen lost from the muscle stores during stress. Cori (1940) clearly established that adrenaline accelerated the reaction: glycogen lactic acid in muscle, and the reaction: glycogen glucose in the liver.

The Use of Tranquilizers to Prevent Antemortem Stress

According to Himwich (1955), an animal, when subjected to a stressful situation, responds with a number of physiological changes which are controlled by mechanisms in the hypothalamus. The sympathetic nerve centers located in the posterior part of the hypothalamus are aroused and nerve impulses are carried to the body and the vascular system and the organs involved in metabolism. The adrenal medulla receives secretory impulses through the splanchnic nerves which then cause the discharge of adrenaline and noradrenaline.

By suppressing the sympathetic portions of the autonomic nervous system the animal does not respond to a stressful situation. According to Huber (1958) tranquilizers act on the lower brain center to alloy or diminish anxiety and agitation. When animals are subjected to stress the adrenal pituitary axis is involved. Tranquilization reduced the intensity of the "alarm reaction" and enabled the animal to adjust adequately without going into a stage of hyperexcitement. There is wide variability among different animals as to the dosage which can be tolerated without various side effects becoming evident. The side effects of overdosage of tranquilizers are muscle tremors, bradycardia, and increased urination.

Reserpine is a white crystalline compound which is

produced from the root of the Rauwolfia plant. According to Leake (1955), the characteristic effects of reserpine come on rather slowly, but are quite prolonged. The results of the administration of reserpine are lowered blood pressure, and tranquilization. In addition, it combats aggressiveness and lowers tension.

Saniz (1956) stated that reserpine reduced the quantity of the cerebral response, lowers anxiety but does not directly depress motor function.

Meyers (1956) reported that reserpine acts through decreased sympathetic activity and not by heightened parasympathetic influence. Effects of injections of reserpine are: (1) sedation; (2) depression of supra-medullary activity; (3) vasodilation causing decreased blood pressure; (4) depression of respiratory rate; and (5) release of antidiuretic hormone. Most of these effects are due to the depression of sympathetic outflow which follows diencephalic depression. Total alkaloids from several species of Rauwolfia do block the effects of adrenaline if sufficient time is allowed to elapse before testing.

Sturkie *et al.* (1958) stated that dosages of reserpine in fowl which were sufficient to cause tranquilization also caused a decrease in blood pressure, heart rate and body temperature.

Himwich (1957) reported that one action of reserpine

and chlorpromazine is to inhibit the hypothalamus which contains areas taking part in the mobilization of the organism for fight or flight.

Leake (1955) reported that chlorpromazine, which was developed in France, depresses motor activity, abolishes response to a conditioned stimulus, lowers temperature and relaxes smooth muscles. Chlorpromazine can cause jaundice due to an infiltration around biliary ducts with allergic swelling.

Dobkin et al. (1954) reported that small doses of chlorpromazine block all sympathetic vasopressor reflexes, while large doses block vagal reflexes. Chlorpromazine seemed to produce an untroubled, serene, drowsy and relaxed patient. There was also a reduction in alarm responses and reflexes. Chlorpromazine suppressed the release of adrenaline, acetycholine and histamine. The animals were more quiet and more sleepy.

Ohler et al. (1954) stated the inhibition of stress by chlorpromazine induces adrenal ascorbic acid depletion. This represents an inhibition of stress-induced ACTH secretion and is not due to direct action on the adrenal cortex. Chlorpromazine may also act to inhibit the pituitary level or other portions of the central nervous system.

Anonymous author, (1954) reported that chlorpromazine acts as a depressant of neuronic activity at the control

and hypothalamic levels and also acts peripherally. Acting by itself, the chlorpromazine produces apathy and emotional indifference and reduced emotional tension, agitation and overactivity.

Lehmann and Harrahan (1954) reported that the effects of chlorpromazine persist for about forty eight hours after treatment. After injection of this drug a human patient will feel cold, drowsy, faint and weak. Chlorpromazine has a very pronounced effect on the central nervous system in both animals and humans. In animals, it produced a type of depression which increases progressively with the dosage. Other effects are strong antiemetic properties, inhibition of the secretion of gastric juice and production of hyperthermia.

Grenell (1957) stated that the effect of chlorpromazine is very selective, with its primary seat of metabolic action in the hypothalamus. This drug seems to affect, primarily, subcortical structures concerned with maintenance of psychomotor drive and wakefulness. Chlorpromazine causes a very great increase of ATP in the tissue of the hypothalmic area, and ATP levels and activity levels are directly related. It is suggested that some of the effects of chlorpromazine are explainable on the basis of molecular shifts and interactions in the cell membranes. The associated intracellular biochemical changes may be related to structural and volume changes interferring with

electron transfer.

Lancaster and Jones (1954) found that chlorpromazine had no significant effect on fasting blood sugar level, on immediate fall in blood sugar level following an insulin injection or on prolonged fall in blood sugar level following administration of insulin. It was found that patients were appreciably calmer and more manageable when on combined insulin and chlorpromazine than when on chlorpromazine alone.

Smith et al. (1955) stated that thorazine has a unique depressive action on the central nervous system.

Ritchie (1958) reported that chlorpromazine was injected into swine with no indication of pain or irritation at the site of injection. When given intravenously, the animal appeared dazed and its respiratory rate was increased for about fifteen minutes. If undisturbed, it would pass into a deep sleep from which it could be aroused only by vigorous stimuli. When given intramuscularly, the effect was marked, with little change in respiratory rate and a temperature decrease of 2.4 degrees in thirty to forty minutes after injection. Animals became docile after injection of chlorpromazine. This was true even in the case of vicious boars.

CHAPTER III

MATERIALS AND METHODS

Animals Used in the Study.

Yearling cattle and lambs which had been fed in dry lot were used in this study. A more detailed description of the cattle and the management procedures followed while the cattle were on experiment will be discussed in Chapter IV along with the results obtained from each individual trial.

Methods Used to Stress Animals.

Animals were stressed by excitement and by the use of adrenaline. The animals which were excited were prodded periodically with an electric hot-shot. They were confined in a small holding pen during excitement to avoid the possibility of injury.

Injections of adrenaline were given subcutaneously in order to simulate a prolonged period of stress. Hedrick (1958) found that administration of adrenaline intramuscularly would cause edema in the area of the injection. He further stated that it was not practicable to administer adrenaline intravenously to cattle to simulate a prolonged period of stress because adrenaline in the blood stream is quickly absorbed and dialyzed in the tissue. The injection was made on the foreshank about two and one half inches above the knee on the lambs and in the loose skin on the shoulder of the cattle.

The preparation used was an aqueous solution of adrenaline, chlorobutanol, sodium chloride and sulfur dioxide saturated with carbon dioxide (each milliliter contained one milligram of adrenaline). The dosages and time intervals at which the adrenaline was administered will be discussed in Chapter IV with the results obtained.

Methods of Counteracting Stress.

Protamine zinc insulin was administered to counteract stress in cattle which were subjected to stress. The insulin was injected intramuscularly in the shoulder. The dosages and time intervals at which the insulin was administered will be discussed in Chapter IV with the results obtained.

Injections of synthetic phenothiazine derivatives and a pure crystalline alkaloid of Rauwolfia root were given intramuscularly in the shoulder. Administration of these compounds was in the form of Thorazine HCL (Chlorpromazine HCL), Diquel (Phenothiazine HCL) and Serpasil. The dosages and time intervals at which the tranquilizers were injected will be given in Chapter IV with the results obtained.

Collection of Blood Samples and Methods of Analysis.

A bleeding needle was used to take blood samples from the jugular vein in the cattle and sheep. The blood was collected in a test tube containing a small amount of heparin to prevent coagulation. Duplicate one milliliter aliquots of blood were immediately placed in fifty

milliliter test tubes to which 9.5 milliliters of 0.03N. barium hydroxide was added as the test tube was rotated. After adding the barium hydroxide, 9.5 milliliters of a five per cent zinc sulfate solution was added. The tube was then inverted to mix the barium and zinc solutions. The content of each test tube was then filtered through Whatman No. I filter paper or its equivalent. A fraction of a drop of toluene was then added to the protein-free filtrate to preserve the sample. The samples were stored at 45° F. until they were analyzed.

The Nelson-Somogyi method for blood glucose as published by the American Association of Clinical Chemists (1953) was followed in making the blood sugar determinations. The absorbance was measured at five hundred forty millimicrons by means of a Bausch and Lomb Spectronic 20 Colorimeter using a blank for setting the zero. A standard glucose curve was used to calculate the amount of glucose present in the sample.

Determination of Muscle pH.

Muscle pH was determined twenty four to forty eight hours postmortem on the cattle and lambs. A Beckman pH meter, Model G, was used to make the determinations. The instrument was standardized to pH 7.0 prior to each reading using a phosphate buffer. In the beef and lamb the glass electrodes were inserted into the longissimus dorsi muscle at the twelfth rib and readings taken to the nearest

0.05 pH unit. A one inch square was removed from the flank muscle of the lambs and was placed in a Waring blender to which ten milliliters of distilled water was added. The pH determination was taken after the muscle tissue and distilled water had been subjected to the action of the Waring blender for one minute.

Determination of Muscle Color.

The beef carcasses were ribbed between the twelfth and thirteenth rib after at least a twenty four hour chilling period. Color of the longissimus dorsi muscle was determined subjectively thirty minutes after the carcass was ribbed using Munsell Color Paddles, Meat Scale A. The color value for the lamb was determined on the flank muscle at least twenty four hours after slaughter. This determination was made in the intact carcass using a Hedonic color scale. (See Appendix) The values ranged from 1 (very pale pink) to 7 (dark red) and were given without knowledge of treatment or the pH of the carcass by the panel. The muscle was given the color score before the pH determination was made.

CHAPTER IV

RESULTS AND DISCUSSION

The effects of antemortem stress on subsequent carcass characteristics of sheep and cattle were studied in this investigation. Measures taken to prevent stress included rest and administration of tranquilizers and insulin. The procedures and results obtained are presented and discussed in latter portions of this paper.

I. THE EFFECTS OF ANTEMORTEM ADRENALINE INJECTIONS ON SUBSEQUENT CARCASS CHARACTERISTICS

Previous investigators have established that increased adrenaline secretion will rapidly deplete the glycogen stores of the body. It was reported by Dukes (1955) that intravenous injections of adrenaline would cause increased conversion of liver glycogen to lactic acid. Dragstedt and Hoffman (1928) observed that adrenaline promoted the breakdown of muscle glycogen to lactic acid. Hedrick (1957) reported that adrenaline caused hyperglycemia and glycosuria. This condition could be caused by the animal being subjected to stress causing increased secretion of adrenaline or by injections of adrenaline intramuscularly or subcutaneously.

