MYOPATHY PRODUCED BY THE PHENOTHIAZINE-DERIVED TRANQUILIZERS

by

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INTRODUCTION

In the fall of 1957, a group of steers from the Iowa State University herd in Ankeny were sent by truck to the University Meat Laboratory in Ames for slaughter. As a matter of general interest, 10 ml. of a tranquilizer (chlorpromazine hydrochloride) was administered intramuscularly into the gluteal region of each animal shortly before loading. Slaughter was performed approximately 20 hours later, and a severe muscle reaction to the drug which resulted in the loss of nearly two pounds of flesh per animal was encountered.

Data obtained from the 1959 Annual Report of the Division of Animal Industry, Board of Agriculture and Forestry, Hawaii (5), indicated that the use of ethyl isobutrazine-10 phenothiazine on steers being shipped to slaughter had resulted in the loss of as much as nine pounds of flesh in a single animal. A dosage of 600 mg. had been injected into the round of each animal, and the district veterinarian's investigation had eliminated any infection due to faulty or unsanitary equipment or methods as the cause of the muscle reaction found at slaughter.

Furthermore, information received from R. K. Somers, Chief Staff Officer for Procedures and Training, Meat Inspection Division, Agricultural Research Service, United States Department of Agriculture, indicated that meat inspectors have found extensive tissue damage as the result of the use of tranquilizers intramuscularly. No data were received as regards the incidence of the lesions or the monetary losses sustained by their use.

Because of these findings and the increasing use of tranquilizers

in the transportation and in the adaptation of cattle to new environments, this project was initiated to determine more precisely the myopathy produced by them. Economic reasons prohibited the use of cattle; hence the dog was selected as the experimental animal. It was believed that the canine muscle reaction to the tranquilizer drugs would be the same as that produced in the bovine species.

REVIEW OF THE LITERATURE

Although the literature is replete with articles concerning the tranquilizers and their many toxic side effects in humans, none were found which described the myopathy produced by them. Paget and Scott (8) state that "it is surprising, in view of the frequency with which drugs are administered by intramuscular injection, that few studies have been published on the amount and type of injury produced by substances likely to be given by intramuscular injection therapeutically."

Likewise, the veterinary literature is lacking in information on this subject. Jones' statement (6, p. 196) that a five percent solution of chlorpromazine hydrochloride is somewhat irritant when injected intramuscularly in the horse was, in fact, the only item uncovered which even hinted that the tranquilizers produced a toxic reaction in muscle.

The verbal and written reports obtained during this study merely indicated that a severe reaction was found with the loss of several pounds of meat at slaughter. Cattle were the only animals mentioned in this respect.

METHOD OF PROCEDURE

Since the phenothiazine-derived tranquilizers comprise the group most commonly used in veterinary practice, it was decided that the project would be limited to the use of these drugs and the study of the myopathy produced by them. Four such tranquilizers were selected: (1) chlorpromazine hydrochloride, (2) ethyl isobutrazine (ethyl-3-dimethyl amino-3' methyl 2' propyl)-10 phenothiazine, (3) perphenazine, and (4) gamma dimethylamino-n-propyl phenothiazine.[1]

The experiment was divided in two parts, the first involving the use of crystalline drugs in varying concentrations (15 mg., 25 mg., 35 mg., 50 mg., and 65 mg.) in a constant volume of diluent (sterile physiological saline) as well as fixed concentrations in varying volumes of diluent (0.5 ml., 1.0 ml., and 2.0 ml.) as shown below.

Diluent in milliliters	Drug concentration in milligrams				
0.5	15	25	35	50	65
1.0	15	25	35	50	65
2.0	15	25	35	50	65

Except for perphenazine each of the drugs was soluble in physiological saline at the selected concentrations and volumes. Perphenazine

[1]The tranquilizers were obtained through the courtesy of Pitman-Moore Co., Jensen-Salsbery Laboratories, Inc., Schering Corporation and Fort Dodge Laboratories, Inc.

was insoluble in water (7, p. 703) yet was used in this manner nonetheless for the sake of uniformity. With this drug the tubes containing the undissolved tranquilizer were agitated briskly before each withdrawal, and a 20-gauge hypodermic needle was used for the intramuscular injections. It was probable that full doses of this drug were not administered because of the settling which occurred in the hypodermic syringe before injection was completed. At 24 hours, undissolved crystals were noted in the muscle grossly, but they were not found at 96 hours and eight days nor after fixation and sectioning at any stage.

