# SOME ANTEMORTEM AND POSTMORTEM EFFECTS OF CHLORPROMAZINE IN THE BOVINE

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# SOME ANTEMORTEM AND POSTMORTEM EFFECTS OF CHLORPROMAZINE IN THE BOVINE

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# INTRODUCTION

Tranquilizers replaced words of reassurance in the treatment of human fear, anxiety and problems of adjustment several years ago. The medical profession has successfully used the many types of tranquilizers for treatment of a variety of conditions ranging from hiccups to mental disorders. These compounds can also be found on the veterinary drug shelf and have been used extensively in the restraint and treatment of both large and small animals.

Webster defined "tranquilizer" as that which renders calm and undisturbed or allays the agitation of. Black's Medical Dictionary, 23rd edition, defined a "tranquilizer" as a drug which induces a mental state free from agitation and renders the patient calm, serene and peaceful.

Recently, livestock producers have expressed an interest in tranquilizers and in the potential of these drugs to decrease the excitement and anxiety associated with various livestock management situations. Stress has been cited as a predisposing factor in shipping fever, pneumonia and excessive shrinkage often seen in livestock following handling, shipping or exposure to extreme environmental conditions. Shrinkage, bruises and disease in slaughter animals reduce the value of the finished product of the livestock producer and they result in an appreciable loss to the livestock industry.

If anxiety and excitement contribute to the loss in value due to shrinkage, bruises, dark cutting and disease in animals which have been handled or shipped, the use of tranquilizers to control this anxiety and

excitement should tend to reduce these losses. Since tranquilizers are drugs used in human medicine, preslaughter use of these compounds would require approval by the Food and Drug Administration of the United States Department of Health, Education and Welfare.

Chlorpromazine was selected as the tranquilizer to be investigated in this study for the following reasons. First, this drug exerted a tranquilizing action without apparent disturbance of the essential functions of the body. (Bardens, 1957). The second reason was the availability of assay procedures for chlorpromazine, which permitted the detection of chlorpromazine and certain of its metabolites in the tissues of an animal which had been treated with the tranquilizer. (Salzman and Brodie, 1956) and (Flanagan et al., 1959).

The studies reported here were designed to observe the influence of chlorpromazine on the behavior of cattle under varying conditions. The rate of elimination and form of the drug eliminated were also studied. Location and relative size of residual deposits of chlorpromazine in the tissues of the bovine were determined following administration of the drug at various intervals prior to slaughtering the animal. Study of elimination and residual deposition of the tranquilizer in the bovine may provide information concerning the approval of chlorpromazine administration as a preslaughter treatment by the Food and Drug Administration.

An objective measure of stress by fluorometric determination of the levels of plasma epinephrine and norepinephrine was also attempted in this study.

## REVIEW OF LITERATURE

This review will consider some of the work relative to (1) the economic and practical importance of stress in beef production, (2) the development and pharmacology of chlorpromazine and (3) the utility of chlorpromazine in the management of large animals.

### a. Effect of Stress on Beef Quality

An appraisal of the effect of stress on beef production must be preceded by a definition of the term stress. Selye (1950) stated that anything that endangers life causes stress and adaptive responses. Adaptability and resistance to stress are fundamental prerequisites for life. The ability of living organisms to adapt themselves to changes in their surroundings appeared to depend largely upon genetic factors. Stress is, according to Selye, the biological interaction between damage and defense, as tension and pressure are the physical interactions between force and resistance.

Some of the earliest experiments dealing with the stress concept were reported by Cannon (1914). He observed cessation of activities of the alimentary canal; shifting of blood from visceral areas to the lungs, the heart, the central nervous system and the skeletal muscles; quick abolution of the effects of muscular fatigue and mobilization of carbohydrates in animals when fear, rage or pain caused the adrenal glands to pour forth adrenaline (epinephrine). These changes make the organism more efficient in the fight or flight for life.

Best and Taylor (1955) describe similar changes following the secretion of the adrenal medulla under conditions which call for unusual effort on the part of the body. The secretion of adrenaline reinforces the sympathetic nervous system in times of stress and this humoral-nervous cooperation raises the bodily reactions associated with states of emergency to maximal efficiency. The results are a rise in blood pressure with a shunting of blood to organs and areas associated with flight or fight actions, the mobilization of carbohydrates to provide readily available fuel, a release of red blood cells into the blood stream from the spleen to increase oxygen carrying capacity of the blood, an increased respiration rate and depth providing a greater exchange of gases, a reduction of blood coagulation time and the development of the physical and emotional characteristics which prepare the organism for the state of emergency.

Using rats subjected to varying degrees of tumbling trauma, Young and Gray (1956) found plasma levels of epinephrine and norepinephrine to increase with increasing severity of trauma. This may imply that greater degrees of stress cause the secretion of greater amounts of material from the adrenal medulla. Another possible explanation for their findings might be based upon a decrease in the rate of metabolism of the secretion products of the adrenal medulla, caused by the stress conditions or trauma. In either case, the plasma levels of epinephrine and norepinephrine would be expected to increase.

Goldstein and Ramey (1957) proposed that the central nervous system is a logical candidate for the role of monitor or "switchboard operator" controlling the responses within an organism subjected to stressing

situations. The prevailing concepts of response control are the sympathetic nervous system-adrenal medulla pathway proposed by Cannon and the pituitary-adrenal cortex mechanism outlined by Selye. Responses to stress conditions occur despite the absence of the adrenals; however this absence may result in damage to tissues which are responding to stress. For this reason, the authors stated that the adrenals may be associated with the maintenance of the responding tissues rather than with triggering the response. If this is true, the non-endocrine agents form a framework of mechanisms upon which the endocrine agents act.

The hypothalamus played a key part in mobilizing reactions to stress, according to Himwich (1953). The physiological changes which are associated with a response to stress conditions are triggered by mechanisms in the posterior hypothalamus.

A more complete review of the physiological manifestations observed as a result of stress conditions has been compiled by Hedrick (1957). He reported that antemortem stress, during a period of twenty-four hours prior to slaughter, would result in dark cutting beef carcasses. The use of periodic stimulation with an electric prod or the injection of adrenaline or insulin as stressing agents during this period overloaded the animal's glucogenic mechanism, resulting in a depletion of muscle glycogen which, in turn, contributed to the darkened muscle color. The author stated that dark cutting is dependent upon the susceptibility of the animal to stress and the intensity and duration of exposure to excitement, trauma, fatigue or adverse weather conditions, all of which might be considered as stressing agents commonly encountered in livestock management. Secretion of glucocorticoid hormones, which occurs subsequent

to adrenaline release, enables an animal to withstand and recuperate from stress if a recovery period is permitted. Hedrick found that severely stressed beef animals, when permitted a recovery period, did not cut dark and had normal muscle glycogen levels.

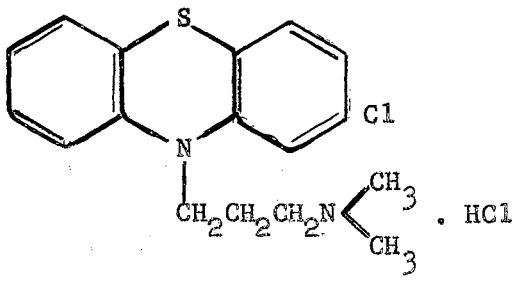
Lawrie (1958), in a comprehensive report on dark cutting in beef, reported that severity rather than duration of the stressing mechanism determined the degree of glycogen depletion in beef muscle. This author found considerable variation in the ability of different animals to withstand stress. Dark cutting may be due to extended overstimulation of the pituitary and adrenal cortex by a hypertensive hypothalamus. Genetic glandular differences could also increase the tendency of particular animals to produce dark cutting carcasses. Lawrie recommended the use of genetics and neuro-endocrinology to prevent dark cutting.

## b. Chlorpromazine

### 1. History and Pharmacology

Chlorpromazine is a product of organic chemistry. Wolley (1958), in his review, reported that Fourneau and Bovet set out to synthesize a drug which would antagonize the pharmacological effects of adrenaline. This work led to the discovery of the first antihistamine in 1933. Alterations and improvements in antihistamines resulted in the production of many derivatives, one of which was phenergan. One of the variations of the structure of phenergan that was investigated proved to be useful as a body temperature reducer and as a means of controlling the nausea of pregnancy. The tranquilizing effect on mental patients of this drug, later to be called chlorpromazine, was discovered accidentally.

Welsh (1958) reported that Charpentier synthesized chlorpromazine in 1950 and it was introduced in Europe as a presurgery medication in 1951. The structure of chlorpromazine is diagramed below as the hydrochloride.




Chlorpromazine hydrochloride

Beckman (1957) stated that chlorpromazine, when parenterally administered, caused cardio-vascular changes that include hypotension, general vasodilatation with decreased peripheral resistance, tachycardia and coronary vasodilatation. The drug inhibits neuronic activity in the cortical and hypothalamic areas of the central nervous system and is a sympatholytic and parasympatholytic ganglionic blocking agent. Chlorpromazine is adrenolytic and exerts a direct effect on peripheral blood vessels.

Hopkin and Lord (1955) cited evidence that the reticular formation and hypothalamic areas of the brain were affected by chlorpromazine. Suggestions that chlorpromazine is a general cell depressant were presented. Such suggestions could be supported by the argument that a cell depressant would produce noticeable effects on reticular formation activity due to the structure of the reticular formation. Evidence of the effects of a cell depressant would be minimized in the activities of the cortical motor and sensory nerves.

Haley (1956) reported that the action of chlorpromazine was on the thalamus and hypothalamus, with the possibility of some action occurring in the cortical areas and brain stem.

Cook (1958) found chlorpromazine to be effective in blocking conditioned responses. Maffii (1959) reported blocking of secondary conditioned responses in 50 percent of the rats used in his study after an oral dose of chlorpromazine (1.75 mg./kg.). This dosage failed to block conditioned responses, but increasing the oral dose to 11.6 mg./kg., resulted in inhibition of 50 percent of the conditioned responses. Unconditioned responses were blocked in 50 percent of the rats when a 33 mg./kg. oral dose of chlorpromazine was administered. Similar work reported by Key and Bradley (1958) showed chlorpromazine to have an effect on the thresholds of auditory induced arousal responses, but no effect on thresholds for arousal evoked by direct stimulation of the brain stem reticular formation. This was interpreted as an indication of the importance of the effect of chlorpromazine on the neural mechanisms regulating the inflow of sensory information normally producing motor responses.

In general, most workers reported decreased body temperature, decreased blood pressure, increased heart rate and a general drowsiness or decreased motor activity in laboratory animals following chlorpromazine administration. The magnitude of these effects is dependent upon the dose and mode of administration.

Himwich (1958) discussed the clinical aspects of tranquilizers as related to human treatment in his review. He also presented some possible explanations for the effects observed following both experimental

and clinical use of chlorpromazine. This drug, in the human, is able to reduce the emotional reaction to a painful stimuli, such as the persisting pain of inoperable cancer. This sedative effect may be due to the ability of chlorpromazine to depress the activity of the midbrain reticular formation. Spontaneous seizure-like waves have been detected in the amygdula area of the brain following chlorpromazine treatment. This disturbance may result in a loss of communication between parts of the brain, which in turn may restrict the general reactiveness of the nervous system and of the individual. Chlorpromazine decreases blood pressure by depressing sympathetic centers controlling blood pressure peripherally. At the same time, impairment of the peripheral parasympathetics which usually have a slowing action on heart rate is caused by chlorpromazine. The combination of the two effects could account for the increased heart rate often seen following treatment with the drug.

Himwich stated that possible interference with serotonin, epinephrine and norepinephrine, all of which are compounds that have been involved in the normal functioning of the nervous system, may result in the tranquilizing effect attributed to chlorpromazine. The phenothiazine nucleus of chlorpromazine resembles the indole nucleus of serotonin when three dimensional structures are considered. The resemblance between the side chain of chlorpromazine and the side chains of epinephrine and norepinephrine is even greater. Due to these similarities in structure, it might be possible for the tranquilizer to replace any one of the three neuro-chemical agents on the respective receptor sites, thereby competitively inhibiting the activity of these agents. Such inhibition would result in a reduction of neuronic activity, characteristic of chlorpromazine treatment.

In an *in vitro* system containing $5 \times 10^{-4}$ M (molar) chlorpromazine, Starbuck and Heim (1959) found 90 percent inhibition of oxygen uptake by homogenates of rat brain tissues as compared to control systems containing no chlorpromazine. The intraperitoneal injection of chlorpromazine (25 mg./kg.) administered to rats which were killed an hour later and brain preparations tested for oxygen uptake showed no significant difference upon comparison with the oxygen uptake of control preparations. From this experiment, the authors concluded that the concentration of chlorpromazine necessary to inhibit brain cell respiration *in vitro* is in excess of the usual therapeutic dose, if uniform distribution in the brain is assumed. The intraperitoneal injection of chlorpromazine did not influence the concentration of serotonin found in the brain.

Further work at the sub-cellular level, reported by Dawkins *et al.* (1958), suggested that chlorpromazine inhibits electron transport between reduced diphosphopyridine nucleotide and cytochrome c by inhibiting an intermediate in oxidative phosphorylation. Guth and Spirtes (1958) suggested that chlorpromazine may have some influence on mitochondrial permeability, however little evidence is presented.