The effects of various levels of subcutaneous adrenaline injections on subsequent carcass characteristics of lambs.

Thirty two Texas wether lambs, varying in weight from

eighty six to one hundred seventeen pounds were used in this study. Twenty four lambs were selected at random and injected with various levels of adrenaline. The levels given were four, six, eight and ten milligrams per one hundred pounds live weight. Eight lambs served as controls and received no injection. The injections were made subcutaneously on the foreshank about two inches above the knee joint or between the foreleg and brisket. The total amount of adrenaline injected was divided into two equal amounts and was given eight and twenty four hours antemortem. The lambs were in full fleece and the weather was mild at the time of slaughter. Feed and water were provided until the time of slaughter. No symptoms of stress were observed in the lambs at any time after the injections of adrenaline.

Color determinations were made on the flank of each carcass by a panel consisting of three members. A hedonic scoring system was used ranging from 1 - very pale pink to 7 - dark red. (See Appendix)

Muscle sections one inch square were removed from the flank muscle and blended in a Waring blender with ten milliliters of double distilled water. The pH of the resulting slurry was then determined. Each carcass was ribbed posterior to the last rib and a pH determination made by placing the electrodes directly into the longissimus dorsi muscle. The pH value and color

determination was made thirty to thirty six hours after slaughter. The amount of adrenaline given, live weights, carcass grades, pH values of flank and loin muscle, and color scores of flank muscle are presented in Table I.

Data presented in Figure 1 indicates that adrenaline injections had a pronounced effect upon the color of the musculature of lambs. The color of the flank muscle of lambs receiving four milligrams per one hundred pounds body weight was significantly darker (.05 level) than the control group, whereas the color of the groups receiving six and eight milligrams per one hundred pounds was significantly darker (.01 level) than the control group. Flank muscles of the group receiving ten milligrams per one hundred pounds was significantly darker (.05 level) than the controls. However, it should be noted that the group receiving ten milligrams per one hundred pounds consisted of only two lambs. The number of lambs involved influenced the test of significance. There were no significant differences in color of flank muscle between the groups which received different levels of adrenaline.

The effect of adrenaline injections on the pH value of the longissimus dorsi muscle of lamb carcasses is presented in Figure 2. (The analysis of variance F-ratio among groups was significant at the .01 level.) The pH value of the longissimus dorsi muscle of lambs receiving four milligrams of adrenaline per one hundred pounds body

TABLE I

The Effect of Adrenaline Administered Subcutaneously on Subsequent Characteristics of Individual Lamb Carcasses

Lamb No.	Live Weight	Adrenaline, mg. /100 lb.	Grade	Color of Flank	pH of Flank	pH of Loin
36	107	0	Good -	3	6.2	5.7
37	100	0	Good -	1	5.85	5.5
43	111	0	Good	2	5.85	5.4
46	112	0	Good -	3	6.25	5.8
54	102	0	Good	6	5.9	5.5
57	84	0	Good -	2	6.25	5.3
63	101	0	Good -	3	5.8	5.55
64	91	0	Good	3	6.3	5.8
38	117	4	Good	3	6.65	5.45
40	87	4	Utility	5	6.55	5.6
51	93	4	Good	6	6.7	5.7
56	88	4	Good	4	6.2	5.8
58	102	4	Good	6	6.75	6.0
59	102	4	Good	6	7.15	6.6
34	104	6	Good -	5	6.85	6.0
41	94	6	Good	3	7.05	5.85
47	99	6	Good	5	6.85	6.2
48	93	6	Good	5	6.65	5.8
53	96	6	Good	5	6.8	6.3
55	89	6	Good -	5	6.4	5.55
60	103	6	Good -	7	6.9	6.2
62	96	6	Good -	4	7.15	6.9
35	98	8	Good -	5	7.1	6.5
39	102	8	Good	4	7.0	6.15
44	104	8	Good	5	6.8	6.1
45	111	8	Good	7	7.05	6.5
50	106	8	Good -	4	7.05	6.4
52	86	8	Good	5	6.85	6.1
61	99	8	Good	5	6.65	5.8
65	90	8	Good	4	6.6	5.8
42	104	10	Good	5	7.1	6.15
49	89	10	Good	7	7.05	6.8

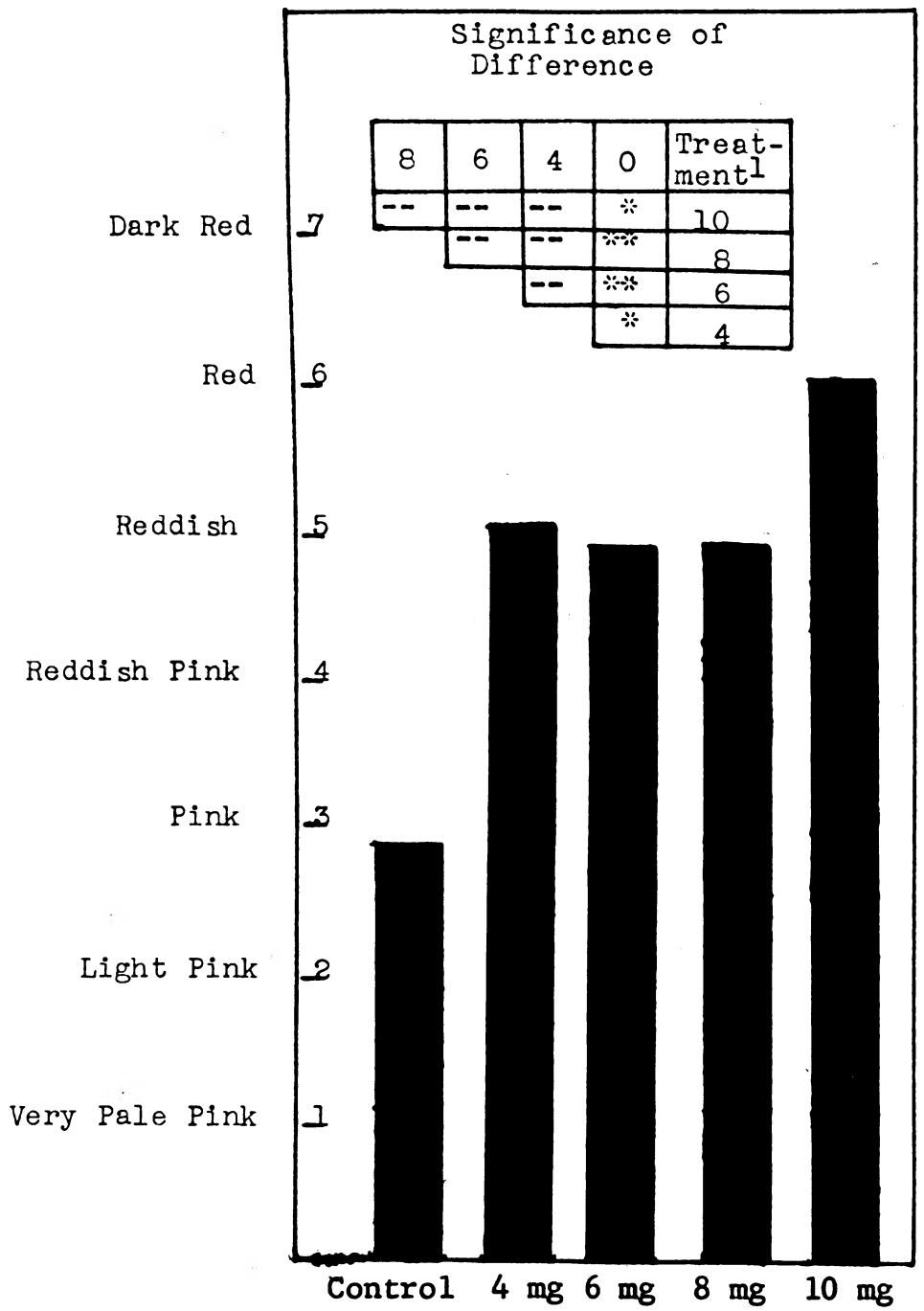

Figure 1. Effect of Subcutaneous Injections of Adrenaline on the Subsequent Color of the Flank Muscle of Lamb Carcasses.

* Significant at .05 level
** Significant at .01 level
1 Treatment given in milligrams/100 pounds live weight.

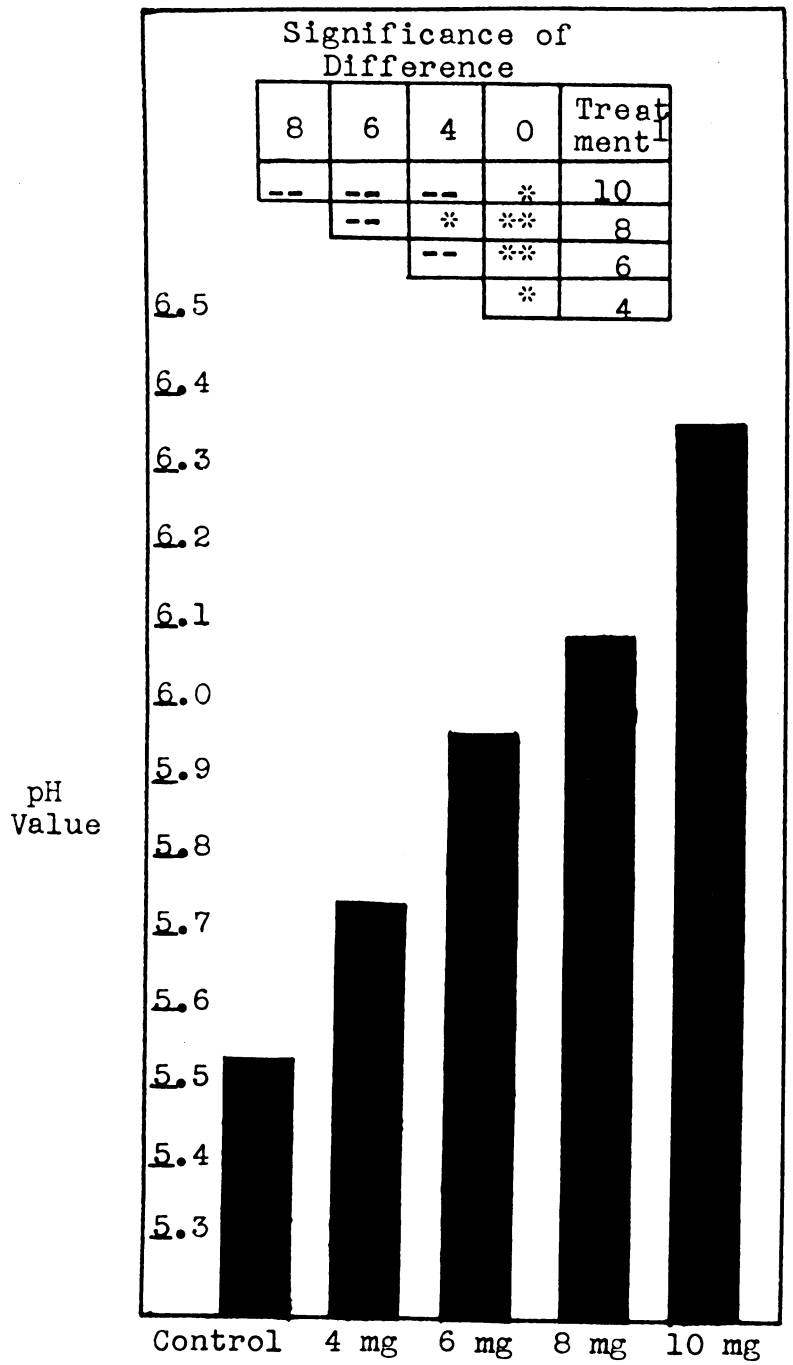

Figure 2. Effects of Subcutaneous Injections of Adrenaline on the Subsequent pH Value of the Longissimus Dorsi Muscle of Lamb Carcasses.