The dogs used for this experiment ranged from six months to eight years of age and included pedigreed as well as mongrel animals. Nineteen were males, eleven females; and their weights varied from approximately 20 pounds to 60 pounds. The larger breeds with well-fleshed and long backs were selected in order to distribute the injections as widely as possible. For the most part they appeared to be healthy, but thorough physical examinations were not made to determine their exact status.

Twenty-four dogs were used in the initial phase of this study. Six were assigned for the testing of each drug, and of these six, two were used for each of the three time intervals selected: 24 hours, 96 hours and eight days. Each pair of dogs received all combinations of volumes and concentrations described above or a total of 15 tranquilizer inoculations. In addition, they received five control injections, two using the hypodermic needle alone, and the other three with 0.5 ml., 1.0 ml., and 2.0 ml. of sterile physiological saline.

In the second phase of the experiment, the four tranquilizers were used as commercially available. Six animals were subjects, with two for

each of the time intervals. Here the concentrations selected were the same as those made previously, but there was no variation in the amount of diluent. Hence, each drug required only five injection sites (0.6 ml., 1.0 ml., 1.4 ml., 2.0 ml., and 2.6 ml. of a 25 mg./ ml. preparation); and each dog served as a test for two drugs, one being given along one side of the back and the other on the opposite side.

Chlorpromazine hydrochloride and perphenazine were obtained and used in a concentration of 25 mg./ml. Ethyl isobutrazine-10 phenothiazine and gamma dimethylamino-n-propyl phenothiazine, however, came in bottles containing 50 mg. of drug per milliliter, and they were diluted to 25 mg./ml. with ion-exchange distilled water having a neutral pH. As determined with a Beckman Zeromatic pH Meter, gamma dimethylamino-n-propyl phenothiazine had a pH of 4.45; and when an equal quantity of distilled water was added as explained above, it was raised to 4.62. Sufficient 0.1 N hydrochloric acid was added to return it to 4.45. Ethyl isobutrazine-10 phenothiazine registered a pH of 2.75, and when diluted with distilled water, 3.20. This was likewise readjusted to the original reading by the addition of 0.1 N hydrochloric acid. Chlorpromazine hydrochloride and perphenazine registered pHs of 4.65 and 5.00 respectively.

The dilution of the drugs to 25 mg./ml. was made in order to coincide with the concentrations of chlorpromazine hydrochloride and perphenazine. In addition, it was believed that the larger volume required for the injection of a given drug concentration would result in greater accuracy.

Except as noted previously, injections were made into the longis-

simus dorsi (thoracis and lumborum) muscles approximately two inches apart using sterile 5/8-inch, 26-gauge hypodermic needles and two or five cubic centimeter sterile syringes. Five sites were selected on each side of the vertebral column or a total of ten per dog. A generous area extending from the neck to the tail was first clipped with No. 10 and No. 40 Oster small animal clipper blades, then shaved, scrubbed with detergent and disinfected with 70 percent ethyl alcohol and tincture of merthiolate. The sites were marked with Carter's Marks-A-Lot ink, black for dogs with white skin and red or yellow for those with black or brown skin. Where it was necessary, further inking of these areas was made on subsequent days to preserve the original site. The identifying mark consisted of a small circle, about 1.25 cm. in diameter, into the center of which the hypodermic needle was inserted (Figure 1).

As a means of pin-pointing the site of intramuscular injections, Paget and Scott (8) used a stock suspension of carbon black, 1:600, in the solutions they tested. While this served as an effective means of locating the injection sites, their results too often indicated that polymorphonucleated white blood cells and macrophages could be seen particularly in the region of the carbon granules as well as in areas of edema. Because it would have been difficult to determine how much of the reaction was the result of the drug and how much the result of the presence of carbon particles (or other identifying substance), their procedure and other similar ones considered were not followed.