2. Metabolism of Chlorpromazine

Salzman and Brodie (1956) injected dogs with chlorpromazine and studied the distribution of the drug in various body tissues and the elimination of the drug from the body. From the rapid decrease observed in plasma levels of chlorpromazine in the interval following injection, they deduced that the localization of the drug in various organs and tissues was rapid and extensive. Plasma levels are low at all

intervals tested after the intravenous injection of the 20 mg./kg. dose of chlorpromazine. Tissues were analyzed from animals sacrificed at intervals of one, three and seven hours following the intravenous injection. Brain tissue had the greatest concentration of chlorpromazine and the plasma the lowest. The tissues analyzed, listed according to the amount of chlorpromazine residual detected, were: brain, lung, spleen, kidney, liver, heart, skeletal muscle, perirenal fat and plasma. In general, the concentrations within the tissues decreased with time following injection. However, considerable chlorpromazine was still distributed throughout the tissues of the dog sacrificed seven hours after injection. Perirenal fat did show an increase in chlorpromazine concentration as time increased.

Chromatographic examination of urine samples from dogs receiving 20 mg./kg. doses of chlorpromazine revealed spots identified as chlorpromazine and three metabolites of chlorpromazine by Salzman and Brodie. The main metabolite was identified as chlorpromazine sulfoxide, the other two remained unidentified. Only 1 to 1.5 percent of the administered dose was eliminated unchanged in 72 hours. Chlorpromazine sulfoxide, eliminated in the urine within 72 hours following an injection of 20 mg./kg., accounted for 10 to 15 percent of the injected chlorpromazine.

Berti and Cima (1957) studied rates of elimination of chlorpromazine and metabolite formation following chlorpromazine administration in several species. Mice, rats, guinea pigs, rabbits and dogs were treated with chlorpromazine by subcutaneous injection of 100 mg./kg. An oral dose of 100 mg. was administered to humans in order to study the pattern of drug and metabolite elimination. Rats, guinea pigs and rabbits

eliminated approximately 20 percent of the administered dose in the urine with the maximum elimination of chlorpromazine and its metabolites occurring between 24 and 48 hours following injection. Elimination of the drug and its metabolites had nearly ceased by the fourth day after injection. In the dog, the rate of elimination reaches a peak between 24 and 48 hours after injection also, but may continue for 8 days. As a result of this extended period of elimination, it was found that dogs eliminated 40 percent of the injected chlorpromazine in either the unchanged form or metabolites. Oral doses in humans resulted in elimination of approximately 20 percent of the dose, with the peak elimination occurring during the first 24 hours. Little or none of the material was eliminated after 72 hours.

These workers also demonstrated chromatographically, the presence of chlorpromazine plus three metabolites in the urine of animals after they had received chlorpromazine injections. Chlorpromazine sulfoxide was the fraction found in greatest concentration in all species. All the metabolites detected were found to contain a halogenated phenothiazine nucleus. Berti and Cima, by comparing relative amounts of the various fractions of chlorpromazine eliminated in the urine to the physiological effect of chlorpromazine on animals of a given species, found a direct relationship between the ability of a species to metabolize chlorpromazine and the resistance shown by that species to the tranquilizing action of the drug. In mice, a greater proportion of the material eliminated was chlorpromazine and the mice were the species most effected by the tranquilizer. From these results, the authors concluded that the significant differences in sensitivity to the tranquilizer could not be

explained by differences in type of detoxification. The sensitivity to chlorpromazine was related to the degree and rapidity of detoxification attained by the species studied.

Chlorpromazine labelled with $S^{35}$ was injected intraperitoneally (80 mg./kg.) into mice by Christensen and Wase (1956) in an effort to study the distribution and elimination of radioactive material. They precipitated tissue sulfur as sulfate and counted the precipitate for radioactivity in the brain, spleen, heart, liver, lung, kidney, gastro-intestinal tract and blood. They also counted a precipitate from the remaining material which they termed, the rest of the body. The brain, spleen and lung exhibited the greatest activity during most of the five day period following injection. The rate of uptake and duration of relatively high concentration within a tissue varied from tissue to tissue. The gastro-intestinal tract and the rest of the body had consistently lower counts than any other tissues. The kidney indicated extremely high activity at 12 and 24 hours after injection. This may coincide with the fact that these authors found that peak elimination of $S^{35}$ in the urine occurred at 12 hours in the mouse. A small amount of activity was observed in the feces. The kidneys were credited with clearing the plasma of the labelled material rather rapidly, but prolonged metabolism and slow release from the tissues resulted in an extended duration of activity in the urine.

The distribution of chlorpromazine in the tissues of several species, following administration of the drug, was studied by Besson and Leder (1955). They found deposits of chlorpromazine in the liver, kidney, lungs, brain, heart, spleen, skin, muscle and urine of guinea pig,

mouse and dog. The lungs showed high levels in all species studied.

Lin et al. (1959) suggested that hydroxylation of chlorpromazine and subsequent conjugation with glucuronic acid is an important route of detoxification of the drug in man. They reported the isolation of several metabolites of chlorpromazine from human urine which they characterized as glucuronic acid derivatives or conjugates.

3. Use of Chlorpromazine in Large Animals

The veterinary profession has utilized chlorpromazine extensively as an aid in treating both large and small animals. The veterinary literature contains many case reports of use of the tranquilizer prior to or in combination with other treatment, other drug therapy or surgery, with or without anesthesia. Only a few examples of the many clinical reports will be cited here.

Chlorpromazine lends itself well to use by the veterinarian due to two of its actions as reported by Bardens (1957). First, it provides a chemical restraint without deceleration of physiological processes or functions. The second desirable feature is the ability of chlorpromazine to tranquilize by the elimination of fear and apprehension.

Estrada (1956) reported from clinical experience that chlorpromazine had an antiemetic action, a central depressant action and an enhancing effect on other drugs when used in the treatment of dogs. Dose levels of 25 to 50 mg., usually given intramuscularly, corrected shyness or unfriendliness in most of his patients. The author used chlorpromazine to successfully control swimming action observed after a dog had received phenobarbital anesthesia.

Troughton et al. (1955) used chlorpromazine hydrochloride for a number of diverse treatments and found it successful in all cases. No

post operative vomiting or running action was observed in dogs which had been anesthetized if chlorpromazine was used as a preanesthetic agent. Less anesthesia was required for satisfactory sedation. Treatment with chlorpromazine prevented car sickness. When dressings were applied or eye treatment was necessary, an injection of chlorpromazine would sedate the animal and decrease rubbing and pawing at the treated area.

In large animal treatment, the authors used chlorpromazine to relax and sedate horses suffering from tetanus. This was thought to be of importance in that the patient could eat and drink and thereby maintain its condition while recovering. Five cases of spasmodic colic in horses were treated by intramuscular injections of chlorpromazine (1.5 mg./kg.) and within 30 minutes symptoms of colic had disappeared. Troughton and his associates recommend that only intramuscular injections of chlorpromazine be used for large animals and that a waiting period of 40 minutes be allowed after the intramuscular injection or 5 to 8 minutes be allowed after intravenous injection for the drug to be effective.

Several authors have reported that chlorpromazine was effective in producing relaxation of the penis of the bull. (Matera and Stopiglia, 1955) and (Lundvall and Campbell, 1957). The treatment of an infection or physical deformity sometimes makes it necessary to observe and manipulate the penis. Under normal conditions, the bull tended to keep the penis in the sheath and struggled vigorously when exposure or manipulation was attempted. Intravenous injections of chlorpromazine at levels of 0.125 to 0.5 mg./kg. have resulted in favorable relaxation of retractor muscles and sedation of the bull.

Related work by Herrick (1958) indicated that chlorpromazine injected intramuscularly in small doses, 0.1 mg./lb., aided collection of semen from bulls by electroejaculation. Some bulls became nervous when the electroejaculator was used. Due to this nervousness, these bulls usually failed to serve an artificial vagina. Intravenous injection of chlorpromazine resulted in relaxation of the penis when ejaculation started. Erection did not occur or ejaculation occurred in the sheath which was unsatisfactory. By using small intramuscular injections the nervousness was overcome, at the same time allowing normal collection of the semen samples. The author detected no effect on semen quality due to chlorpromazine treatment.

An instance in which chlorpromazine was successfully used to sedate a large animal was cited by Cartmell (1956). He injected 250 mg. of chlorpromazine into an 800 pound cow to sedate her while dislodging a short piece of stalk which had been caught in her esophagus. The stalk was removed with a minimum of effort on the part of both the doctor and the patient.

The effect of chlorpromazine on horses was studied by Martin and Beck (1956). They used intramuscular injections in all cases and dose levels ranged from 0.5 to 2.5 mg./kg. The effects on heart rate varied between individual horses. The breathing rate was decreased, but breathing became more regular and deeper following chlorpromazine injections. Body temperature was decreased in all cases and remained so for at least 4 hours following injection. In most cases, the decrease was less than 1.0° F., but following a dose of 2.5 mg./kg., decreases of 2.2° F. and 3.0° F. were observed. A dose of 2.0 mg./kg. resulted in a decrease in

motor activity in most cases, but increased activity did occur. Repeated daily injections of chlorpromazine over a 3 or 5 day period resulted in a decrease in red blood cell count and a fall in hemoglobin concentration. In some cases, normal levels were not reached until 13 days after treatment.

Visible depression was observed in horses as soon as 20 minutes after receiving a dose of 2.5, 3.0 or 4.0 mg./kg. chlorpromazine. The degree of depression increased through the first and second hours after injection with evidence of depression remaining for periods up to 6 hours. In a few instances, depression was evident 24 to 48 hours following injection. The animals had a dull, sleepy appearance with heads hanging low. Despite the depressed attitude, horses could be easily aroused by noises. In two cases, the animal went down, but did not struggle. Within a short time, the down animal was able to regain its feet, unassisted. Some periods of excitement were observed, with pawing and moving about in the stall indicating a short period of restlessness. Smaller doses usually produced some depression, except in the case of 0.5 mg./kg. where the horses were quiet, but did not appear to be visibly depressed. Doses of chlorpromazine between 0.5 and 2.5 mg./kg. produced some variation in degree of depression.

Clinically, treatment of a mare which refused to let her foal nurse with 1.0 mg./kg. body weight of chlorpromazine resulted in acceptance of the foal. The mare had first rejected the colt three days after foaling and had continued to prevent its nursing, unless restrained, for three days before treatment was initiated. Following the single treatment, the problem did not reoccur. In at least two cases where animals

were excited prior to treatment, injection of 2 mg./kg. doses of chlorpromazine failed to sedate the animals visibly.

The standard method of treatment of tetanus in large animals using large injections of antibiotics and tetanus antiserum may not be effective due to the failure to overcome the prolonged muscle tetany which occurs during treatment. For this reason, Tait and Ryan (1957) were interested in the muscle relaxing powers of chlorpromazine in relation to treatment of tetanus in the horse. They reported their experience in two cases of tetanus, one in a three year old stallion, the other in a nine day old foal. In both cases, treatment with chlorpromazine permitted muscle relaxation during the treatment with antibiotics and antiserum. The horses were both able to eat and drink, eliminate fecal material and carry on normal bodily functions which, in most cases, is impossible during part of the treatment period at least. Intramuscular injections of chlorpromazine were used with a dose of 500 to 700 mg./day for the three year old and a dose of 100 to 150 mg./day for the colt. There was evidence of swelling and soreness in the muscle where injections were made, but this cleared up in a few days and apparently had no lasting effect.

In the treatment of swine, little use of chlorpromazine appeared in the literature, however Ritchie (1957) reported observations on swine injected both intravenously and intramuscularly with chlorpromazine. Doses of 0.5 mg./kg. to 3.3 mg./kg. were used for intravenous injections while intramuscular injections had dose ranges of 2.0 to 4.4 mg./kg. No sign of pain or irritation at injection sites was observed. Squealing stopped before intravenous injection was completed and upon release, the

animals appeared dazed, had increased respiratory rates and if undisturbed would go into a deep sleep. As reported with other species, arousal from the sleep was not difficult. Intramuscular injections produced less immediate responses and a less pronounced degree of tranquilization than intravenous injections. In most cases, swine were made more manageable for further treatment by intravenous injection of chlorpromazine, however unpredictable variation in the effect of the drug did occur. Intramuscular injection of chlorpromazine seemed to make vicious boars more docile when approached.

Intramuscular injections of chlorpromazine calmed farrowing sows which resulted in the acceptance of pigs, allowing pigs to nurse and uneventful completion of farrowing. (Kristjansson, 1957). Similar results were observed following intravenous injection of 75 to 200 mg. of chlorpromazine in frenzied sows during farrowing by Hibbs (1958). Sows ate and drank normally while under the influence of the tranquilizer. Both of these authors reported that no recurrence of the tendency to destroy pigs was observed in the sows after the single treatment.