* Significant at .05 level
** Significant at .01 level
1 Treatment given in milligrams/100 pounds live weight.

weight was significantly higher (.05 level) than the pH of the controls. The groups receiving six and eight milligrams of adrenaline per one hundred pounds body weight had a highly significant increase (.01 level) in the pH of the longissimus dorsi muscle as compared to the controls. Due to the small number involved in the ten milligrams of adrenaline per one hundred pounds group the rise in pH of the longissimus dorsi was only significant at the .05 level when compared to the controls. The increase in dosage of adrenaline from four to eight milligrams per one hundred pounds body weight caused significant increase (.05 level) in the pH of the longissimus dorsi muscle. When an analysis of variance was calculated to determine differences between the four milligram and the ten milligram treatment the F-ratio was 4.67 (5.99 required for significance at the .05 level). There was a close relationship between the levels of adrenaline injected antemortem and the subsequent pH value of the longissimus dorsi muscle. As the level of adrenaline injected was increased the hydrogen ion concentration of the longissimus dorsi muscle decreased. This is in agreement with the work of Hedrick (1957) who reported that as the level of adrenaline injected in cattle antemortem was increased, the subsequent pH value of the carcass was correspondingly higher. According to Dragstedt and Hoffman (1928), Dukes (1955) and Hedrick (1957), this is the result of the breakdown of muscle glycogen to

glucose, thereby leaving insufficient glycogen in the muscle for conversion to lactic acid after the animal is slaughtered.

The data presented in Figure 3 shows the effect of adrenaline injected antemortem on the subsequent pH of the primary flank muscle. An analysis of variance gave an F-ratio among groups which was significant at the .01 level. The pH of the groups receiving four, six and eight milligrams per one hundred pounds live weight was significantly higher (.01 level) than the control group, while the pH of the group receiving ten milligrams per one hundred pounds was only statistically significant at the .05 level. The effect of adrenaline treatment on the subsequent carcass, whether in the longissimus dorsi or the primary flank muscle, resulted in a definite increase in the ultimate pH values. Data presented in Figures 1, 2 and 3 indicates there is a definite relationship between the pH value of a muscle and the color of the musculature of the carcass.

Data presented in Figures 4 and 5 show a definite relationship between the color of the flank and the hydrogen ion concentration of the longissimus dorsi and the primary flank muscle. As the hydrogen ion concentration decreases, the color of the muscle becomes darker. This data is in agreement with the work reported by Hedrick (1957).

A photograph of rib chops taken from representative

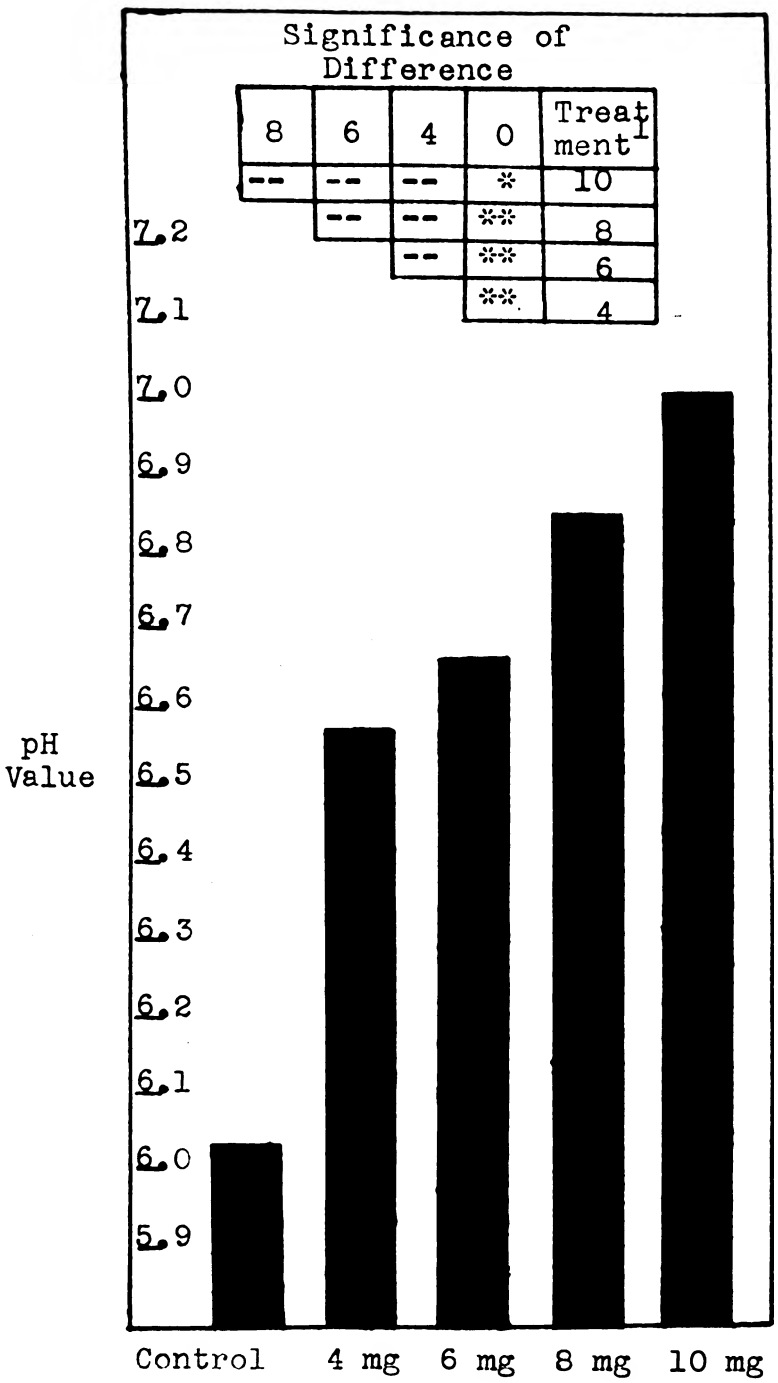

Figure 3. Effects of Subcutaneous Injections of Adrenaline on the pH Value of the Primary Flank Muscle of Lamb Carcasses.

* Significant at .05 level
** Significant at .01 level
1 Treatment given in milligrams/100 pounds live weight.

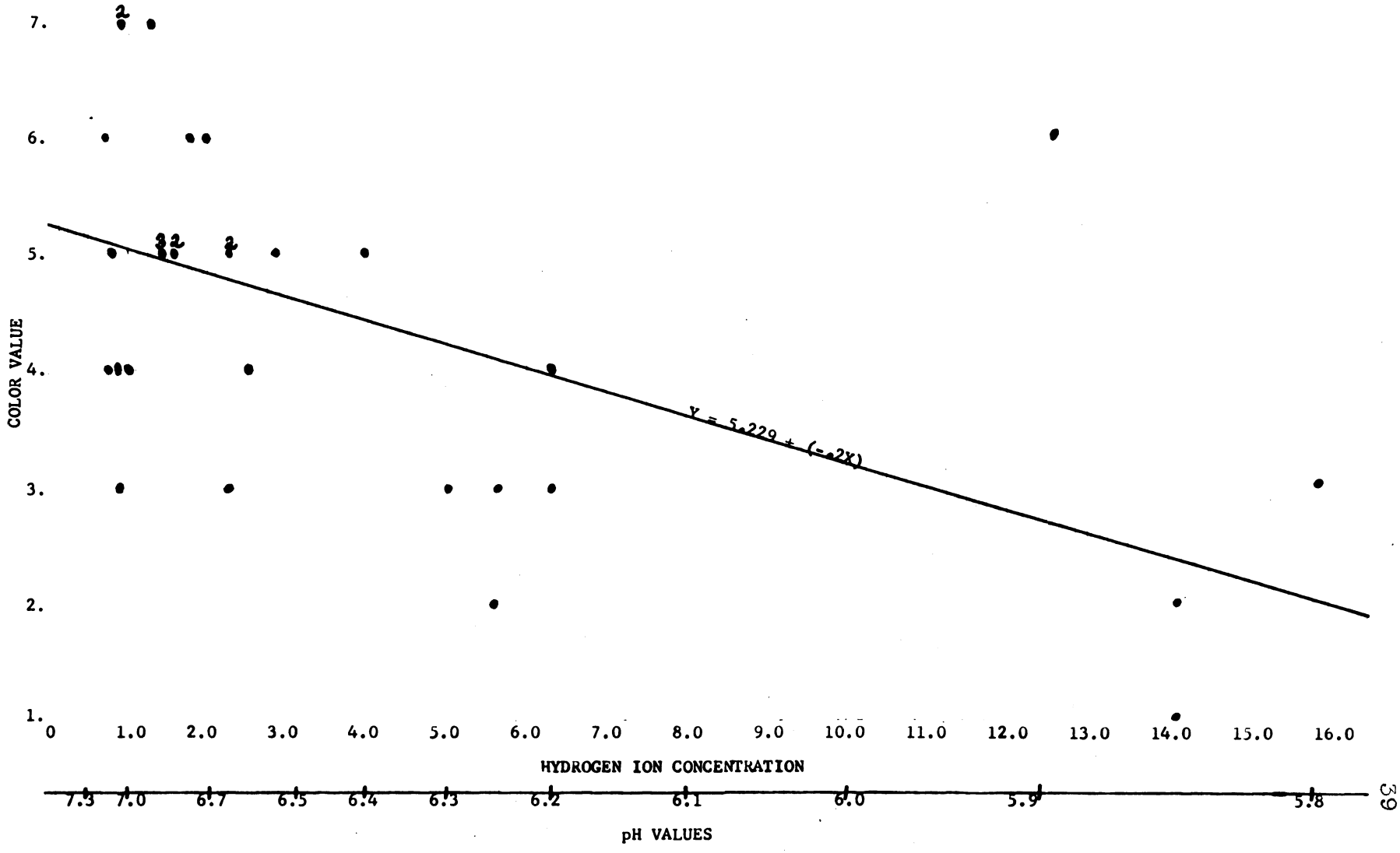

Figure 4. Relationship of pH Value and Color Score of Primary Flank Muscle of Lamb Carcasses.

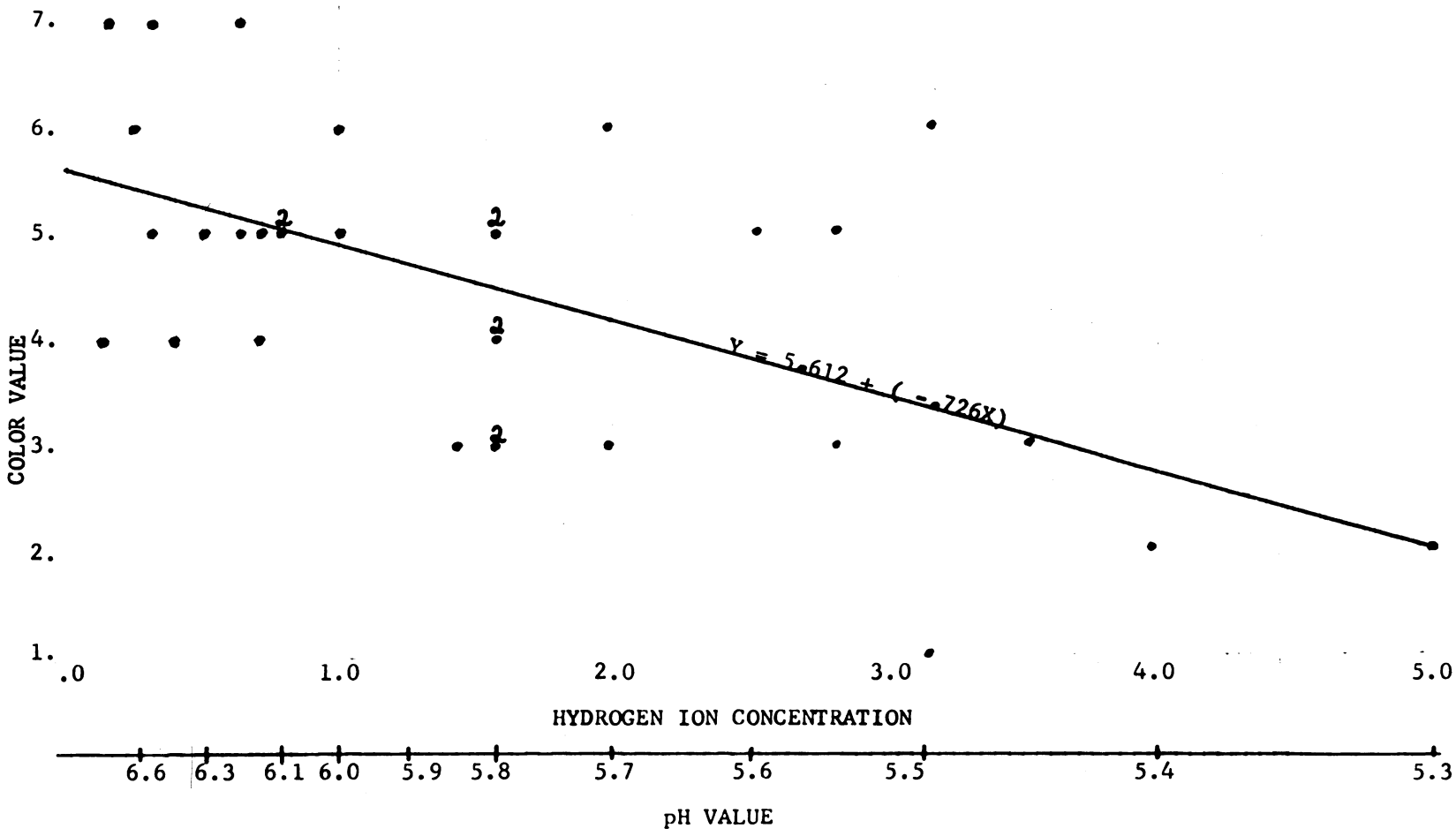

Figure 5. Relationship of pH Value of Longissimus Dorsi Muscle to the Color of the Primary Flank Muscle of Lamb Carcasses.

carcasses is presented in Figure 6. A photograph of the primary flank muscle taken from carcasses receiving the different treatments is presented in Figure 7.

Mean values of color scores, and pH values of the primary flank and longissimus dorsi muscles are presented in Table II.

The effects of subcutaneous adrenaline injections, administered antemortem, on postmortem carcass characteristics of beef.

Cattle are subjected to many stress producing situations when marketed. The kinds of stress which might be encountered are: inclement weather, trauma, fatigue, abusive handling, etc. Animals vary in degree of susceptability to various kinds of stress. If an animal is susceptable to stress, there is a possibility the subsequent carcass will cut dark. According to Dragstedt and Hoffman (1928) and Hedrick (1957), stress causes an adrenaline secretion increase, which in turn promotes the breakdown of muscle glycogen. Hedrick (1957) reported that dark cutting carcasses could be produced by either injecting adrenaline approximately twenty four hours antemortem or by periodic excitement during this period.

Ten yearling Shorthorn cattle were used to study the length of time required to replenish the muscle glycogen level required for bright cutting beef after the animal had been subjected to stress.

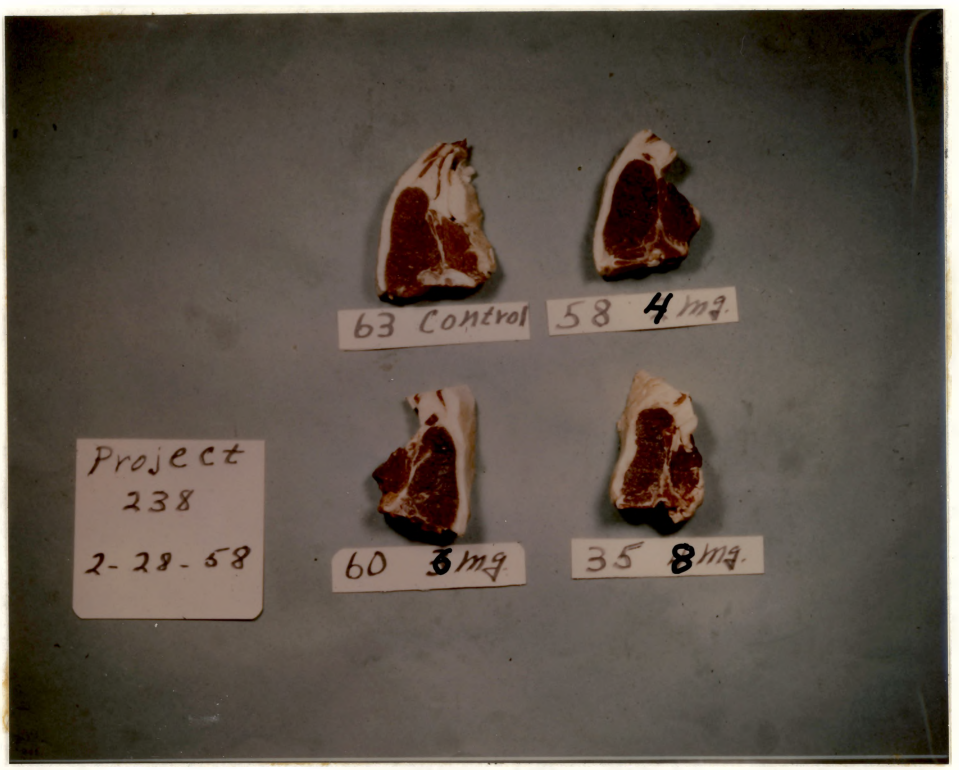

Figure 6. The Effect of Adrenaline Induced Stress on the Color of Lamb Loin Chops.

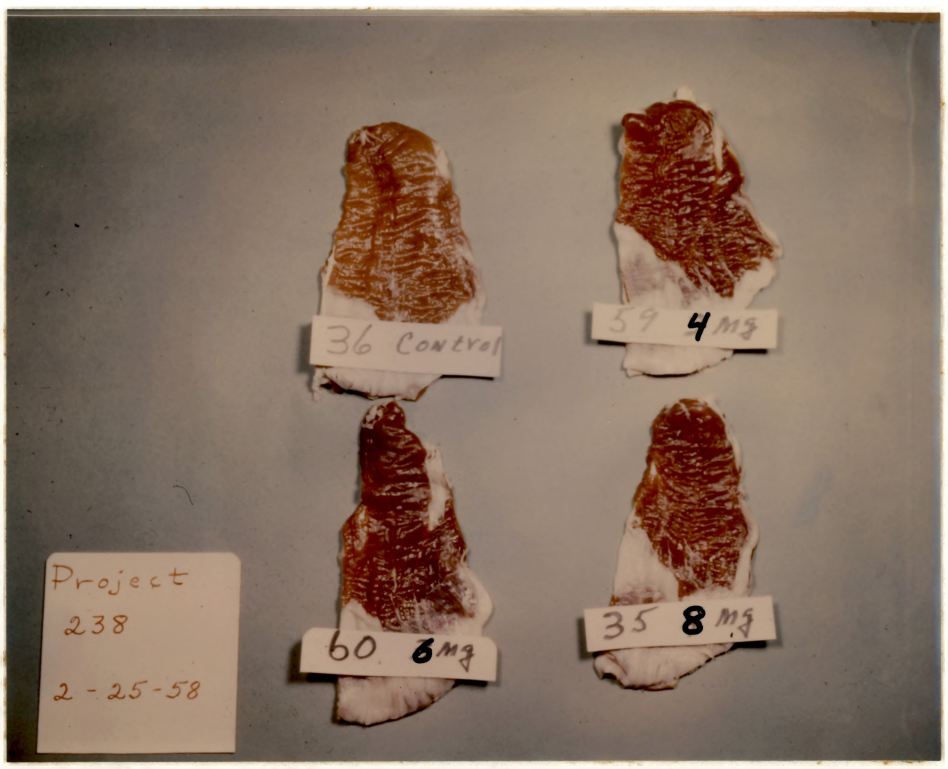

Figure 7. The Effect of Adrenaline Induced Stress on the Color of the Primary Flank Muscle of Lamb Carcasses.