The use of tranquilizers to control shrinkage due to shipping, handling and changing environmental or management conditions of livestock has been advocated by the producers and distributors of these compounds. Hoerlein and Marsh (1957) treated calves, which were to be weaned and hauled from their native pasture to feed lots, with intramuscular injections of chlorpromazine. Trials indicated that 1.0 mg./kg. was the dose level which produced sufficient sedation, but did not produce ataxia severe enough to cause cattle to go down in the trucks while they were being transported. Larger doses resulted in rather serious

loss of control and in-co-ordination, especially of the hind legs. The authors found chlorpromazine to decrease anxiety in calves which had been weaned and moved by truck to a new location. After moving, the treated calves resumed eating and drinking more readily than untreated controls and were much quieter, physically and vocally. In most cases, treated calves lost less weight or gained more weight in the weigh period following weaning and transfer than did untreated calves.

### c. Drug Residues in Meat Animals

An announcement relative to the status of drug materials under the food additive amendment to the Federal Food, Drug and Cosmetic Act was published by the U. S. Department of Health, Education and Welfare (1959). A veterinary drug may become a food additive through addition to the animals' feed or drinking water, whether or not residues of the drug become a component of human food derived from the animal. A veterinary drug may become a food additive, regardless of the route of administration if, as a result of its use, residues of the drug or its conversion products become a component of human food derived from the animal. Such a substance may be approved for animal use as a drug, provided that any residue of the drug or any conversion product falling within the meaning of the act does not become a component of human food derived from the treated animal, and provided further that the substance is not administered as a component of feed, including the drinking water supply. Such substances cannot be permitted in animal feed or water supplies, whether or not residues of the drugs or their conversion products become a component of meat, milk or eggs of the treated animals.

More latitude was implied in the statements made by Chicci (1959) relative to drugs used in animals. Any residues or conversion products found in the meat, milk or eggs of treated animals must be shown to be safe for human consumption, according to this author's discussion at a recent meeting of Food Technologists. This would imply that the residues are acceptable if proven safe.

# EXPERIMENTAL

This study utilized three experiments in an effort to evaluate several aspects of chlorpromazine treatment in beef cattle. Each of the experiments will be discussed individually since the techniques varied significantly, and the observations reported are, in most cases, dissimilar.

Several factors common to the three experiments will be considered prior to a discussion of the individual experiments. The complete study involved thirteen head of beef cattle obtained from the Oklahoma Agricultural Experiment Station herds.

The chlorpromazine used throughout this study was in solution, marketed under the trade name, "Thorazine". It was supplied by the manufacturer, the Smith Kline and French Laboratories. The chlorpromazine hydrochloride concentration of this product is 25 mg./ml. Chlorpromazine hydrochloride was the active form of the drug in the injection solution and was one of the two forms of the drug detectable by the analytical procedures used. In the discussion of the experiments, the term chlorpromazine will be used to denote chlorpromazine hydrochloride.

The term tranquilization and tranquility will be encountered frequently in the text that follows. Physical symptoms used as indicators of tranquilization were; a relaxed stance, partially closed or droopy eyelids and a generally drowsy or sleepy appearance. The tranquilized animal had a tendency to move in a slow, relaxed manner.

## EXPERIMENT I

Preliminary investigations were designed to study the effects of three routes of injection of chlorpromazine and several dosages of the drug in the bovine. Also included in Experiment I was a study of the rate and amount of elimination of chlorpromazine in the urine of beef cattle following the injection of the tranquilizer.

### 1. Materials and Procedure

Four animals, one Shorthorn cow, weighing 845 pounds, and three well finished Angus heifers, averaging 620 pounds, were used in this experiment. The cattle were kept in stanchion type stalls at the Oklahoma State University Veterinary Clinic during the period of treatment and observation. Each animal was injected with chlorpromazine, in the form of "Thorazine", by one of three routes; intramuscular, intraperitoneal and intravenous. The dose was varied, depending upon the type of injection used. After injection, each animal was observed for at least eight hours and behavioral effects attributable to the tranquilizer were noted.

Analysis of the urine collected from two of the four animals provided information concerning the metabolism and the rate of excretion of the injected drug. Urine was collected and the volume of each collection measured during a 10 to 12 hour period following injection. Samples of each collection or of the combined volume of several collections were placed in flasks and frozen until analysis could be completed.

Urine was analyzed for chlorpromazine and chlorpromazine sulfoxide by the method developed by Salzman and Brodie (1956). Using the Salzman and Brodie method, the samples of urine were treated with 10 percent NaOH and 25 ml. of heptane containing 1.5 percent isoamyl alcohol. After shaking and centrifuging, 20 ml. of the heptane phase was transferred to a bottle containing 0.1 M acetate buffer, pH 5.6, and shaken again. The phases were separated and an aliquot of the acid phase added to a bottle containing 0.1 M HCl. This was shaken and centrifuged. The optical density of an aliquot of the organic phase was read in the Beckman D. U. Spectrophotometer at 255 and 270 millimicrons. Standards were made by dissolving crystalline chlorpromazine hydrochloride in water and making final dilutions in 0.1 N HCl.

Chlorpromazine sulfoxide was separated from chlorpromazine in the above procedure by shaking the heptane phase with the acetate buffer. Chlorpromazine sulfoxide concentration was determined by acidifying an aliquot of the buffer phase after it had been shaken and spun. The optical density of this aliquot was read at 275 millimicrons in the Beckman Spectrophotometer.

2. Results and Discussion

The Shorthorn cow was injected intravenously with 150 mg. of chlorpromazine (0.186 mg./lb.). Tranquilization was evident immediately. She responded only slightly to cutaneous trauma with a sharp needle and was not irritated by flies. When led, the cow exhibited slight in-coordination of the rear legs. She had a sleepy appearance while standing quitely. Feed and water were available, but she failed to eat or drink.

Sensitivity to flies was observed within thirty minutes and all visual evidence of tranquilization had disappeared within one hour and thirty minutes following the injection. The cow was eating, drinking, and had urinated at this time. Her eyes were bright and alert and she seemed to respond to changes in her environment.

The three Angus heifers were treated at the same time and were observed in an effort to compare three routes of chlorpromazine administration; intravenous, intramuscular and intraperitoneal injection.

The brief duration and limited degree of tranquilization observed in the Shorthorn cow indicated that a larger intravenous dose of chlorpromazine would be necessary under practical conditions. One of the three Angus heifers, No. 667, was injected intravenously with a slightly larger chlorpromazine dose (0.25 mg./lb.). Tranquilization was evident immediately following injection. Although the heifer appeared to be rather sleepy, she was easily led and well co-ordinated 15 minutes following the injection.

Heifer No. 667 lay down, one hour after the injection, and although rather severe methods were employed, she could not be stimulated to stand. She was still down two hours after the injection and was not roused by stimulation with an electric prod. She made an attempt to stand 15 minutes later, but after rising to her rear feet and fore knees, she went down again. In a second attempt, 10 minutes later, she stood without any stimulus and apparently without difficulty. Her temperature was normal at this time.

Some tranquilization was evident in No. 667 three hours following injection, although she appeared to be annoyed by flies and had begun to

chew her cud. She first ate hay four and one-half hours after the chlorpromazine had been administered. All visible evidence of tranquilization had disappeared eight hours after the injection of a 0.25 mg./lb. dose of chlorpromazine.

Chlorpromazine was injected, intramuscularly, into the left shoulder, infraspinatus muscle area, of the Angus heifer No. 663. The dose was 0.408 mg./lb. Within thirty minutes, a decrease in response to the electric prod was observed, indicating some tranquilization. There was no evidence of in-co-ordination. Although some tranquility was observed, the degree of tranquility was less than that seen in either the Shorthorn cow or heifer No. 667.

Evidence of some discomfort or nervousness was observed in No. 663, approximately two hours following the intramuscular injection. The heifer lay down, then stood again after remaining down only two or three minutes. Similar activity was observed one hour later when the heifer lay down for five minutes, stood and repeated the process, returning to a standing position. Visible evidence of tranquilization was limited four and one-half hours following injection and had disappeared completely seven and one-half hours after the tranquilizer was injected intramuscularly.

Angus heifer No. 681 was injected, intraperitoneally, with a chlorpromazine dose of 0.471 mg./lb. and observed for a period of ten hours. There were no visible symptoms of tranquilization observed at any time during this period.

The urine eliminated by the Shorthorn cow and Angus heifer No. 667 was collected during the observation period following chlorpromazine

injection. Control samples were obtained from each of the two animals by use of a catheter prior to injection. Assay of the urine samples indicated that metabolism of chlorpromazine to chlorpromazine sulfoxide was extensive in the bovine or that chlorpromazine sulfoxide was more easily eliminated by the kidneys than chlorpromazine. Figures 1 and 2 show the pattern of elimination in the Shorthorn and the Angus, respectively, of the two forms of the drug studied. From the figures, it is evident that the sulfoxide form represents a large portion of the elimination products detected. The data also indicated that the peak concentration of chlorpromazine compounds in the urine occurred after five and one-half hours following injection in the Shorthorn and between four and one-half and twelve hours following injection in the Angus. Chlorpromazine sulfoxide was still present in the urine of the Angus when a collection was made twenty-four hours after chlorpromazine treatment.

Table I shows the volume of each collection, the length of the intervals between collections, the chlorpromazine and chlorpromazine sulfoxide concentrations, and the total amount of chlorpromazine and sulfoxide in each urine collection. The percentage of the administered dose of the drug that was eliminated in each urine collection was calculated and included in Table I. The urine eliminated by the Angus heifer between twelve and twenty-four hours following injection was not collected. The sums representing total amount of the drug eliminated and the percent of the administered dose do not account for chlorpromazine that may have been eliminated during this period.

The data indicated that approximately 12 percent of the administered dose of chlorpromazine was eliminated in the ten to twelve hour period

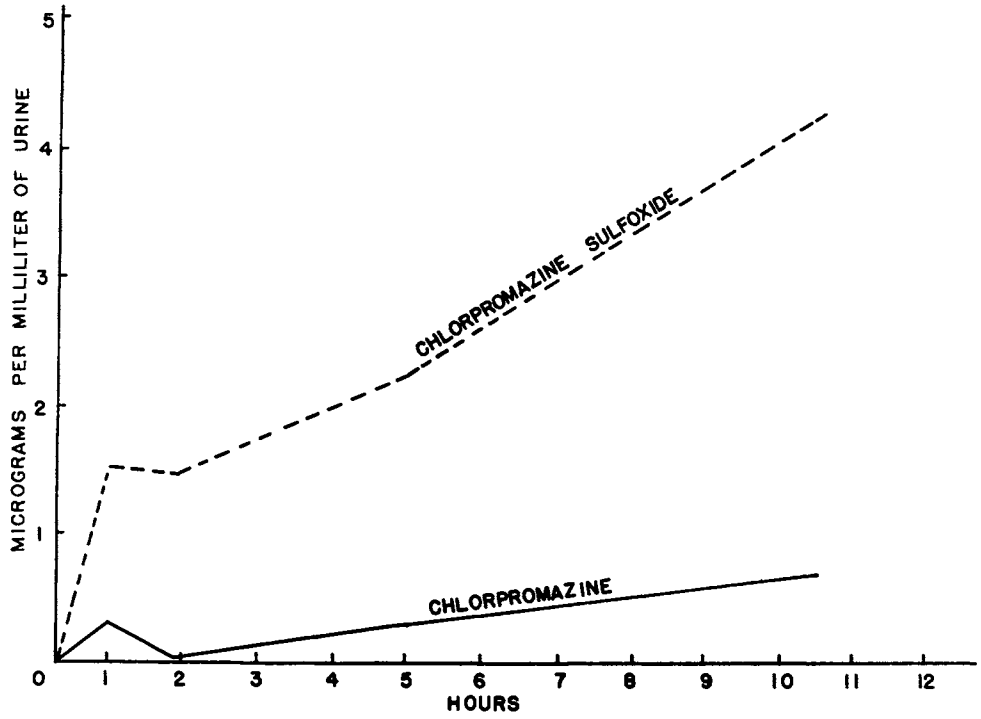



Figure 1. Levels of Chlorpromazine and Chlorpromazine Sulfoxide in the Urine of a Shorthorn Cow at Intervals Following I.V. Chlorpromazine Administration.

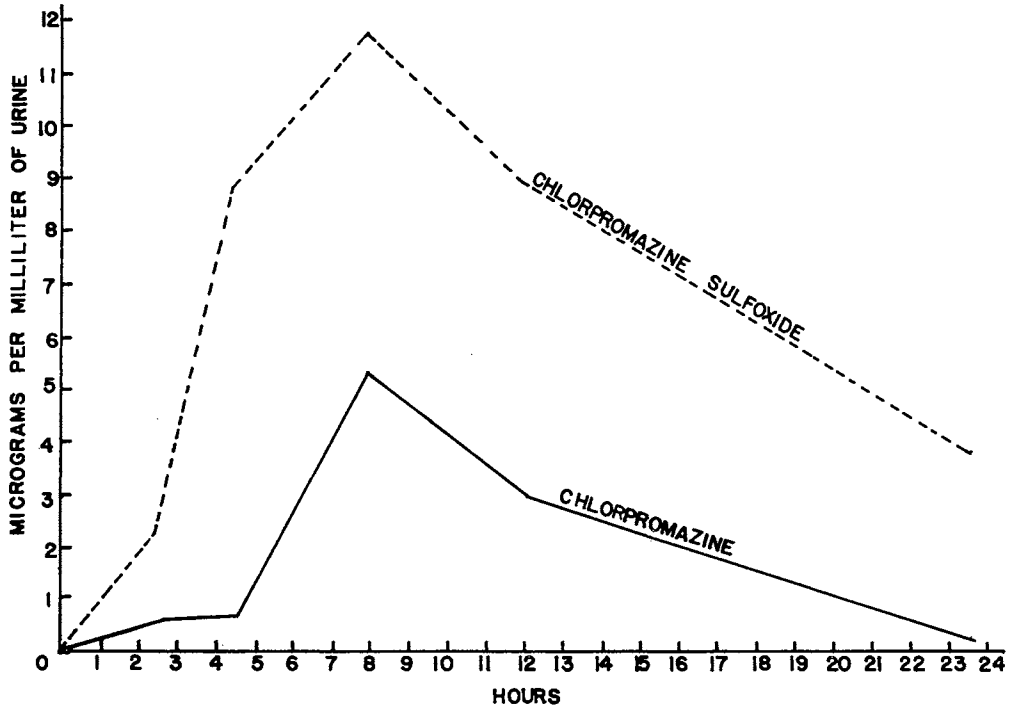



Figure 2. Levels of Chlorpromazine and Chlorpromazine Sulfoxide in the Urine of an Angus Heifer at Intervals Following I.V. Chlorpromazine Administration.