TABLE II

The Effect of Varied Levels of Adrenaline Administered Subcutaneously on Subsequent Lamb Carcass Characteristics

Adrenaline mg./100 lb. body weight	No. of Animals	Mean Color Value	Mean pH of Flank*	Mean pH of Longissimus Dorsi*
0	8	2.9	6.03	5.54
4	6	5.0	6.58	5.74
6	8	4.9	6.66	5.96
8	8	4.9	6.85	6.09
10	2	6.0	7.07	6.36

* Calculated from mean hydrogen ion concentration

Eight cattle were injected subcutaneously with three milligrams of adrenaline per one hundred pounds live weight and two, serving as controls, received no injection. Two animals were slaughtered at each of the following time intervals after receiving adrenaline: twenty four, forty eight, seventy two and ninety six hours. The controls and the injected cattle were taken off feed twenty four hours before slaughter and given free access to water until the time of slaughter. Forty eight hours after the cattle were slaughtered the carcasses were ribbed and pH values taken with a Beckman Model G pH meter. The pH values were taken by placing the electrodes in direct contact with the cut surface of the longissimus dorsi muscle. Color values were taken one hour after the carcass had been ribbed. Color of the longissimus dorsi muscle was taken subjectively using Munsell Color Paddles, Meat Scale A as a reference standard. All carcasses graded Choice. The data collected from these cattle is presented in Table III.

The control carcasses had the lowest color value, i.e., the lightest, brightest color. A photograph of rib steaks taken from carcasses of each time interval is presented in Figure 8. The pH values of the controls were lower than the injected cattle. The cattle which were slaughtered twenty four hours after injection exhibited the darkest color, highest pH value, and stickier gummier cut surface of the muscle. These carcasses were typical of dark

TABLE III

The Effect of Adrenaline Administered to Beef Cattle on Subsequent Carcass Characteristics

Animal Number	Live Weight	Carcass Grade	Interval between Injection and Slaughter[1]	Color of Muscle[2]	pH of Muscle[3]
801	790	Choice -	Control	A-4	5.4
807	700	Choice -	Control	A-4	5.4
828	735	Choice -	24 hours	A-9	6.0
829	760	Choice	24 hours	A-9	6.2
802	890	Choice	48 hours	A-6	5.75
803	780	Choice	48 hours	A-7	5.95
819	825	Choice -	72 hours	A-8	5.95
806	750	Choice -	72 hours	A-9	6.0
809	755	Choice	96 hours	A-6	5.55
810	735	Choice -	96 hours	A-8	5.9

1 All animals except 801 and 807 were injected subcutaneously with three milligrams of adrenaline per one hundred pounds bodyweight.

2 Munsell Color Paddles, Meat Scale A.

3 Determined in the longissimus dorsi at twelfth rib.

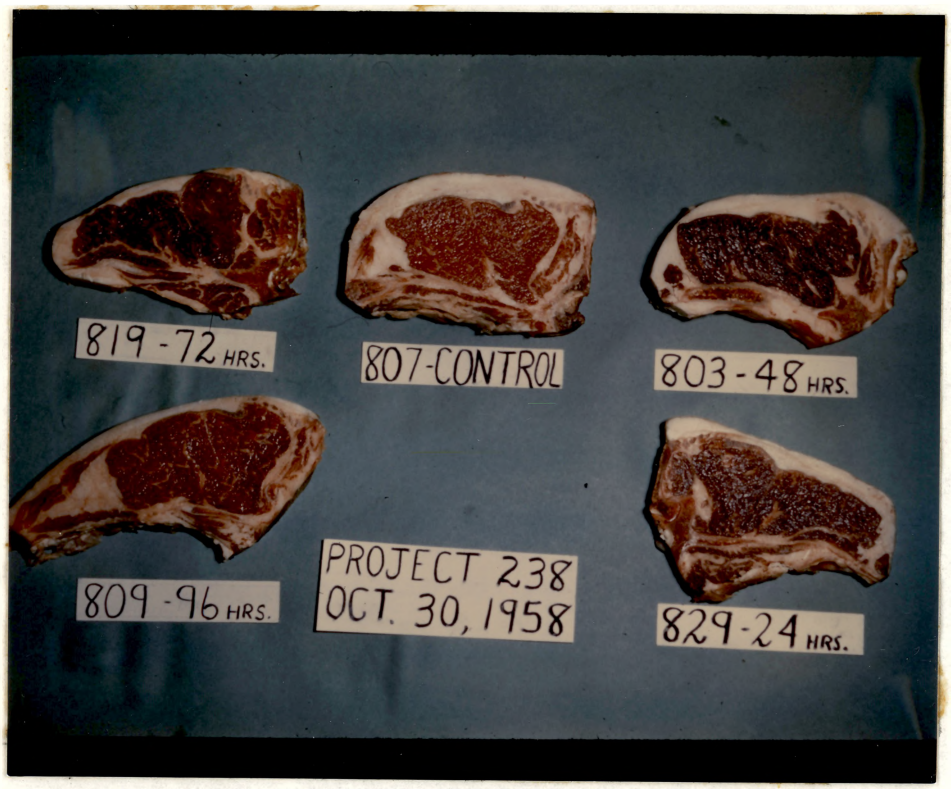

Figure 8. The Effect of Adrenaline, Injected at Varying Time Intervals Antemortem, on the Color of the Longissimus Dorsi Muscle of Beef.

cutting beef. There was a gradual lowering of the color values and a decrease in pH value as the period of time between injection and slaughter was lengthened. The cattle which were injected ninety six hours antemortem were not as light and bright in color or as low in pH value as the controls. This indicates that muscle glycogen replenishment is a much slower process than that of depletion. It would seem impractical to consider holding cattle after they have been shipped, as a preventive measure for dark cutting beef.

Effects of various levels of subcutaneous injections of adrenaline on carcass characteristics of Utility grade beef.

Nine steers of mixed dairy breeding were used in this experiment to determine the effect of varied levels of adrenaline on subsequent carcass characteristics. Hedrick (1957) observed that highly finished animals were more adversely affected by a given level of adrenaline than animals with less finish. Adrenaline affects the glycogen stores of the muscle and liver, but has no effect on the fat deposits of the body. When adrenaline is given on a body weight basis, animals with more finish receive more adrenaline per unit of muscle.

All cattle used in this experiment were Utility grade except number 7 which was low Standard. The animals were

divided into three equal groups, two animals received injections and one served as a control. The level of adrenaline injection varied from three milligrams to six milligrams per one hundred pounds live weight. (See Table IV)

Blood samples were taken from animals one through six and analyzed for blood sugar content using the Nelson-Somogyi method for sugar determination. The blood glucose levels of cattle at various periods after injection with adrenaline are presented in Table V.

Color value determinations were made of the longissimus dorsi muscle with Munsell Color Paddles, Meat Scale A. The carcasses were ribbed forty eight hours postmortem and the longissimus dorsi muscle was allowed to brighten for one hour before the color determinations were made. The pH values were taken immediately after the carcasses were ribbed by inserting glass electrodes into the cut surface of the longissimus dorsi muscle.

Data presented in Table IV shows the effects of various levels of subcutaneous adrenaline injections on the subsequent carcass characteristics of low grade beef. The animals in Group I had darker color values and higher pH values than the Control. However, the color value is not as high as that of the cattle receiving the higher levels of adrenaline in Group II. Although the color value of muscle from the cattle injected with adrenaline was higher in Group II than in Group I, the pH values were

TABLE IV

The Effects of Various Levels of Subcutaneous Adrenaline Injections on the Carcass Characteristics of Low Grade Beef

Animal Number	Time of Antemortem Injection (Hrs.)	Treatment (mg./100 lb.)	Color	pH of Loin Eye
Group I:				
1	No injection	Control	A-7	5.5
2	24 hours	3.5	A-9	5.9
3	24 hours	3.5	A-9	5.8
Group II:				
4	24 and 12 hrs.	6.0*	A-10	5.9
5	24 and 12 hrs.	6.0*	A-10	5.9
6	No injection	Control	A-5	5.5
Group III:				
7	24 hours	3.0	A-5	5.45
8	24 and 12 hrs.	6.0*	A-10	5.8
9	No injection	Control	A-7	5.4

* One-half given at each time interval.

TABLE V

Effects of Subcutaneous Injections of Adrenaline on the Blood Sugar Content of Utility Grade Cattle

Animal Number	Treatment*	Blood Sugar (mg./100 ml.)	Hours after Injection
Group I:			
1	Control	57.0	0
		57.0	4
		57.0	24
2	3.5	48.5	0
		181.0	4
		52.0	24
3	3.5	47.0	0
		157.0	4
		57.0	24
Group II:			
4	6.0	55.0	0
		205.0	5
		95.0	12
		52.4	24
5	6.0	57.0	0
		130.0	5
		80.0	12
		65.0	24
6	Control	75.0	0
		75.0	5
		75.0	12
		75.0	24

* Treatment presented as milligrams of adrenaline per 100 pounds liveweight.

essentially the same. The higher color value denotes darker color. The animal receiving three milligrams in Group III had a low pH value which was much the same as the pH value of the controls in all three groups. The data in this table indicates that the minimum amount of adrenaline required to produce dark cutting carcasses in low grade cattle is approximately 3.5 milligrams per one hundred pounds body weight.

Data presented in Table V indicates the rise in blood glucose will reach a peak four to five hours following injection with adrenaline. This increase is the result of adrenaline promoting the breakdown of liver and muscle glycogen to blood glucose. This data corresponds with that reported by Dragstedt and Hoffman (1928) and Drury and Wick (1958). These workers found adrenaline injections to have the same effect on the blood glucose level of dogs as is presented in Table V.

II. The Use of Insulin and Tranquilizers as Measures to Prevent Stress

Injection of Thorazine and insulin as a measure to prevent stress in cattle subjected to periodic excitement with an electric hot-shot.

Nine Utility grade steers of mixed dairy breeding were used in this study. The cattle were divided into three groups of three animals each. One animal in each group was

injected subcutaneously with one unit of insulin per pound body weight, one was injected intramuscularly with one milligram Thorazine per pound body weight and the remaining animal served as a control and received no injection. After injection the animals were subjected to periodic excitement with a hot-shot while confined in a small holding pen. The stimulation was not continuous but was of such a nature as to keep the animals moving in the holding pen. The animals received no feed or water during the antemortem stress period.

The cattle were slaughtered twenty four hours following the beginning of the stress period. Blood samples were taken prior to injection, prior to stress and at various periods during stress. The carcasses were ribbed and pH values of the longissimus dorsi muscle were taken forty eight hours after slaughter.

Group I consisted of three Jersey steers weighing 745 to 780 pounds. Before injection all animals were of similar temperament. Stress consisted of periodic excitement beginning twenty four hours antemortem for three and one half hours; eighteen hours antemortem for twenty minutes; sixteen hours antemortem for twenty minutes; and thirteen hours antemortem for twenty minutes. The animals were allowed to rest between periods of excitement.