TABLE I

CHLORPROMAZINE HYDROCHLORIDE AND CHLORPROMAZINE SULFOXIDE ELIMINATED IN THE URINE

| Animal | Urine Sample No. | Collection: Time after Injection (hours) | Collection: Urine Volume (mls.) | Concentration in Urine: Chlorpromazine (mcg./ml.) | Concentration in Urine: Chlorpromazine sulfoxide (mcg./ml.) | Amount Excreted: Chlorpromazine (mcgs.) | Amount Excreted: Chlorpromazine sulfoxide (mcgs.) | Percent of Injected dose |
|---|---|---|---|---|---|---|---|---|
| Shorthorn cow[1] | S-1 | 0 | 100 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | S-2 | 1 | 1000 | 0.29 | 1.51 | 290.00 | 1510.00 | 1.20 |
| | S-3 | 2 | 750 | 0.012 | 1.48 | 9.00 | 1110.00 | 0.75 |
| | S-4 | 3 - 5 | 2055 | 0.31 | 2.26 | 637.00 | 4644.30 | 3.52 |
| | S-5 | 6 - 10.5 | 1870 | 0.72 | 4.34 | 1346.40 | 8115.80 | 6.30 |
| | Total | --- | 5775 | ---- | ---- | 2282.40 | 15380.10 | 11.77 |
| Angus No. 667[2] | A-1 | 0 | 75 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | A-2 | 2.5 | 1168 | 0.53 | 2.30 | 619.04 | 2686.40 | 1.93 |
| | A-3 | 4.5 | 755 | 0.64 | 8.92 | 483.20 | 6734.60 | 4.22 |
| | A-4 | 8.0 | 275 | 5.27 | 11.75 | 1449.25 | 3231.25 | 2.74 |
| | A-5 | 12.0 | 400 | 2.97 | 8.92 | 1188.00 | 3568.00 | 2.78 |
| | A-6 | 24.0 | 300 | 0.25 | 3.80 | 75.00 | 1140.00 | 0.71 |
| | Total | --- | 2973 | ---- | ---- | 3814.49 | 17360.25 | 12.38 |

1/ Shorthorn cow received 0.186 mg./lb. dose of chlorpromazine hydrochloride, intravenously.
2/ Angus No. 667 received 0.250 mg./lb. dose of chlorpromazine hydrochloride, intravenously.

following injection as unchanged chlorpromazine or chlorpromazine sulfoxide. It was interesting to note, that although the Shorthorn cow eliminated almost twice the volume of urine eliminated by the Angus during this period, the percent of the administered dose eliminated by the two animals was nearly the same. Ten and one-half hours after injection, the Shorthorn cow had eliminated 11.77 percent of the administered dose of 0.186 mg./lb. and twelve hours after injection, the Angus heifer had eliminated 11.67 percent of the 0.25 mg./lb. dose. The analysis of the urine for chlorpromazine and chlorpromazine sulfoxide during the first twelve hours following chlorpromazine injection failed to account for approximately 88 percent of the original dose. The data indicated that limited amounts of chlorpromazine and chlorpromazine sulfoxide were present in the urine twenty-four hours after injection in the Angus.

The first urine collected from Angus heifer No. 667 was noticeably darker in color than the control sample collected before injection. An occult blood test using "Hematest" reagent tablets sold by the Ames Company indicated the presence of hemoglobin in the urine. (Gradwohl, 1956). Samples of urine from the other two Angus heifers were tested following chlorpromazine treatment to determine whether hemoglobinuria was a result of chlorpromazine treatment, or confined to the individual, Angus 667. Hemoglobin was detected in the urine of both heifers.

The period of tranquilization observed in the four animals used in this experiment did not exceed eight hours in any case. A longer period of tranquilization would be desired in many management situations. It is possible that some effects of the drug are longer lasting than the behavioral symptoms that were used to estimate tranquility. These longer

lasting effects could result in decreased excitability of the animal under abnormal conditions. Intramuscular injection of chlorpromazine might be expected to result in a longer period of tranquilization than intravenous injection. Intramuscular injection would reduce the rate of absorption into the blood stream, thereby prolonging the effect of a given amount of the drug injected. The reduced absorption rate may increase the time necessary for the body to inactivate or metabolize a dose of the compound. When a comparison of the observations on Angus No. 667 and Angus No. 663 were made, it was apparent that intramuscular injection did not extend the period of tranquility, despite the use of a larger intramuscular dose. The time between injection and observation of the first evidence of tranquilization was increased by using an intramuscular injection as compared with the intravenous injection.

The degree of tranquilization varied, depending upon the dose and route of injection. When intravenous injections were used, tranquilization was evident immediately. The increase in chlorpromazine dose from 0.186 mg./lb. to 0.25 mg./lb. resulted in a marked increase in degree of tranquility. No information is available to determine whether a portion of the increase in tranquilization was due to differences in individual animal response or whether the total increase in tranquilization was due to the increase in dose. Intramuscular injection of chlorpromazine at a level of approximately 0.4 mg./lb. resulted in a limited degree of tranquilization. Blood levels probably do not achieve the same magnitude as produced by intravenous injection when absorption of the material from an intramuscular injection site is necessary. The lower blood levels of the drug may be less than the threshold levels required to produce some

of the visible symptoms of tranquilization. This would explain the limited degree of tranquilization seen following an intramuscular injection and may also be an important factor in the failure of intramuscular injection to extend the duration of tranquilization. Although chlorpromazine was still present at the injection site, absorption from the site into the blood stream may not have been rapid enough to maintain threshold levels of chlorpromazine in the blood.

Intraperitoneal injection of chlorpromazine failed to produce any evidence of tranquilization. The reason for this is not known. Failure to absorb the drug from the peritoneal cavity before it was metabolized may be the explanation. If the injection was made into omental fat within the cavity, absorption would be slow due to the poor blood supply to this tissue. The observation of hemoglobinuria following intraperitoneal injection indicated that some chlorpromazine entered the blood stream and caused lysis of the red blood cells.

Several unexpected results of chlorpromazine injection were observed. At least one of these results, the detection of hemoglobin or blood protein in the urine appeared to be a commonly occurring side effect of this drug in the bovine. The literature reviewed contained no report of hemoglobinuria following chlorpromazine treatment in large or small animals. The lay-and-stand activity observed in Angus No. 663 following intramuscular injection appeared to be a hypertensive type of action. Two possible explanations for this action have been considered. The pharmacological action of chlorpromazine upon the neuro-humoral system of this particular animal may have resulted in the restless activity. The inflammatory effect of the injection of the drug into the shoulder muscle

may have increased the irritability of the heifer and resulted in discomfort when she tried to lay down. This would stimulate her to stand. Martin and Beck (1956) reported periods of excitement in horses receiving intramuscular injections of chlorpromazine.

Even with the limited data available here, it was obvious that the bovine metabolized a large portion of chlorpromazine before the drug was eliminated. Assuming that chlorpromazine sulfoxide is the main metabolite of chlorpromazine in the mammal as indicated by Salzman and Brodie (1956), only a small portion of the injected material was eliminated within the period of tranquilization. A large portion of the drug is still unaccounted for after twenty-four hours.

The results of Experiment I indicated that an attempt to detect residual deposits of the drug within the tissues of the bovine, as proposed at the outset of the overall study was advisable. Since a large portion of the drug injected into the system was not accounted for, it may have been deposited in the body tissues. If these deposits are localized in the muscle or fat tissues, the use of chlorpromazine immediately prior to slaughter should be limited.

Complete metabolism of the chlorpromazine molecule by the bovine would explain the inability to detect the drug or its metabolites in the urine. If extensive metabolism was occurring, deposition of residuals would be limited. This would also tend to reduce the dangers associated with pre-slaughter chlorpromazine injection.

Intravenous injection appeared to be the most reliable method of administration. Intraperitoneal injection produced no tranquilization, while intramuscular injection resulted in a limited degree of

tranquilization. To draw conclusions in a trial as limited in scope as Experiment I is difficult, but logic would indicate that by direct introduction into the blood stream, better control of the amount of the drug available for elimination, deposition or metabolism is possible.

Adequate tranquilization, useful under practical conditions from the standpoint of degree and duration, requires the intravenous injection of a chlorpromazine dose of at least 0.2 mg./lb.

## EXPERIMENT II

Residual deposition of chlorpromazine and chlorpromazine sulfoxide in the tissues of beef animals was studied in Experiment II. Further information concerning behavioral changes following chlorpromazine treatment was also obtained.

### 1. Materials and Procedure

Five Hereford calves were injected with chlorpromazine and slaughtered at various intervals after injection. A sixth Hereford calf was used as a control and was not injected. Samples of tissues and organs were removed at slaughter and analyzed for chlorpromazine and chlorpromazine sulfoxide to gain information about the extent and distribution of residual deposits of the two forms of the tranquilizer. The calves used in Experiment II were housed in the holding pen in the Meat Laboratory during treatment and observation. The treated calves each received intravenous chlorpromazine injections of 0.4 mg./lb. as a basic treatment.

The interval between chlorpromazine injection and slaughter of the animals varied from four to seventy-two hours. During this interval, the calves were permitted to run in a large pen. Feed and water were available in the instances involving treatment-slaughter intervals of seventy-two hours. The calves were observed closely in the interval between injection and slaughter to study the effects of chlorpromazine on the bovine.

Each calf was slaughtered at the Meat Laboratory using procedures outlined by Deans (1951). At the time of slaughter; tissues, organs and

the carcass were studied closely for evidence of any abnormalities which may have resulted from injection. Tissues which were analyzed for residuals included muscle, fat, liver, heart, lung, brain, tongue, spleen, kidney and blood. Representative samples of the lung, tongue and liver were obtained at slaughter. The heart, brain, spleen and kidneys were removed intact and saved for analysis. In some cases, fresh samples of lean and fat were also removed at slaughter. The samples were wrapped in freezer paper, frozen and stored in a deep-freeze at 0°F. until they were analyzed. Blood samples, collected during slaughter, were placed in flasks and frozen. When available, urine samples were tested for hemoglobinuria using the occult blood test. (Gradwohl, 1952). The carcass was placed in a 34°F. cooler and muscle tissue samples were removed from the carcass after it had cooled, in some instances.

The tissue samples were analyzed for chlorpromazine and chlorpromazine sulfoxide, using the method of Salzman and Brodie (1956). The procedure was the same as outlined in Experiment I, using a tissue homogenate in 0.1 N HCl in the initial step. A second method and a slight revision of it were used to determine the chlorpromazine and chlorpromazine sulfoxide content of the tissues of the last calf slaughtered. The original method was developed by Flanagan et al. (1959) and utilizes an alkaline hydrolysis step to remove the more firmly bound chlorpromazine from the tissue. Prior to analysis, the tissue was thoroughly mixed in a Waring Blender. A sample, 2.0 to 5.0 gm. of the blended material, was placed in a stoppered tube with 5 ml. of water and shaken to produce a homogenous mixture. The diluted homogenate was brought to pH 13 by the addition of 10 N NaOH. Twenty-five ml. of ether were added

and the tube stoppered and shaken for two minutes. Emulsification was broken by centrifugation. The ether layer was transferred to a separatory funnel. The extraction step was repeated five times, combining the extracts. The residue was saved for determination of bound chlorpromazine and chlorpromazine sulfoxide.

The ether extracts were washed with 0.1 N NaOH. The alkali washings were added to the tissue residue. When the alkali layer became clear, the ether layer was washed with water. The ether layer was then back extracted five times with 0.1 N HCl and the adsorption spectrum of a portion of the acid extract was determined over a range of 220 to 320 millimicrons on the Beckman D. U. Spectrophotometer.

The concentration of chlorpromazine was obtained by plotting the adsorption spectrum and drawing a diagonal line connecting the points on the spectrum at 236 millimicrons and 264 millimicrons. The optical density was measured between the point on the spectrum and the point on the diagonal line at 255 millimicrons. The concentration of free chlorpromazine sulfoxide was determined by subtracting the optical density at 315 millimicrons from the optical density at 275 millimicrons. Sample concentrations were determined from a standard curve prepared for each of the two compounds by obtaining absorption spectra of standard solutions and plotting optical density, determined as described above, against concentration.

To determine bound chlorpromazine and sulfoxide, the residue, saved from the determination of free chlorpromazine and chlorpromazine sulfoxide was first gently warmed to vaporize any remaining ether. The residue was then hydrolyzed by addition of NaOH and heating in a boiling water

bath for one hour. Five ether extractions of the hydrolyzed residue were accomplished using a separatory funnel, pooling the ether extracts. The pooled extracts were handled in the same manner as the pooled ether extracts in the determination of free chlorpromazine and chlorpromazine sulfoxide.

A slight revision of the Flanagan et al. method was used in determining total chlorpromazine and chlorpromazine sulfoxide. By using this revision, it was not necessary to extract and read two sets of aliquots from the same tissue sample to determine total chlorpromazine and chlorpromazine sulfoxide. The macerated tissue sample was suspended in a solution of NaOH and heated for one hour in a boiling water bath. This material was extracted with ether five times, combining the ether extracts. The pooled ether extract was carried through the remaining portion of the procedure as outlined by Flanagan et al. The results of this determination were termed "total" chlorpromazine and "total" chlorpromazine sulfoxide.

2. Results and Discussion

A Hereford bull calf (calf No. I), weighing 352 pounds, was the first animal used in Experiment II. An intravenous chlorpromazine dose of 0.4 mg./lb. was injected and calf No. I was observed for a seventy-two hour interval between injection and slaughter. Tranquilization was observed immediately after the injection. Stimulation of the calf produced some response, usually an attempt to avoid the stimulating agent. Noises did not disturb the animal while he was lying down, but he could be easily roused. Although some evidence of in-co-ordination of the rear legs was observed, the calf contentedly chewed his cud two hours following the

injection. Within three hours after the injection, this animal was alert and aware of activity in the area and seven hours after the injection, all evidence of tranquilization had disappeared. The activity and appearance of calf No. I were normal from seven hours after injection until slaughter.

Calf No. I was slaughtered and a thorough inspection of the tissues and organs revealed no abnormalities which might be attributed to the chlorpromazine treatment. Samples of the organs and tissues were removed and prepared for analysis as indicated earlier. A positive test for the presence of hemoglobin in the urine was the only deviation from normal conditions detected at the time of slaughter. After a forty-eight hour chill, a composite sample of muscle from the round, rib, and chuck was removed, mixed and ground. This ground sample was wrapped and frozen to be stored until analyzed. Assay of the samples from calf No. I, by the Salzman and Brodie method, indicated no detectable residual chlorpromazine or chlorpromazine sulfoxide in any of the tissues.

A wild Hereford heifer, weighing 332 pounds, was injected with the standard 0.4 mg. of chlorpromazine per pound of body weight, intravenously. This heifer was slaughtered eight hours after the chlorpromazine treatment. The degree of tranquility was not as great as had been observed previously in calf No. I. Tranquilization was definitely observed following injection although there was little or no evidence of tranquility remaining at the time of slaughter.

No adverse effects of the tranquilizer treatment could be determined from observation of the carcass and tissues. The test for hemoglobin in the urine was positive. Tissues and organs were sampled at the time of

slaughter and samples of fat and lean were removed from the carcass following a twenty hour chilling period. Tissue residues of chlorpromazine were detected in brain, heart, lung and tongue while residues of the sulfoxide were found in brain, kidney and lung tissue. Table II presents data on the amount and location of the residues detected.

The control calf was slaughtered the same day that calf No. II was treated and slaughtered. The two Hereford heifer calves were handled in a similar manner. They were kept together in the holding pen at the Meat Laboratory prior to slaughter and were slaughtered under similar conditions. After slaughter, samples of brain, blood, heart, kidney, liver, lung and tongue were obtained for assay. These samples, along with samples of muscle and fat from the carcass of the control calf, were used to determine if the Salzman and Brodie procedure was able to extract material from any of these tissues which may influence the chlorpromazine or chlorpromazine sulfoxide determination. No interference was detected.

The third portion of Experiment II involved two wild Hereford heifers; calf No. III, weighing 275 pounds, and calf No. IV, weighing 306 pounds. The two heifers were brought to the holding pen at the same time and were treated concurrently.

Animal No. III was observed for a four hour interval between treatment and slaughter. The standard 0.4 mg./lb. dose was injected intravenously. Chlorpromazine treatment resulted in a different reaction pattern than that observed in the animals discussed earlier. The degree of initial tranquilization following chlorpromazine injection was limited as opposed to a rather marked tranquilization observed in most cattle

immediately following intravenous administration. This very wild heifer was obviously tranquilized immediately, but to a limited extent, since she resisted restraint vigorously and ran when approached. One and one-half hours following the injection, she was lying down. She made attempts to stand when approached and had the desire to run, but apparently lacked sufficient co-ordination to permit her to stand. One hour later, she was able to stand and walk, but she could be approached easily and showed evidence of marked tranquilization. Animal No. III was excitable, immediately prior to slaughter, but at the same time her actions indicated laziness or fatigue.

Examination of the carcass and other tissues of calf No. III following slaughter revealed no abnormal effects of chlorpromazine treatment. Hemoglobinuria was detected as the only factor which could be attributed to the tranquilizer treatment. Tissue and organ samples were obtained at slaughter. Samples of muscle were taken from the hot carcass at slaughter and from the chilled carcass forty-eight hours after slaughter. The sample of fat was taken from the hot carcass. The only residual chlorpromazine detected in the samples from animal No. III was found in the fat as shown in Table II.

Hereford heifer No. IV, treated at the same time as heifer No. III, received an initial chlorpromazine injection of 0.4 mg./lb., intravenously. Moderate tranquility was evident almost immediately. The heifer was alert and usually came to her feet, if lying down, as soon as the door to the pen was opened. If startled, she ran.

The results of analysis of tissues from animals No. I and No. II indicated that a 0.4 mg./lb. dose of chlorpromazine resulted in residuals

which, if detectable at all, approached the minimum amount detectable by the Salzman and Brodie method. To more accurately evaluate the distribution of the residual material, larger residuals would be an advantage. This could be accomplished by increasing the dose, which could result in complete sedation and this was not desired. Since animal No. IV exhibited evidence of only a mild degree of tranquilization following the standard injection, it was possible to give a subsequent injection with little danger of overdosing. For this reason, a second chlorpromazine injection of 0.4 mg./lb. was given in two stages, two and one-half hours following the initial injection. In-co-ordination of her movements and a visible sleepiness were observed in animal IV following the second injection. She remained standing and moved around the pen during a fifteen minute observation period following the injection. The calf lay down when left alone in the pen, but when the pen door was opened, her head would come up and she watched the intruder in an alert manner. If she did not sense danger, she would close her eyes and put her head down on her flank, assuming the position observed throughout this study to be characteristic of a resting tranquilized animal. Four hours after the second injection, animal No. IV was able to stand and run with noticeable in-co-ordination.

The animal was slaughtered four hours after the second injection or six and one-half hours following the initial injection. Tissues and carcass were normal at slaughter. Samples for analysis were removed and frozen. The urine in the bladder was almost coffee-colored due to the presence of hemoglobin. Chlorpromazine residuals were detected in all tissues sampled; excluding muscle, tongue and kidney; as indicated by Table II. No residual chlorpromazine sulfoxide was detected.

TABLE II

RESIDUAL CHLORPROMAZINE AND CHLORPROMAZINE SULFOXIDE IN BEEF TISSUES

| Animal No. | Dose mg./lb. | Injection-Slaughter interval (hours) | Form of Residual | Concentrations in Tissues Analyzed for Residual Chlorpromazine and Sulfoxide (mg./100g. fresh tissue) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Muscle | Fat | Liver | Heart | Lung | Brain | Tongue | Spleen | Blood | Kidney |
| I. | 0.4 | 72 | Chlorpromazine | 0[1] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | CPZ Sulfoxide | 0[2] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| II | 0.4 | 8 | Chlorpromazine | 0 | 0 | 0 | 0.09 | 0.10 | 0.05 | 0.06 | -- | 0 | 0 |
| | | | CPZ Sulfoxide | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -- | 0 | 0.08 |
| III | 0.4 | 4 | Chlorpromazine | 0 | 0.09 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | CPZ Sulfoxide | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IV | 0.8[3] | 6.5[4] | Chlorpromazine | 0 | 0.17 | 0.07 | 0.06 | 0.18 | 0.15 | 0 | 0.17 | 0.06 | 0 |
| | | | CPZ Sulfoxide | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

1/ 0 indicates concentration is less than 0.01 mg./100 g., the minimum sensitivity of chlorpromazine analysis.

2/ 0 indicates concentration is less than 0.03 mg./100 g., the minimum sensitivity of sulfoxide analysis.

3/ Two 0.04 mg./lb. doses injected intravenously in a two and one-half hour period.

4/ Slaughtered 6.5 hours after the initial injection, 4 hours after the second injection.

Treatment of the last calf used in Experiment II was designed to increase the amount of drug injected, in an effort to produce detectable residuals of chlorpromazine or its sulfoxide in the muscle tissue of the carcass. A relatively large initial intravenous injection of chlorpromazine was used. A dose of 0.62 mg./lb. was injected into the extremely wild, 350 pound Hereford heifer. Fifteen minutes later, an additional 0.20 mg./lb. was injected, making the total dose 0.82 mg./lb. This large intravenous dose was supplemented by four intramuscular injections of 2 mg. of chlorpromazine per pound of body weight. The intramuscular injections were spaced over the seventy-six hour interval between the initial injection and slaughter of calf No. V.

After receiving the intravenous injections, a total dose of 0.82 mg./lb., calf No. V was tranquilized as evidenced by a slightly unsteady gait, poor co-ordination, heavy lidded eyes and lack of normal alertness. Despite the comparatively high dosage, none of the more severe symptoms, such as the inability to stand, serious loss of control of extremities or extra-ordinary dullness were apparent. She was difficult to handle and fought vigorously against the halter even after receiving the large dose of tranquilizer. She was still on her feet and would run when approached an hour after injection.

Seven and one-half hours following the initial injection, animal No. V received an intramuscular chlorpromazine injection of 2 mg./lb. into the musculature of the left shoulder area. One and one-half hours later, she was able to run, but her front feet had a tendency to knuckle under and her rear leg motion was awkward. She remained sleepy and dopey for at least six hours following the intramuscular injection.

Animal No. V was alert and wild twenty-two hours after the treatment had begun. Difficulty in catching and restraining her prior to injection of the second 2 mg./lb. intramuscular dose of chlorpromazine demonstrated this. To decrease the possibility of excessive irritation in a single area, the intramuscular injections were introduced into the right and left shoulders alternately. The first intramuscular injection was into the left shoulder, therefore the second was injected into the right shoulder. Within twelve hours after the second intramuscular injection, animal No. V was active and ran when approached, but at the same time appeared listless and acted very tired when allowed to stand quietly. The longer interval between the second and third intramuscular injections allowed calf No. V an opportunity to recover from the tranquilized condition to insure that she ate and drank.

Following the rest interval, and fifty-three hours after the initial intravenous injection, the third intramuscular injection was made into the left shoulder. By this time, the heifer was more easily caught and more co-operative while being prepared and held for the injection. Dullness and a listless attitude were observed for at least six hours following the third intramuscular injection.

The fourth intramuscular injection was made into the muscle of the right shoulder seventy-one hours after treatment had been initiated. The calf stood quietly when approached and offered little resistance to handling at the time of this injection. She was active and had been eating hay prior to the injection. During the interval between the fourth intramuscular injection and slaughter, calf No. V could be

approached and would stand while a hand was placed on her head or back.

Calf No. V was slaughtered seventy-six hours after treatment was initiated. The tissues and carcass appeared normal. Samples were removed and stored for analysis.

A study of the possible degradation of residual chlorpromazine in muscle during the chilling and aging period was attempted, using the muscle tissue of the shoulder areas of calf No. V. Since large amounts of chlorpromazine had been injected into the tissue, relatively large residuals would be present. At the time of slaughter, the muscle tissue of the shoulder and neck area was removed, boned, ground and mixed thoroughly. When the muscle was removed from the carcass, extensive necrotic areas were observed where chlorpromazine had been injected intramuscularly. The meat from each side was treated separately and placed in a separate tray. Samples from each tray of ground muscle were removed and frozen for analysis, and the remainder of the tissue from each shoulder area was placed in the cooler at $34^{\circ}$ F. Samples were removed from both trays at intervals during a period of fourteen days following slaughter.

Fresh muscle and internal fat samples were obtained from the loin of the carcass of animal No. V. Subsequent samples were removed from the loin to determine if degradation of the residual occurred in the intact carcass muscle during a forty-eight hour chilling period.

Three different analytical procedures were used to evaluate the residual chlorpromazine and chlorpromazine sulfoxide concentration of the various samples obtained from the tissues and carcass of calf No. V.