Definite differences were noted in the response of the individual animals when they were stimulated with the

hot-shot. During the initial period of excitement, the control steer (Number 16) became completely exhausted before the steer receiving Thorazine became excited. The steer receiving insulin appeared to become excited somewhat slower than the control, but more rapidly than the steer receiving Thorazine. The control steer became extremely excited as indicated by bawling when touched, violent kicking at any moving object, attempting to jump out of the holding pen, rearing on hind legs and jumping against the side of the adjacent building, and frequent urination. Respiratory rate of the control animal increased much more rapidly than the tranquilized steer. This could be explained in part by increased activity. After a period of one and one half hours of excitement the control steer would lie down whenever possible and when stimulated with the hot-shot would bawl, but make little or no attempt to arise. It was only with much effort that this steer could be made to stand. The steer receiving insulin exhibited the same indications of excitement, however, they were less pronounced than that of the control. After a period of two hours of stress the insulin injected steer would lie down, but differed from the control in that he would get up when stimulated. The tranquilized steer had a dopey, listless appearance and when stimulated with the hot-shot would move away but did not become as excited and nervous as the other steers. After one and one fourth

hours the tranquilized steer began to exhibit the same reactions as the other two steers. The tranquilized steer showed indications of tiring at the end of the initial period but was not completely exhausted as were the other steers. During each of the subsequent periods of excitement the steer receiving the tranquilizer appeared to have more energy than the other two animals. At the time of slaughter the tranquilized steer appeared dopey, but responded quickly to stimulation from the hot-shot. The other two animals in this group were much slower in their response to stimulation.

As can be noted from the data presented in Table VI, there was very little difference in the pH values of the longissimus dorsi muscle of the carcasses in Group I. All carcasses were dark cutters. A photograph of short loin steaks taken from each of the three carcasses and an unstressed control is presented in Figure 9. The period of excitement was much longer than necessary to cause subsequent carcass of the control to cut dark. The severity of antemortem stress was possibly such as to overcome any stress inhibitory effects of the Thorazine or insulin.

The cattle in Group II ranged in weight from 710 to 725 pounds. The injections given were the same as those for Group I. Stress consisted of periodic excitement beginning twenty four hours antemortem for one and one-half hours.

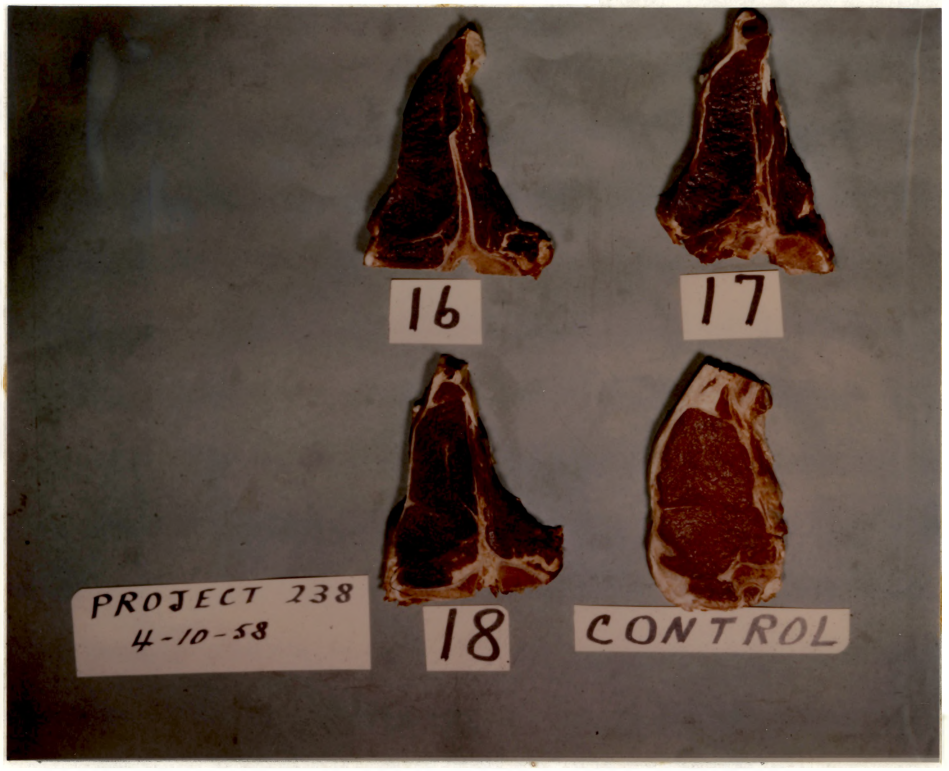

Figure 9. The Effect of Excitement on the Color of Short Loin Steaks of Beef.

TABLE VI

The Effect of Excitement on Subsequent Carcass Characteristics of Utility Grade Cattle Previously Injected With Insulin or Tranquilizer

Animal Number	Preventive Treatment	Period of Stress[1]	pH of Muscle[2]
Group I:			
16	Control, no injection	4.5 hrs. during 24 hr. antemortem period	6.5
17	1 unit insulin/lb. injected subcutaneously 28 hrs. antemortem	4.5 hrs. during 24 hr. antemortem period	6.35
18	1 mg. Thorazine/lb. injected intramuscularly 28 hrs. antemortem	4.5 hrs. during 24 hr. antemortem period	6.3
Group II:			
19	Control, no injection	1.5 hrs. at beginning of 24 hr. antemortem period	6.7
20	1 unit insulin/lb. injected subcutaneously 30 hrs. antemortem	1.5 hrs. at beginning of 24 hr. antemortem period	5.7
21	1 mg. Thorazine/lb. injected intramuscularly 30 hrs. antemortem	1.5 hrs. at beginning of 24 hr. antemortem period	6.35

TABLE VI (Cont'd)

Animal Number	Preventive Treatment	Period of Stress[1]	pH of Muscle[2]
Group III:			
23	Control, no injection	40 min. at beginning of 24 hr. antemortem period	5.9
24	1 unit insulin/lb. injected subcutaneously 24 hrs. antemortem	40 min. at beginning of 24 hr. antemortem period	6.8
22	1 mg. Thorazine/lb. injected intramuscularly 24 hrs. antemortem	40 min. at beginning of 24 hr. antemortem period	6.0

1 Stress was periodic stimulation with an electric hot-shot.

2 Determined in the longissimus dorsi at the twelfth rib.

There were definite variations in temperament of the animals in this group prior to treatment. The animal receiving Thorazine was nervous and easily excited. The animal receiving insulin was the opposite, being very sluggish and docile. It should be noted that the steer receiving insulin had an unusually thick hide. During the excitement period the control and tranquilized steers responded similarily to the control and insulin injected steers of Group I. The control and tranquilized steers became very excited, however, the tranquilized steer was slower to respond to stimulation. After twenty minutes of stimulation the tranquilized steer became very excited and would lunge against the fence and wall of the adjacent building. Although this steer showed indications of fatigue, he would still respond to stimulation with the hot-shot. During the excitement period the control steer showed signs of exhaustion and would lie down if given an opportunity. The animal which received the insulin did not become excited when stimulated with the hot-shot but showed signs of fatigue at the end of the excitement period. When this animal was stimulated he would walk to the opposite side of the pen. Often other steers would bawl when shocked; however, this steer made no sound or other indication that he was fully affected by the shock.

All carcasses in Group II cut dark, however the carcass from the animal receiving insulin was not as dark

as the control or the one receiving Thorazine. The data presented in Table VI shows the range in pH values of the longissimus dorsi muscle. A photograph of short loin steaks taken from each of the three carcasses and an unstressed control is presented in Figure 10.

As in Group I, the period of excitement for the control appeared to exceed the minimum required to cause dark cutting beef. Due to the nervous disposition of the steer receiving Thorazine, it is possible that the dosage level was not sufficient to cause tranquilization. The steer receiving insulin was not as susceptible to stress as the control or the steer receiving the tranquilizer, probably because of docile disposition and extremely thick hide.

The animals in Group III ranged in weight from 565 to 645 pounds. The animals in Group III were handled different from those in Groups I and II in that they were allowed free access to water during the evening and night following the stress period. The excitement period was forty minutes in duration at the beginning of the twenty four hour antemortem period. The animals in this group were of the same temperament. Animals 23 and 24 were Holstein and 22 was a Guernsey. At the beginning of the stress period, the tranquilized steer was definitely groggy and appeared tranquilized but became excited when stimulated with the hot-shot. All three steers in this group reacted similarly when stimulated with the hot-shot.

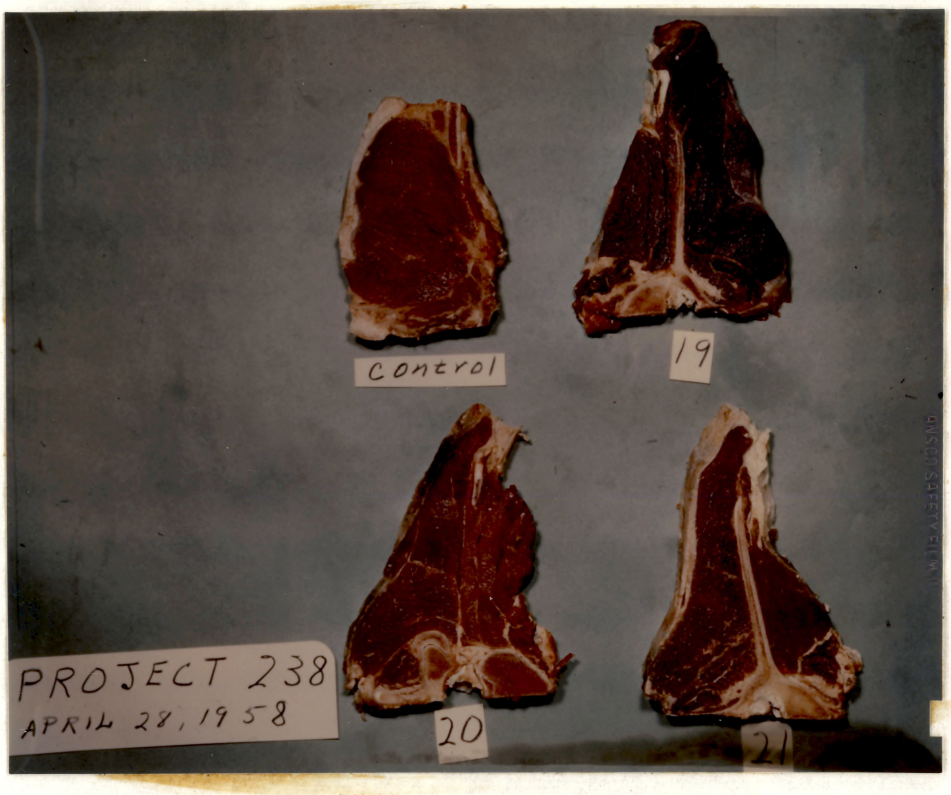

Figure 10. The Effect of Excitement on the Color of Short Loin Steaks of Beef.

These steers were beginning to show signs of fatigue when the stress period was ended.

At the time of slaughter there were wide variations in appearance and reactions of the steers in Group III. Steer 24 which received insulin was very aggressive and alert. In contrast steer 22 was almost completely sedated, and appeared to have no interest in his surroundings. Steer 23 appeared normal.

The data presented in Table VI shows the variation in pH values of the carcasses in this group. Although there were variations in the color of the longissimus dorsi muscle, all carcasses were dark cutters. A photograph of short loin steaks taken from each of the three carcasses is presented in Figure 11.

Under the conditions of this study Thorazine did not protect the animals from the stress imposed upon them. The pH values of the animals receiving tranquilizers were near that of the controls regardless of the period of stress. However, there were indications that Thorazine would prolong the time required for the animal to reach maximum excitement.

The use of insulin appeared to have little or no effect on the behavior of the animal or consistently improve the carcass characteristics as compared to the control. This is contrary to the results reported by Howard and Lawrie (1956) in which they used massive doses of

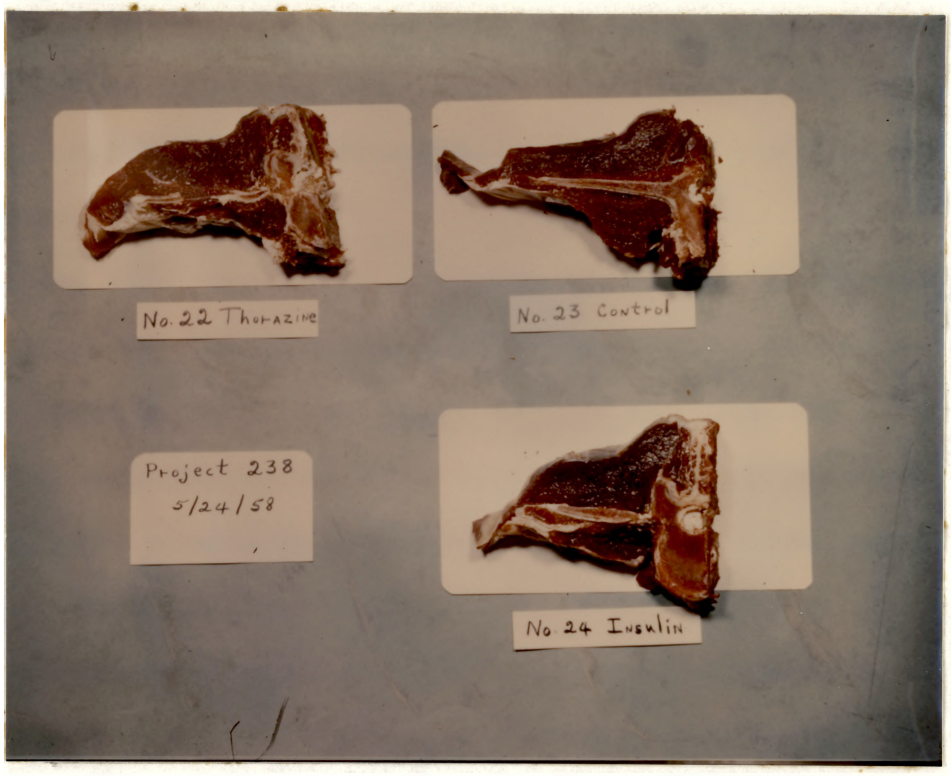

Figure 11. The Effect of Excitement on the Color of Short Loin Steaks of Beef.

insulin to produce dark cutters. The difference in results can be attributed to the great differences in the dosage level.

Many deleterious side effects were encountered from Thorazine at the dosage level used. Outstanding among these effects were large areas of edema at the site of injection. In addition, the animals were listless, dopey, ears dropped, closure of the palpebral fissure and moderate ataxia.

The data presented in Table VII shows the effects of various periods of excitement with a hot-shot on the blood sugar levels of Utility grade steers injected antemortem with insulin and Thorazine. In all instances, the steers receiving Thorazine had the highest blood sugar level at the end of the excitement period. The steer in each group which received insulin had the lowest blood sugar level.

It is possible that the blood sugar of the controls had reached a peak and began to decline prior to the time the blood samples were taken. While in the case of the steers receiving Thorazine, the tranquilizer prolonged the time required to reach maximum state of excitement and therefore the blood samples possibly were taken when the blood sugar level was at the highest peak. In the case of the steers receiving insulin, the dosage level given should promote glycogen deposition and retard glycolysis.

TABLE VII

The Effects of Varying Periods of Excitement With a Hot-Shot on the Blood Sugar Levels of Utility Grade Cattle Injected Antemortem With Various Compounds to Inhibit Stress

Animal Number	Antemortem Treatment	Blood Sugar Level (mg./ 100 ml.)	Hours After Injection
Group I:			
16	Control	81	0
	No injection, 4.5 hrs.	83	4
	excitement with hot-shot	95	9
	during 24 hr. antemortem	67	16
	period	52	23
		71	28
17	1 unit insulin/lb.	76	0
	subcutaneously, 4.5 hrs.	69	4
	excitement with hot-shot	69	9
	during 24 hr. antemortem	36	16
	period	36	24
		23	28
18	1 mg. Thorazine/lb.	84	0
	intramuscularly, 4.5 hrs.	52	4
	excitement with hot-shot	138	9
	during 24 hr. antemortem	62	16
	period	71	23
		52	24
Group II:			
19	Control	61	0
	No injection, 1.5 hrs.	79	5.5
	excitement with hot-shot	128	7.0
	at beginning of 24 hr.	74	24.5
	antemortem period	79	29.5

TABLE VII (Cont'd)

Animal Number	Antemortem Treatment	Blood Sugar Level (mg./ 100 ml.)	Hours After Injection
20	1 unit insulin/lb. subcutaneously, 1.5 hrs. excitement with hot-shot at beginning of 24 hr. antemortem period	56	0
		52	5.5
		74	7
		39	24.5
		70	29.5
21	1 mg. Thorazine/lb. intramuscularly, 1.5 hrs. excitement with hot-shot at beginning of 24 hr.	70	0
		74	5.5
		177	7
		70	19.5
		74	24.5
		74	29.5
Group III:			
23	Control, no injection 40 min. excitement with hot-shot at beginning of antemortem period	29	5
		97	6
		16	13
24	1 unit insulin/lb. subcutaneously, 40 min. excitement with hot-shot at beginning of 24 hr. antemortem period	29	5
		86	6
		14	13
22	1 mg. Thorazine/lb. intramuscularly, 40 min. excitement with hot-shot at beginning of 24 hr. antemortem period	34	5
		124	6
		34	13

The effects of thirty minutes excitement with a hot-shot on subsequent carcass characteristics of cattle previously injected with varying levels of Diquel.

Three steers used in this experiment were Utility grade and were predominantly of dairy breeding. The steers were of similar disposition, being rather quiet and easy to handle. This group of steers ranged in weight from 660 to 760 pounds live weight.

Varying levels of Diquel were injected intramuscularly four hours prior to the excitement period. The levels given were: steer 25 received one milligram, steer 26 three-fourths milligram, and steer 27 one-half milligram Diquel per pound body weight respectively. These steers were stimulated for thirty minutes at the beginning of the twenty four hour antemortem period with a hot-shot.

During the excitement period steer 25 appeared dopey and listless. Steer 26 would lie down and arise only when shocked. Neither steer 25 nor 26 appeared to become excited when stimulated with the hot-shot, however, they would move quickly. In contrast steer 27 became very excited and would react rapidly when stimulated with the hot-shot.

Blood samples were taken at the time of injection, prior to excitement and immediately following the excitement period. The data presented in Table VIII shows the blood sugar level for each animal. Steer 27 is the only

TABLE VIII

The Effects of a Thirty Minute Period of Excitement With Hot-Shot on the Blood Sugar Level of Utility Grade Cattle Which Had Been Injected With Diquel Four Hours Previously

Animal Number	Antemortem Treatment	Blood Sugar Level (mg./ 100 ml.)	Hours After Injection
25	1 mg. Diquel/lb. intramuscularly, 30 minutes excitement with hot-shot 24 hrs. antemortem	43 56 56	0 5.5 6.5
26	3/4 mg. Diquel/lb. intramuscularly, 30 minutes excitement with hot-shot 24 hrs. antemortem	43 43 56	0 5.5 6.5
27	1/2 mg. Diquel/lb. intramuscularly, 30 minutes excitement with hot-shot 24 hrs. antemortem	52 56 119	0 5.5 6.5

animal which had a definite increase in blood sugar level. It can be concluded from this study that the higher levels of Diquel inhibited the stress mechanisms of the animal responsible for increasing the blood sugar level during excitement.

The data presented in Table IX shows the effects of stress on the carcass characteristics of beef previously receiving intramuscular injections of Diquel. The pH value of steer 25 was comparable to that of bright cutting beef, while the values of steers 26 and 27 were higher than bright cutting beef. A photograph of rib steaks taken from the carcasses of each animal is presented in Figure 12. The color of the muscle ranged from a cherry red color of steer 25 to a very dark purplish black color of steer 27. The muscle of steer 26 is shady in color.

Diquel at the one milligram per pound dosage level appeared to give protection against stress resulting in the subsequent carcass having a brighter color as compared to those animals receiving lower levels. The side effects of Diquel were the same as previously described for Thorazine. Extensive edema was present in the area surrounding the site of injection.

The Physiological Effects of Serpasil on Cattle:

Five cattle were injected intramuscularly with varying dosage levels of Serpasil to determine the physiological effects this compound has on the animal. Dosage levels

TABLE IX

The Effects of Thirty Minutes Hot-Shot Treatment on the Carcass Characteristics of Utility Grade Cattle Four Hours After Injection With Diquel

Animal Number	Antemortem Treatment	Period of Stress*	pH of Loin
25	1 mg. Diquel/lb.	30 minutes	5.4
26	3/4 mg. Diquel/lb.	30 minutes	5.7
27	1/2 mg. Diquel/lb.	30 minutes	5.65

* Stress was administered at the beginning of 24 hour antemortem period.