The lean from the loin area, removed at slaughter and at intervals during a forty-eight hour chill, was analyzed using the Salzman and Brodie method. Samples of fat removed from the carcass at slaughter and at intervals during the forty-eight hour chill along with samples of the ground lean material prepared from the muscle tissue of the right shoulder area were analyzed by the method to determine free and bound chlorpromazine and chlorpromazine sulfoxide as outlined by Flanagan et al. (1959). The revision of the Flanagan et al. method which determines total chlorpromazine and total chlorpromazine sulfoxide was used to analyze samples of loin muscle removed from the carcass at slaughter, samples of ground material prepared from the musculature of the right and left shoulder areas and samples of the organs and tissues removed at slaughter.

The results of these analyses can be seen in Table III. No chlorpromazine or sulfoxide was detected in muscle samples from the loin by either the Salzman and Brodie technique or the determination of total chlorpromazine and its sulfoxide. Similarly, the Flanagan et al. method failed to detect any residual in the fat tissue removed from the carcass. All of the detected chlorpromazine in the ground muscle from the right shoulder area was of the bound form (Table III). No sulfoxide was found in the shoulder tissue. Relatively large concentrations of total chlorpromazine were located in the lung and kidney samples of animal No. V. Comparison of this data and data from previous animals is not possible since the method of analysis was not the same in both cases.

The response to chlorpromazine treatment observed in the five treated calves of Experiment II was, in general, similar to the response seen

TABLE III

CHLORPROMAZINE CONCENTRATIONS IN TISSUES FROM CALF NO. V FOLLOWING A COMBINATION OF I.V. AND I.M. INJECTIONS

| Tissue | Cooler storage time after slaughter (hours) | "Free"[1] Chlorpromazine (mg./100g.) | "Bound"[1] Chlorpromazine (mg./100 g.) | "Total"[2] Chlorpromazine (mg./100 g.) |
|---|---|---|---|---|
| Muscle from Shoulders[3] | | | | |
| Right | 0 | less than 0.25 | 3.0 | --- |
| Right | 16 | less than 0.25 | 4.1 | --- |
| Right | 48 | less than 0.25 | 5.1 | --- |
| Right | 100 | less than 0.25 | 3.9 | --- |
| Right | 260 | --- | --- | 5.6 |
| Right | 329 | --- | --- | 6.6 |
| Left | 260 | --- | --- | 3.7 |
| Left | 329 | --- | --- | 3.6 |
| Brain | 0 | --- | --- | less than 0.25 |
| Tongue | 0 | --- | --- | less than 0.25 |
| Spleen | 0 | --- | --- | less than 0.25 |
| Lung | 0 | --- | --- | 0.69 |
| Kidney | 0 | --- | --- | 0.55 |
| Liver | 0 | --- | --- | less than 0.25 |

1/ Analyzed by the Flanagan et al. (1959) procedure.
2/ Analyzed by the revision of the Flanagan et al. (1959) procedure.
3/ Samples from trays of the ground muscle tissue removed from the areas receiving I.M. injections.

in the preliminary work done in Experiment I. In every case, tranquilization was observed, and in-co-ordination occurred in varying degrees following injection of chlorpromazine. Although the calf had a drugged appearance after the second intravenous injection, animal No. IV became alert when activity or noise in the pen startled it out of its drowsiness. Even when in-co-ordination was so great that calf No. III could not stand, she became nervous and attempted to move away when approached. Such observations indicate that the "fright and flight" reaction is not seriously depressed by chlorpromazine as used in this study. The observations reported here indicate also that motor activity is decreased following injection of chlorpromazine under conditions where the animal does not encounter stimuli likely to cause "fright or flight" reactions. When left alone, the treated calves stood or lay quietly and appeared to be sleepy. Movements were slow and lazy, when observed under normal conditions.

The variation in response to a given dose is made apparent in Experiment II. Calf No. III was too un-co-ordinated to stand following an intravenous chlorpromazine injection of 0.4 mg./lb. while her pen-mate, calf No. IV, displayed little, if any, in-co-ordination following a similar dose. A second dose of 0.4 mg./lb. was required to produce comparable reactions in animal No. IV.

The ability to withstand large chlorpromazine doses appeared to be related to the temperment of the animal. One of the arguments for this theory was seen in the data from calf No. V. The wildest of the animals used in the whole study, calf No. V was injected intravenously with a chlorpromazine dose of 0.82 mg./lb. Despite this large dose, calf No. V

exhibited none of the serious symptoms of chlorpromazine treatment shown by Angus 667 in Experiment I after receiving only 0.25 mg./lb. and calf No. III in Experiment II, after receiving 0.4 mg./lb. Close observation of a larger number of animals of varying temperment following chlorpromazine treatment would be necessary to determine the influence of temperment on the response to chlorpromazine, but the observations reported here hint that such a relationship may exist. Since the literature is vague about the pharmacological activity of chlorpromazine, any physiological explanation of this relationship would be difficult at this time.

Comparison of limited observations reported in Experiment I following intravenous injection of chlorpromazine with similar observations in Experiment II, showed similar degrees of response resulting from doses which differ widely in strength. Again, the response appeared to be related to temperment. Both of the animals in Experiment I which received intravenous injections were quiet, halter broken cattle while the calves used in Experiment II were wild. One other difference not stressed earlier was the difference in degree of fatness of the Angus heifer compared to the Hereford calves used in Experiment II. The Hereford calves were thin and carried very little finish, whereas the Angus heifer had a fair amount of finish and was classified in the Good grade for slaughter cattle. The increased fatness of the Angus might tend to increase the effective dose of an intravenous injection. Blood volume in the fatty tissue is limited, therefore the rest of the body would be exposed to a larger relative dose of tranquilizer than would the organs and lean tissue of a thin animal of the same weight receiving an equal dose of the drug. This may explain why the response of the Angus to a

dose of 0.25 mg./lb. was as great or greater than the response observed in the less fat Hereford calves. Age may be a factor also, with the younger animals being more resistant to the effects of the tranquilizer.

When urine was available, hemoglobinuria was detected following treatment with chlorpromazine. Commercial chlorpromazine hydrochloride (Thorazine) has a pH of approximately 4.5. The low pH of the drug injected into the blood stream may have caused lysis of red blood cells. This was the most apparent explanation for the hemoglobinuria. The low pH was also thought to be a contributing factor in the tissue damage observed at the site of the intramuscular injections in the shoulders of calf No. V.

Beef muscle tissue from the animals slaughtered contained no detectable residual chlorpromazine or chlorpromazine sulfoxide as determined by the Salzman and Brodie method in this experiment. The large total dose administered to animal No. V was an attempt to produce residuals in muscle tissue, but no residual was detected by either of the two methods used to analyze the muscle tissue.

Chlorpromazine was detected in the fat of one animal, calf No. IV, following the injection of a dose of 0.8 mg./lb. Residuals of chlorpromazine were detected in brain, spleen, kidney, tongue, liver, heart, lung and blood of one or more of the animals used in this experiment. Chlorpromazine sulfoxide was detected in kidney, brain and lung tissue when animal No. II was slaughtered eight hours after injection of a chlorpromazine dose of 0.4 mg./lb. Lung, kidney and brain tended to show greater residual concentrations than the other tissues. This agreed with work reported by Christensen and Wase (1956) and Salzman and Brodie (1956).

Detection of residuals in the tissues of calf No. II showed that a 0.4 mg./lb. dose of chlorpromazine was capable of producing residuals in the tissue eight hours after injection. Three possible explanations may be offered for the failure to detect the residuals after injecting animal No. I with an equal dose and slaughtering seventy-two hours later. First, there may never have been detectable residual deposits of chlorpromazine in the tissues of calf No. I due to individual differences in ability to metabolize, eliminate and deposit the drug. Secondly, if residuals had been deposited, the time interval between deposition and slaughter may have been of sufficient length to permit the tissue deposits to be reabsorbed, gradually, into the blood stream and eliminated or metabolized at some central point within the body. The third possibility is that the time interval may have allowed intracellular metabolism of the tissue in which the residual was deposited to alter the drug, making detection of the residual impossible by the Salzman and Brodie method. Failure to detect residuals in the tissues of calf No. III is more difficult to explain. Deposition of detectable residuals may not have occurred within four hours after injection of the drug. The chlorpromazine may not be detected in the blood stream in this event since the dose used would, if distributed uniformly in the blood, result in a concentration less than or approaching the minimum sensitivity of the analysis method.

No degradation of residual chlorpromazine during chilling and aging was shown by the analysis of samples of the ground shoulder muscle of calf No. V.

A limited study of the effect of cooking on chlorpromazine introduced into samples of raw beef produced variable results. Chlorpromazine

was introduced into two pounds of thinly sliced beef. The beef was ground, mixed thoroughly and formed into five patties. Four patties were cooked at temperatures varying from 120° F. to 180° F. and the fifth was a raw control. The patties were wrapped and frozen until they could be analyzed by the method for determining total chlorpromazine and chlorpromazine sulfoxide. The above experiment was repeated later. Results of the two trials are shown in Appendix Table I.

3. Summary

The following statements are a brief summary of the results of Experiment II. Animal variation in response to injected chlorpromazine was observed. This variation may be associated with differences in temperment of the animals. Tranquilized animals are capable of exhibiting manifestations of the "flight and fright" reaction to noxious stimuli, but demonstrate decreased motor activity under normal conditions. In-coordination, especially of the rear extremities, was observed in all cases of chlorpromazine treatment and was serious enough in some cases to be of practical importance.

No undesirable physical alterations in the organs, tissues or carcass of treated animals were observed following tranquilizer treatment. Hemoglobinuria was observed following chlorpromazine injection in all samples tested. Muscle tissue failed to deposit detectable residuals of chlorpromazine or its sulfoxide following treatment with relatively large doses of the drug. Residuals were detected in edible organs and tissues, other than muscle, at intervals following a 0.4 mg./lb. dose of chlorpromazine, injected intravenously. Tissue damage was observed in muscle tissue following intramuscular injections. No degradation of injected chlorpromazine in the above muscle tissue was detected.

## EXPERIMENT III

This experiment was designed to study the influence of chlorpromazine on the concentration of epinephrine and norepinephrine in the plasma of animals subjected to stress conditions. Observations of the behavioral response to stress conditions were made while the animals were in both the tranquilized and the non-tranquilized states.

### 1. Materials and Procedure

Three steers, one Angus, one Hereford and one Shorthorn, ranging in weight from 650 to 800 pounds, were used in this experiment. Stimulation with an electric prod or "hot shot" was used as the stress mechanism. A pen, approximately 15 feet wide and 40 feet long, in the Oklahoma State University Beef Barn housed the steer during the stressing periods. Since only one animal was stressed at each session, the pen permitted the steer enough room to move about freely, preventing, to some extent, bruising which may have occurred in more confined quarters. In one instance, a slightly larger outside pen was used.

The frequency of stimulation used was standardized as that necessary to keep the steer active throughout the eight hour stress period. When labored breathing and ataxia indicated excessive fatigue or overheating, the stimulation was ceased for a time, permitting a short rest period. Water was available in the pen and the steer was not disturbed while drinking. If it could be prevented, the steer was not permitted to lie down during the stress period.

Each animal was subjected to a stress period of eight hours in an attempt to observe the normal pattern of epinephrine and norepinephrine concentrations in the plasma under stress conditions. Following a rest period of approximately two weeks, each animal was again subjected to a stress treatment. Intravenous injection of a chlorpromazine dose of 0.4 mg./lb. preceded the second stress period. The injection was made into the jugular vein after a control blood sample had been collected. The epinephrine and norepinephrine concentrations following treatment were determined and compared with the results of the first stress period in the same animal.

Each steer was observed throughout the stress periods. The reaction to stress was noted in both the normal and tranquilized state. Influences of chlorpromazine on the general behavior were also observed.

Blood samples were collected at intervals during the stress periods. A control blood sample was obtained prior to stress or tranquilizer treatment. A second sample of blood was taken within thirty minutes after stress or treatment was begun and a third within an hour after initiation of the treatment. A two hour sample and subsequent samples, taken at two hour intervals during the remainder of the stress period, made up the complete series of samples obtained in each stress period. Deviations in the schedule did occur. The steer was placed in either a stanchion-type stock or in a chute with a head-gate to facilitate bleeding. Blood was taken from the jugular vein using an 18 gauge needle and a 30 ml. syringe. The syringe contained five ml. of the sodium fluoride-sodium thiosulfate anticoagulant-preservative solution as used by Mangan and Mason (1958b) in their analysis procedure for plasma epinephrine and

norepinephrine. Fifteen ml. of blood were collected, making a total of twenty ml. The blood was placed in tubes and refrigerated immediately. Samples were held under refrigeration less than ninety minutes before being subjected to the first phase of the analysis procedure.

The method used to evaluate the epinephrine and norepinephrine levels in the plasma was reported by Mangan and Mason (1958b). The method was a revision of the original fluorometric method developed by Weil-Malherbe and Bone (1952).

Blood samples, obtained as outlined earlier, were centrifuged and the plasma decanted. The plasma was diluted with an equal volume of 0.2 M sodium acetate buffer adjusted to pH 8.4. The diluted plasma was raised to pH 8.4 by addition of a few drops of 0.5 N sodium carbonate solution. The buffered plasma solution was added to an acid washed alumina column. Following the plasma-acetate mixture, five ml. of the acetate buffer and five ml. of distilled water wash were passed through the column. The filtrates obtained to this point were discarded. The epinephrine and norepinephrine were eluted from the column by passing five ml. of 0.2 N acetic acid through the column, followed by five ml. of water. The combined acetic acid elution and water wash filtrate (10 ml.) was treated with ethylene diamine dihydrochloride and ethylene diamine and incubated at 50° C. for 20 minutes. A condensation of ethylene diamine and a form of the epinephrine occurs during the incubation. The product of this condensation reaction is fluorescent. Following the incubation and cooling, the samples were saturated with NaCl and extracted with 6 ml. of isobutanol on a mechanical shaker.

Standard solutions, reagent blanks and column blanks were carried through the condensation and extraction phases with the samples.

Standard solutions were prepared containing 0.02, 0.06, 0.08, 0.10 and 0.20 micrograms of epinephrine in ten ml. of 0.10 N acetic acid. Sample tubes containing ten ml. of 0.10 N acetic acid were used as reagent blanks. Column blanks were prepared by passing five ml. of 0.20 N acetic acid and five ml. of water through an alumina column. Aliquots of the isobutanol phase were placed in fluorometer tubes following extraction and the fluorescence determined in the Farrand Photoelectric Fluorometer, Model A.

The sensitivity of the instrument was adjusted so that a reading of 100 was obtained on the galvanometer with the 0.20 microgram epinephrine standard. When the fluorescence of the 0.10 microgram standard exceeded the fluorescence of all the samples in a series, the 0.10 standard was used to adjust the sensitivity. The fluorescence of the remaining standard solutions, samples and blanks was determined with this sensitivity setting on the instrument. The above procedure was followed using filters 5433 and 3384 in the secondary and was repeated using filter 2418 in the secondary. The primary filter system for both readings consisted of filters 5113 and 3389. Standard curves were plotted, using the fluorescence of the set of standards run with each set of samples. A curve was plotted from the fluorescence readings of the standard solutions for each secondary filter system using the least squares method. The average fluorescence of the column blanks was subtracted from the fluorescence of each sample and the concentration read from the appropriate standard curve. This concentration may be referred to as apparent epinephrine concentration in the sample as determined by the filter system used.

The purpose of using two sets of filters and obtaining two sets of readings on each sample was to differentiate the epinephrine and

norepinephrine in the sample. The ratio of epinephrine to norepinephrine fluorescence was determined for each set of filters by constructing fluorescence curves of standard solutions of epinephrine and norepinephrine. By dividing the slope of the epinephrine curve by the slope of the norepinephrine curve this ratio was determined. Using filters 5433 and 3384 in the secondary, a peak transmission was obtained at 510 millimicrons, according to Mangan and Mason (1958b). The ratio of epinephrine to norepinephrine using filters 5433 and 3384 was 1.01. Therefore, the following relationship was established: $A + \frac{N}{1.01} = b$ where A = concentration of epinephrine, N = concentration of norepinephrine and b = apparent epinephrine concentration measured with filters 5433 and 3384 in the secondary. The corresponding epinephrine to norepinephrine ratio for filter 2418, which transmits at 600 millimicrons, was 4.64 for the filter and instrument used in this trial. Therefore, $A + \frac{N}{4.64} = c$ where c = apparent epinephrine concentration of the sample measured with filter 2418 in the secondary. The two equations were solved simultaneously.

$$A + \frac{N}{1.01} = b$$

$$B + \frac{N}{4.64} = c$$

$$N = \frac{4.64 \times 1.01}{4.64 - 1.01} (b - c) = K (b - c) = 1.29 (b - c)$$

$$A = b - \frac{N}{1.01} = c - \frac{N}{4.64}$$

From the b and c values, N and A were calculated using the appropriate equation.

A conversion factor to put the A and N values calculated as above on the basis of the original blood sample was developed. The column

filtrate subjected to the ethylene diamine condensation reaction was a ten ml. volume, five ml. of 0.20 N acetic acid plus five ml. of water. This filtrate contained all of the epinephrine and norepinephrine removed from the plasma by the column. The standard solutions subjected to the ethylene diamine condensation were also ten ml. volumes, each containing a given amount of epinephrine. Since the volume of the sample and the standard were the same when extracted and read and the concentration of the standard was expressed in micrograms per ten ml., the A and N values represented the total detected epinephrine and norepinephrine in the decanted plasma sample. The volume of plasma and anticoagulant decanted after the centrifugation of the original blood sample varied from sample to sample. The A and N values were converted and expressed as micrograms of plasma epinephrine or plasma norepinephrine per milliliter of blood. The factor used to convert the A and N values to micrograms of plasma epinephrine or plasma norepinephrine per ml. of blood was determined by the following equation: $F = \frac{P}{S}(B)$ where F = conversion factor, P = volume of plasma-anticoagulant decanted after centrifugation, S = the total volume of blood sample plus anticoagulant and B = the volume of blood collected. To find the concentration in the blood the equations used were: epinephrine concentration = A/F and norepinephrine concentration = N/F.

The experiment dealt with relative concentrations of the hormones in the plasma fraction of the blood. Since it was not essential that the absolute values of the hormone concentrations be determined, no tests of the efficiency of the method in recovering epinephrine and norepinephrine from the blood were made. The determinations were run under the

assumption that recoveries would tend to be constant throughout the experiment. This would permit valid comparisons of relative values. The micrograms of plasma epinephrine and plasma norepinephrine per milliliter of blood reported here may not be an accurate estimate of the actual levels in bovine blood if recoveries in the determination deviated from 100 percent.

2. Results and Discussion

Animal variation in response to the stressing agent was obvious in this trial. The Angus and Shorthorn steers were visibly excited by stimulation with the electric prod, while the Hereford responded to a lesser extent. The Hereford steer would run to avoid the prod, but gave no indication that the stimulation was painful when actually prodded. The Hereford did not appear to be wild or excited when the technician approached with the prod. This steer did seem rather adept at staying just out of reach of the prod during both stress periods. This was accomplished without the violent rushing about or crashing into the walls of the pen observed when the other two animals were approached or stimulated. In all cases, the steers became tired as the period of stress progressed.

The Shorthorn and Angus steers had developed belligerent attitudes by the time the first stress period was completed. In each case, the animal charged the handler while being led to the pen following the collection of the final blood sample. The aggressive response was observed in both of the above animals during the second stress period. The Shorthorn charged the handler eight hours after treatment was initiated in the first phase of the study, but in the second stress

period, after chlorpromazine injection, the steer was consistently charging anyone who entered the pen within one hour after the treatment began. The first charge made by the Angus also occurred after eight hours of stimulation in the first stress period. Nineteen days later, during the second stress period, the Angus tried to charge everyone, whether they were inside or outside the pen, within two hours after the treatment was started. Neither of these animals were aggressive nor had they been known to charge under normal conditions. Whether the more rapid development of the tendency to charge the attendant was due to a training effect, or whether this factor can be associated with tranquilizer could not be determined.

The steers used in Experiment III, when treated with a chlorpromazine dose of 0.4 mg./lb. intravenously, exhibited responses similar to those observed in Experiments I and II. A sleepy appearance and relaxed stance were observed in all cases. In-co-ordination of the rear legs was apparent when the steers walked or moved. A relaxed appearance and some dragging of the rear feet were the only changes observed in the Hereford following the injection of tranquilizer. In the Shorthorn, the tranquilization was more apparent than in either of the other two steers. Within two or three minutes following injection, the Shorthorn became rather sleepy and full effects of the tranquilizer were observed approximately fifteen or twenty minutes following injection. The Shorthorn lay down while being led back to the pen following the collection of blood thirty minutes after the chlorpromazine had been injected. Rear leg in-co-ordination was noted to be serious at this time. This steer appeared to be sleepy and relaxed eight hours after injection, but whether this

could be attributed to the tranquilizer or to fatigue was not determined. Tranquilization was evident in the Angus immediately after injection, but was difficult to assess after two hours due to the aggressive nature of the steer. Little physical evidence of tranquilization was noted after this time.

Fatigue, in these steers, resulting from the extensive activity during the stress period, may have produced behavioral symptoms similar to the symptoms normally observed following tranquilizer injection. For this reason, it was difficult to determine the duration of tranquility.

As mentioned earlier, there may be some relationship between the early development of an aggressive attitude or belligerence and the injection of chlorpromazine.

The results of the fluorometric estimation of plasma epinephrine and norepinephrine are presented in Table IV. Careful examination of this data does not indicate that a relationship between the plasma levels of these catechol amines and the duration of the stress conditions used exists in either the tranquilized state or the normal state. Considerable variation was observed in the concentration of both epinephrine and norepinephrine. The range of plasma epinephrine concentrations varied from 0.0001 microgram per milliliter of blood to 0.0084 microgram per milliliter. The range of plasma norepinephrine concentrations in the three steers was from 0.0034 to 0.0175 microgram per milliliter of blood. The epinephrine concentrations detected in the Shorthorn during the first stress period are obviously higher than the epinephrine concentrations during the stress period following administration

TABLE IV

CONCENTRATIONS OF PLASMA EPINEPHRINE AND NOREPINEPHRINE OF TRANQUILIZED AND NORMAL ANIMALS WHEN SUBJECTED TO STRESS CONDITIONS

| Animal | Treatment | Blood Sample No. | Time Stressed (hours) | Concentration of Plasma Hormone (mcg./ml. of blood)[1] | |
|---|---|---|---|---|---|
| | | | | Epinephrine | Norepinephrine |
| Hereford | Stressed | 1 | 0 | 0.0009 | 0.0083 |
| | | 2 | 0.5 | 0.0008 | 0.0065 |
| | | 3 | 1 | 0.0004 | 0.0053 |
| | | 4 | 2 | Sample lost in assay | |
| | | 5 | 4 | 0.0006 | 0.0081 |
| | | 6 | 6 | 0.0008 | 0.0079 |
| | | 7 | 8 | 0.0009 | 0.0065 |
| Hereford | Chlorpromazine[2] and Stressed | 1 | 0 | 0.0019 | 0.0134 |
| | | 2 | 0.5 | 0.0002 | 0.0039 |
| | | 3 | 1 | 0.0023 | 0.0175 |
| | | 4 | 2 | 0.0012 | 0.0149 |
| | | 5 | 4 | ---[3] | 0.0145[4] |
| | | 6 | 6 | ---[3] | 0.0149[4] |
| Shorthorn | Stressed | 1 | 0 | 0.0033 | 0.0072 |
| | | 2 | 0.5 | 0.0084 | 0.0024 |
| | | 3 | 1 | Sample lost in assay | |
| | | 4 | 2 | 0.0034 | 0.0117 |
| | | 5 | 4 | 0.0038 | 0.0095 |
| | | 6 | 6 | 0.0050 | 0.0135 |
| | | 7 | 8 | 0.0065 | 0.0118 |
| Shorthorn | Chlorpromazine[2] and Stressed | 1 | 0 | 0.0013 | 0.0126 |
| | | 2 | 0.5 | 0.0034 | 0.0136 |
| | | 3 | 1 | 0.0030 | 0.0126 |
| | | 4 | 2 | 0.0018 | 0.0137 |
| | | 5 | 4 | 0.0006 | 0.0094 |
| | | 6 | 6 | 0.0013 | 0.0100 |
| | | 7 | 7 | 0.0015 | 0.0097 |
| Angus | Stressed | 1 | 0 | 0.0013 | 0.0034 |
| | | 2 | 0.25 | 0.0001 | 0.0061 |
| | | 3 | 1.25 | 0.0003 | 0.0082 |
| | | 4 | 3.75 | 0.0005 | 0.0088 |
| | | 5 | 4.75 | 0.0016 | 0.0149 |
| Angus | Chlorpromazine[2] and Stressed | 1 | 0 | 0.0022 | 0.0159 |
| | | 2 | 0.5 | 0.0024 | 0.0078 |
| | | 3 | 1 | 0.0008 | 0.0094 |
| | | 4 | 2 | 0.0013 | 0.0100 |
| | | 5 | 4.5 | 0.0030 | 0.0144 |

1/ Based on volume of blood originally collected and centrifuged.
2/ Chlorpromazine dose, 0.4 mg./lb., administered intravenously.
3/ Sample fluorescence greater than fluorescence of standard used to adjust maximum sensitivity of the instrument.
4/ Estimate of concentration (see 3/).

of tranquilizer to the Shorthorn. The maximum epinephrine concentrations achieved by the Shorthorn during both stress periods were greater than maximums observed in either the Hereford or the Angus.

The failure to detect changes in epinephrine and norepinephrine concentrations in the plasma at different intervals during the stress period in this study is probably due to the rapid removal or neutralization of the hormones by the peripheral vascular system. Mangan and Mason (1958a), in recent work, and others have shown that injection of epinephrine or norepinephrine results in large increases in plasma concentrations of the hormones when measured approximately thirty seconds after injection. This concentration increase deteriorates rapidly however, and within five minutes after injection, most of the injected hormones have disappeared and the plasma concentrations have returned to levels approaching the normal level. Mangan and Mason observed this rapid removal when dogs were repeatedly injected with epinephrine at ten minute intervals. When splanchnic nerves were stimulated in the dog, the epinephrine and norepinephrine levels were increased markedly, and the rate of disappearance of the endogenous hormone was comparable to that seen when exogenous hormones were introduced into the system.