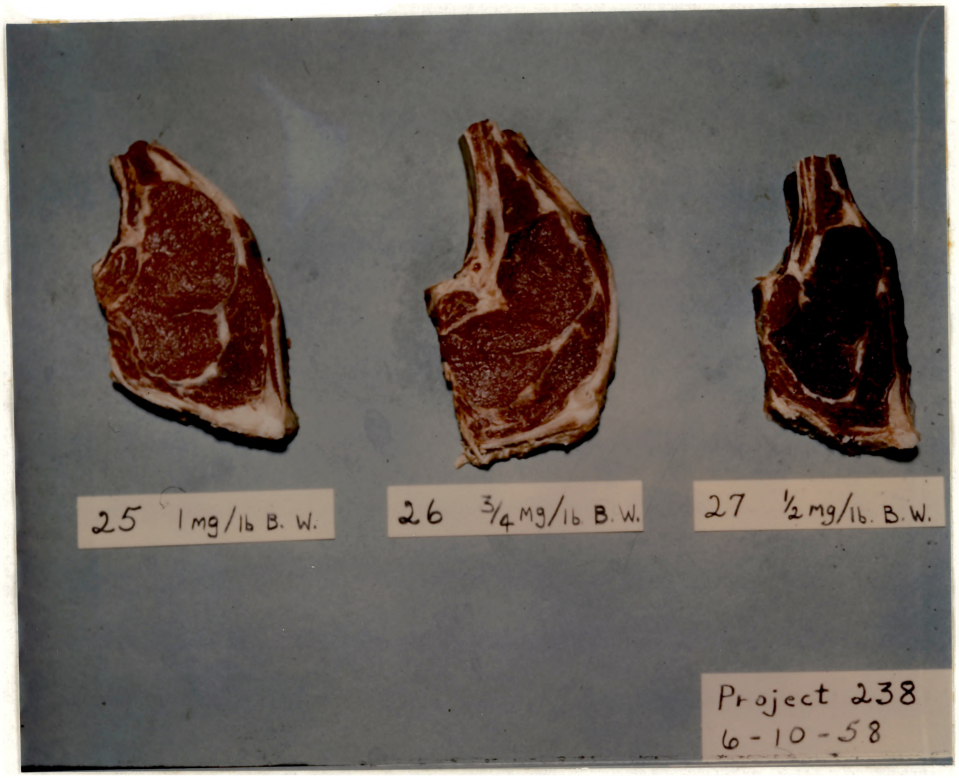

Figure 12. The Effect of Excitement on the Color of Rib Steaks of Beef Which Received Varying Levels of Diquel.

given are presented in Table X. The cattle had access to feed and water during the treatment period. Observations were made over a period of thirteen days.

Two hours after treatment steer 134 appeared normal except for a looseness of the feces. This condition continued to worsen and eight hours post-treatment a watery discharge from the eyes and nostrils was noted. At this time the animal appeared to be slightly sedated. Twenty-four hours post-treatment showed a return to normal in the condition of the feces, however, there was still a discharge from the eyes and nostrils. The animal appeared to be normal forty eight hours post-treatment. At no time was the animal off feed.

Within the first eighteen hours post-treatment steers 25,46 and 35 appeared normal in every respect. However, at twenty four hours post-treatment steer 35 appeared very sick. The eyes were red and swollen, there was a pronounced nasal discharge and breathing was short and rapid. The bowels were very loose and watery and the animal would not eat or drink. Muscle tremors were in evidence and the animal was very nervous. The rectal temperature was 103° F. Thirty two hours post-treatment showed little improvement. The animal was markedly depressed with the eyes remaining red and swollen. The nasal discharge was still very marked and there was a loss of saliva from the mouth. The rectal temperature was 105° F.

TABLE X

Dosage Levels of Serpasil Administered Intramuscularly to Beef Cattle

Animal Number	Live Weight (lbs.)	Treatment (mg./100 lbs.)	Total Dosage (mg.)
25	590	0.5	3.0
46	600	1.0	6.0
134	750	1.0	7.5
35	600	2.0	12.0
95	660	3.0	19.8

Forty eight hours post-treatment showed some improvement in the condition of steer 35; however, he was still deeply depressed and breathing was still short and rapid. The animal ate a little hay and drank some water; however, this appeared to require a great deal of effort. Seventy two hours post-treatment the animal appeared to be making good recovery. There was very little nasal discharge and the condition of the eyes was approaching normal. The condition of the feces was firm and the animal was eating hay and grain. Breathing was still rapid and the animal's movements were slow and deliberate.

Seven days post-treatment steer 35 had returned to normal. During this period steers 25 and 46 were normal and showed no sign of tranquilization, even when prodded with a hot-shot.

Steer 95 appeared normal two hours after receiving Serpasil. Four hours post-treatment this animal appeared to be in a state of sedation, was very docile, had muscle tremors, short rapid breathing and diarrhea. Five hours post-treatment in addition to the above conditions the steer's eyes were red, watery and swollen and saliva was flowing from the mouth. Seven hours post-treatment the steer way lying down and had to be urged to rise. The steer walked with a staggering gait, appeared very dazed, had a pronounced nasal discharge, and a rectal temperature of 105° F. Eleven hours post-treatment the steer was lying

down in a position characteristic of an animal with tetany and no amount of urging would cause the steer to rise. The rate of breathing was abnormal and the rectal temperature was 106.2° F.

On the following day steer 95 could stand up only for a short period, because of ataxia. There was no improvement in any of the conditions noted above. The animal would not eat or drink. The next day some improvement was noted in the above conditions and the steer attempted to eat. During the next few days the animal continued to show improvement. On the thirteenth day the animal appeared to be approaching normal physiological reactions.

The administration of Serpasil at a level which produced a noticeable degree of tranquilization resulted in deleterious side effects which would preclude the use of this compound as a preventive measure against stress.

CHAPTER V

SUMMARY AND CONCLUSIONS

I. SUMMARY

Thirty two lambs and thirty six yearling cattle were used in this investigation to study the effects of antemortem stress on postmortem carcass characteristics. Animals were stressed by:

(a) periodic stimulation with a hot-shot, or

(b) subcutaneous injections of adrenaline.

Twelve cattle were used to determine the effect of antemortem stress on postmortem carcass characteristics of animals which had previously received intramuscular injections of tranquilizers or insulin. These animals were stressed by periodic stimulation with a hot-shot.

Munsell Color Paddles were used as a reference standard for subjective color evlauation of the longissimus dorsi muscle of the cattle. The color of the primary flank muscle of the lamb carcasses was evaluated by a three-member panel using a hedonic scale. The pH values of the primary flank and the longissimus dorsi muscle of the lambs and the longissimus dorsi muscle of the cattle were determined. Blood sugar determinations were made on eighteen cattle.

Physiological stress induced by excitement during the twenty four hour antemortem period depleted the muscle glycogen of the cattle to an extent that the pH values of

the subsequent carcasses remained high. Thirty minutes of excitement with a hot-shot at the beginning of the twenty four hour antemortem period was sufficient to cause the musculature of the beef animal to become dark and the pH value to remain high. High pH and dark color of the muscle were directly related.

The administration of adrenaline produced hyperglycemia. This induced physiological stress simulated those reactions which occur when an animal is subjected to an environmental stress. Subcutaneous injections of three milligrams of adrenaline per one hundred pounds body weight was sufficient to produce dark cutting beef carcasses. Four milligrams of adrenaline per one hundred pounds of body weight produced lamb carcasses with dark flank and longissimus dorsi muscles. As the level of adrenaline was increased the color of the subsequent muscle of beef and lamb became darker, the muscle pH value higher, and the texture of the muscle became stickier and gummier.

Cattle injected with three milligrams of adrenaline per one hundred pounds body weight twenty four hours antemortem produced dark cutting carcasses. Carcasses from cattle that received the same level of adrenaline ninety six hours antemortem were "shady", i.e., the muscle was darker than normal in color. These results indicate that a much longer period is required for muscle glycogen replenishment than for glycogen depletion. If hold-over

feeding is to be of value in reducing the incidence of dark cutting beef the period of feeding would have to exceed four days.

Under the conditions of this study, intramuscular injections of insulin did not inhibit the effects of physiological stress on postmortem beef carcass characteristics.

The administration of Thorazine and Diquel did not consistently alleviate the effects of physiological stress on subsequent beef carcass characteristics. There was evidence that these compounds did protect animals from short periods of stress. However, when the animals were subjected to prolonged stress the subsequent carcasses were similar in color and pH value as those from animals receiving no tranquilizer. The administration of Serpasil at a level which produced a noticeable degree of tranquilization resulted in deleterious side effects which would preclude the use of this compound as a preventive measure against stress.

II. CONCLUSIONS

From the results of this study it can be concluded that:

The color of lamb and beef muscle is affected by antemortem stress. Prolonged antemortem stress such as excitement or subcutaneous injections of adrenaline depletes the muscle glycogen causing the musculature of the

carcass to be dark. An injection of four milligrams of adrenaline per one hundred pounds body weight produced dark primary flank and longissimus dorsi muscles in lamb. A three milligram injection produced dark cutting carcasses in Choice grade cattle. As the dosage level was increased the color of the muscle became darker in lamb.

Thirty minutes excitement twenty four hours ante-mortem caused the musculature of Utility grade cattle to be dark.

The pH value and dark color of lamb and beef muscle are closely related.

The existing supposition that dark color of lamb muscle is associated with age of animal appears to be erroneous.

At the end of ninety six hours following the injection of three milligrams adrenaline per one hundred pounds body weight the subsequent carcasses of Choice grade cattle cut "shady". However, there was considerable improvement of color as compared to carcasses of animals slaughtered twenty four hours post-treatment. The extent to which the glycogen level will be replenished during the recovery period will depend on the intensity of stress, the duration of the recovery period and the environmental conditions under which the animal is held. The glycogen reserve is depleted much faster during stress than it is replenished during the recovery period.

Administration of one unit insulin per pound body weight to cattle prior to periods of excitement from forty minutes to four and one half hours did not consistently alleviate the effect of stress on subsequent carcass characteristics.

The administration of Diquel or Thorazine at levels up to one milligram per pound body weight to cattle prior to periods of excitement from thirty minutes to four and one half hours twenty four hours antemortem did not consistently alleviate the effect of stress on subsequent carcass characteristics. There were indications that these compounds gave partial protection against excitement for short periods of time. Extensive edema was present at the site of injection.

Administration of Serpasil at a level which produced a noticeable degree of tranquilization resulted in deleterious side effects which would preclude the use of this compound as a preventive measure against stress.

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A P P E N D I X

Number of lamb _____

Weight of lamb _____

Grade of lamb _____

Pen number _____

Very pale pink _____

Pink _____

Reddish pink _____

Reddish _____

Red _____

Dark red _____

Comments

Dosage of lamb _____

Nutritional treatment _____

pH of flank _____

pH of longissimus dorsi _____

Other noticeable characteristics:

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