The time required to catch the steer, lead him to the chute and prepare the syringe for collection of the blood usually exceeded five minutes. This interval would permit the degradation of the hormones as described above if the steer had been stimulated immediately prior to catching him. Therefore, one would anticipate that the plasma levels of the hormones may not necessarily follow a given pattern under the conditions used here. The wide variation observed in epinephrine and norepinephrine

levels may have been the result of sampling error which could include such things as excitement of the animal while restraining him prior to bleeding or while attempting to obtain the blood sample.

To draw conclusions relative to the influence of chlorpromazine on epinephrine and norepinephrine concentrations in the blood under normal or stress conditions from the results of this experiment would be hazardous. If chlorpromazine treatment (0.4 mg./lb., intravenously) does alter the blood levels of the two hormones, the limited data presented here indicates that the change in hormone concentration would probably be small. The hormone concentrations in a given animal were generally within the same range whether the animal had received tranquilizer or not.

To effectively determine the influence of chlorpromazine on plasma concentrations of epinephrine and norepinephrine would require a larger number of comparisons which could be treated statistically. Study of the influence of chlorpromazine on the hormone concentrations under stress conditions could be accomplished only by restraining the animal and inserting a cannula to collect blood samples at short intervals following the use of a stressing agent such as electrical stimulation. In the case of large animals, such as the bovine, the restraint itself may cause sufficient stress to permit evaluation of the influence of chlorpromazine or other tranquilizers on the concentrations of the chemical regulators in the blood.

3. Summary

Experiment III indicated that individual animal variation in response to stress conditions occurred in the bovine. Chlorpromazine injection resulted in varying degrees of tranquilization and some in-co-ordination similar to observations in Experiments I and II. The results

of analysis of blood plasma for epinephrine and norepinephrine did not indicate that changes in the concentration of these humoral agents are related to stress or to the duration of the stress used in this study. If the release of the hormones did occur, the peripheral vascular system was probably capable of removing the epinephrine and norepinephrine from the circulation before blood samples were collected in this experiment. The results indicate that the injection of chlorpromazine had no general influence upon the amount of epinephrine and norepinephrine in the plasma.

# DISCUSSION

The results of the three experiments in this study and the information in the research literature provide some basis for the evaluation of the tranquilizer, chlorpromazine, as a tool in livestock management. The information available indicates that this tranquilizer may have utility in animal industry and at the same time points out some serious limitations of the drug in a practical livestock operation.

The reports of successful clinical utilization of chlorpromazine widely distributed in the veterinary literature are evidence of the value of this tranquilizer in large animal practice. Successful use of chlorpromazine to reduce the severity of after-effects of anesthesia by Troughton et al. (1955), to relax and sedate horses suffering from tetanus by Tait and Ryan (1957), to chemically restrain animals while treatment was effected by Cartmell (1956), Matera and Stopliglia (1955) and Lundvall and Campbell (1957) and to aid in keeping the animal quiet after treatment by Troughton et al. (1955) are only a few of the instances in veterinary practice where chlorpromazine has proved its usefulness.

Problems of livestock management which have been solved or reduced by the utilization of chlorpromazine include the enhancement of semen collection from nervous bulls by Herrick (1958), the reduction of emotional disturbances in females during or following parturition by Martin and Beck (1956), Kristjansson (1957) and Hibbs (1958) and the prevention of secondary effects of changes in management and environment by Hoerlein and Marsh (1957). The majority of the reports of chlorpromazine administration listed above involved the intramuscular injection of the drug.

The primary aim of this study was to investigate the effectiveness of chlorpromazine as a method of reducing the secondary effects of handling prior to the slaughter of meat animals. The effects of chlorpromazine in the bovine were studied and the drug's potential to reduce shrinkage, bruising, dark cutting and disease susceptibility resulting from handling and shipping of slaughter animals was considered. Chlorpromazine was observed to decrease motor activity in animals not receiving external stimuli in Experiment II. However, these calves were alert to activity in the pen and were not easily handled. The response to stress conditions observed in Experiment III was not obviously altered by chlorpromazine injection. The attempt to utilize the fluorometric estimation of plasma epinephrine and norepinephrine as an objective method of measuring the degree of stress experienced by an animal was unsuccessful in this study. Comparison of the results of this fluorometric estimation of epinephrine and norepinephrine levels during the stress periods following administration of tranquilizer and stress periods involving no tranquilizer treatment indicated that no relationship existed between the injection of tranquilizer and the levels of the hormones in the blood. The above observations lead to the conclusion that chlorpromazine may aid in reducing the emotional and metabolic stress experienced by an animal in a holding pen after arriving at the slaughter plant or auction barn, but probably does not reduce the stressing effect of shipment and handling. Chlorpromazine may therefore, slightly reduce the shrinkage occurring between the feed lot or pasture and the slaughter floor, reduce the incidence of dark cutting and reduce the susceptibility to diseases such as shipping fever. Little reduction in bruising would be expected

following chlorpromazine treatment.

The report by Martin and Beck (1956) of decreases in red blood cell count and hemoglobin concentration following repeated daily intramuscular injection of chlorpromazine in horses indicated blood damage by the drug. A positive test for hemoglobinuria was obtained in all instances permitting the collection and testing of the urine of chlorpromazine treated animals used in the experiments reported here. No quantitative estimate of the blood damage was attempted in this study. If this loss of red blood cell material is extensive, the metabolic effects of a reduction in circulatory efficiency may offset any neuro-humoral actions of chlorpromazine, useful in combating the secondary effects of stress.

In the management situations requiring preslaughter chlorpromazine treatment of an animal, intramuscular injections are not advisable due to the presence of injection site residuals of the drug at the time of slaughter. Intravenous injection would eliminate this problem. Animal variation in response to chlorpromazine injection was observed by Ritchie (1957) and Martin and Beck (1956). This variation in response was an outstanding feature in the study reported here with variation being observed in all three experiments. Intravenous injection would have a tendency to magnify this variation. Release of a large amount of tranquilizer directly into the blood stream would probably depress the more susceptible animal to a greater extent then would the slow accumulation of the drug in the blood stream due to absorption from an intramuscular injection site. Selection of the dose to be injected intravenously under practical conditions becomes difficult due to the individual variation in response.

The degree of tranquilization required for preslaughter treatment of meat animals presents another problem in the selection of an optimal dose. The observation of animals used in this study stressed the importance of avoiding large intravenous doses of chlorpromazine in situations requiring the handling or shipping of cattle. Ataxia, observed in this study and others, Hoerlein and Marsh (1957), would tend to increase the amount of bruising and trampling when cattle were shipped. At the same time, smaller doses of chlorpromazine may produce some tranquilization, but this tranquility is of limited duration as observed in Experiment I. The duration of tranquility following any single intravenous injection of chlorpromazine failed to exceed eight hours in this study. No general recommendation can be made relative to intravenous dosage of chlorpromazine when it is to be injected prior to handling and shipping slaughter animals.

Preslaughter use of a drug in meat animals requires approval of the Food and Drug Administration. (U. S. Department of Health, Education and Welfare, 1959). "Such a substance may be approved for animal use as a drug, provided that any residue of the drug or any conversion product falling within the meaning of the act does not become a component of human food derived from the treated animal." The above is a quote from the ruling by the Food and Drug Administration cited earlier. Detection of the total administered dose of the drug in the excretion products of the animal prior to slaughter would indicate that no residuals of the drug were present in the tissues at the time of slaughter. Salzman and Brodie (1956) and Christensen and Wase (1956) reported that the larger portion of the chlorpromazine eliminated was found in the

urine. The above authors and Berti and Cima (1957) studying the elimination of chlorpromazine and its metabolites in the urine, found only a fraction of the injected dose was eliminated from the animal body by this route. Salzman and Brodie (1956) accounted for approximately 12 to 17 percent of the drug in the urine during a seventy-two hour period following chlorpromazine administration to the dog. Berti and Cima (1957) detected 20 percent of the dose in the urine of several species over a period of several days following administration. The elimination of chlorpromazine and chlorpromazine sulfoxide in the urine accounted for approximately 12 percent of the dose injected intravenously to two animals in Experiment I. The data above stimulated the search for residual deposits of the drug in the tissues of the bovine. No residuals were detected in the muscle tissue by the method used for analysis of the tissues. Residuals of chlorpromazine found in some edible internal organs and edible offal tissues would probably prevent the approval of chlorpromazine as a preslaughter drug treatment in beef, according to the letter of the law cited by the U. S. Department of Health, Education and Welfare (1959).

If the interpretation of the law as cited by Chicci (1959) is valid, proof that the residual of the drug in any edible tissue is safe for human consumption would result in the approval of chlorpromazine as a preslaughter treatment. The problem lies in proving such a residual "safe".

Briefly considering chlorpromazine and its practical adaptations in the livestock industry, it may be stated that the drug is a useful tool in livestock management. It has been used to chemically restrain, to

facilitate veterinary treatment, and to reduce undesirable effects of nervous reactions to parturition or other stressing situations. The ability of chlorpromazine to reduce the motor activity of cattle held under strange environmental conditions may make it useful in preventing shrinkage loss and dark cutting carcasses following the handling and shipping of livestock prior to slaughter. Individual animal variation in degree of response to chlorpromazine, the development of in-co-ordination in some animals following chlorpromazine treatment and the limited duration of tranquility observed when small doses of chlorpromazine were used make it difficult to recommend a dose for use in slaughter cattle prior to handling and shipping. Some damage to red blood cells was observed, which may have a tendency to counteract the beneficial effect of the drug during handling and shipping. The failure of chlorpromazine to control excitability of the stimulated animal reduces the value of the tranquilizer in the prevention of bruises during movement and handling. Although residual chlorpromazine was not detected in the muscle tissue, widespread distribution of residual deposits in other tissues of the body would tend to cause the Food and Drug Administration to be hesitant about approving the drug for preslaughter use. More research would be required in this area before such approval could be obtained.

## SUMMARY

Thirteen beef animals were used in a series of three experiments designed to study the effects of chlorpromazine treatment on the live bovine and on the tissues and carcass of the slaughtered animal. Chlorpromazine was observed to decrease motor activity to some extent, depending upon the dose and the route of injection. In most cases, the "flight or fight" reaction to noxious stimuli was retained by the animal following chlorpromazine injection. Obvious differences in reaction to stress conditions were observed in only one animal and this occurred after a three day period of tranquilization. Chlorpromazine treatment resulted in ataxia or in-co-ordination in several of the animals treated. Hemoglobinuria was observed following chlorpromazine treatment.

The results of Experiment I indicated that approximately 12 percent of the chlorpromazine injected was eliminated in the form of chlorpromazine or chlorpromazine sulfoxide in ten to twenty-four hours following the injection of the drug.

No effects of chlorpromazine treatment were detected by inspection of carcasses or offal of slaughter cattle in Experiment II. Residual chlorpromazine deposits were found to be distributed widely in the tissues of animals receiving chlorpromazine injections from six and one-half to eight hours prior to slaughter. No residuals were detected in the tissues of an animal injected seventy-two hours before slaughter and only one of the tissues sampled was found to contain residual deposits of chlorpromazine when injection was made four hours prior to slaughter.

Data were obtained that indicated chlorpromazine residuals in muscle tissue were not altered during the period of chilling or aging a carcass at low temperatures.

Determination of the levels of plasma epinephrine and norepinephrine in the blood of stressed animals with and without the use of chlorpromazine pretreatment failed to show any influence of chlorpromazine on the concentration of these hormones. The technique used in this study did not permit adequate control of the time interval between stress and collection of blood samples. For this reason, the data may not be an accurate estimate of the effect of chlorpromazine on the plasma epinephrine and norepinephrine concentration.

Chlorpromazine has been used by veterinarians successfully in situations requiring chemical restraint and to relieve anxieties in the animal associated with treatment of a number of conditions. The ability of chlorpromazine to reduce motor activity in the bovine would be valuable in reducing some of the secondary effects of shipping and handling slaughter animals. Difficulty in selection of an optimal dose, due to animal variation in response and the frequency of undesirable side effects such as ataxia limit the value of the drug in the prevention of losses encountered in slaughter cattle.

Wide distribution of residual forms of chlorpromazine in the animal body following injection may prevent the approval of the tranquilizer as a preslaughter treatment by the Food and Drug Administration.

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# APPENDIX

## TABLE I

CHLORPROMAZINE RESIDUES IN BEEF COOKED AFTER TREATMENT[1/]

| Sample No. | Baked to Internal Temperature °F | Form of Residue | Concentration mg./100 g. |
|---|---|---|---|
| A-1 | 120 | Chlorpromazine Sulfoxide | 4.5 |
| A-2 | 140 | Chlorpromazine Sulfoxide | 8.8 |
| A-3 | 160 | Chlorpromazine Sulfoxide | 8.2 |
| A-4 | 180 | Chlorpromazine Sulfoxide | 11.5 |
| A-5 | 0 | Chlorpromazine | 17.0 |
| B-1 | 120 | Chlorpromazine | 10.1 |
| B-2 | 140 | Chlorpromazine | 13.4 |
| B-3 | 160 | Chlorpromazine | 8.8 |
| B-4 | 180 | Chlorpromazine | 7.3 |
| B-5 | 0 | Chlorpromazine | 11.8 |

1/ Approximately 25 mg./100 g. Chlorpromazine mixed into the meat.

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