No anesthetic agent was used on the first four dogs; as a result they struggled during the administration of the drugs. It was apparent that the tranquilizers caused a stinging sensation and pain, for the

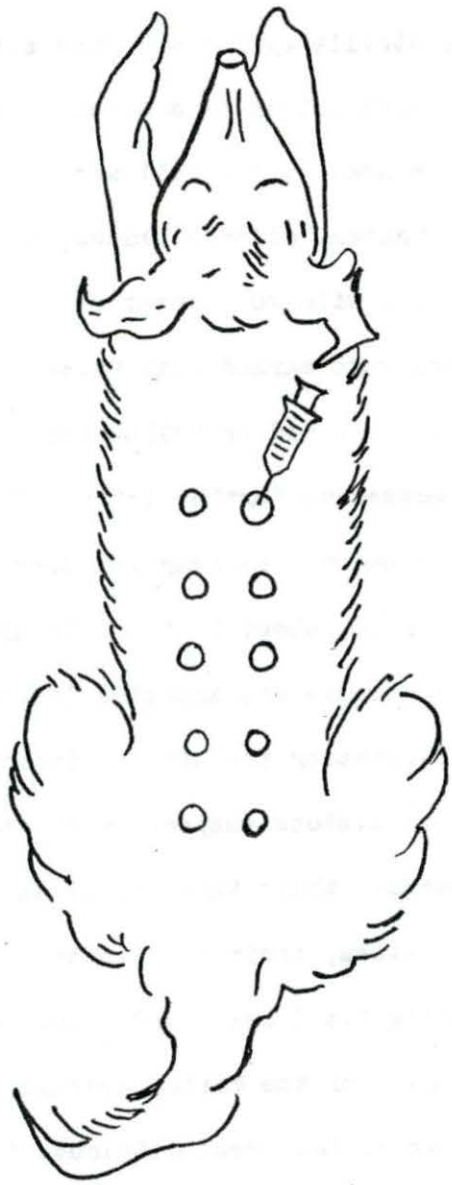


Figure 1. Sketch of dog simulating the position assumed at the time of injection and illustrating the identification of the administrative sites.

animals minded little or not at all when control injections of physiological saline and the hypodermic needle alone were made. In several instances, because the dogs moved about so much when the tranquilizer was being injected, the hypodermic needle had to be reinserted before administration could be completed.

A short acting anesthetic, thiopental sodium, was administered intravenously to the next subject, and since the results proved to be advantageous for both dog and administrator, the use of it was continued throughout the remainder of the project. In all cases, the depth of anesthesia was carried only to a level where the subject was relaxed and unable to respond during the injection period.

On the day following administration, the patients were quiet, the majority remaining in a recumbent position but appearing alert otherwise. The appetite was fair to poor but returned to normal by the third or fourth day. Likewise, there was a noticeable improvement in their disposition on the third day post-injection; and they were essentially normal by the fourth, being able to romp about in their cages at will then.

Although an average of 280 mg. of tranquilizer was administered to 20 dogs in the first part of the experiment and 390 mg. to each of the six dogs in the second, no anesthetic deaths were encountered. While used post-anesthetically here, this nevertheless suggested that there is a wide margin of safety when the phenothiazine-derived tranquilizers are used in conjunction with pentothal sodium.

Palpation and examination of the injection sites revealed tenderness and occasional swellings up to four days later. As regards the swellings,

these were for the most part observed at sites in which the drug was partially or wholly introduced subcutaneously as occurred especially in obese animals. Slight hemorrhage was encountered in nine sites at the time of administration and was probably the result of the accidental rupturing of a cutaneous blood vessel.

All subjects were sacrificed with a combination of pentobarbital sodium and electrocution. Immediately after death, harvesting of the muscle tissues was accomplished by careful dissection in the following manner. The animal was placed in ventral recumbency to simulate the position assumed at the time of drug administration. One-inch straight head-pins were then inserted into each of the ten inked circles at approximately the same angle and depth used during injection. A dorsal midline incision was made with a Bard Parker blade No. 22 from the base of the scapula to the sacrum. The subcutaneous fat and loose connective tissue were dissected free and an incision then made into the heavy fascial sheath (lumbodorsal fascia) surrounding the longissimus dorsi; this incision was extended cranially and caudally. These manipulations resulted in reflection of the skin and loosening of the pins; consequently, at this point, they were withdrawn and reinserted, but this time directly into the muscle at approximately the same sites.

Gentle digital palpation was usually sufficient to locate the larger, swollen lesions. Removal of strips of muscle, 2.5 to 3.7 cm. in length and each containing an injection site, was then carried out by dissecting with scalpel and scissors down along the spinous and transverse processes of the vertebrae and over the rib cage. At times the technique was modified by removing the entire length of longissimus

intact from the cervical region caudally, then cutting into five sections according to the pin sites. During the latter operation, an occasional transverse incision was made directly into a lesion; when this occurred in the 24-hour and 96-hour specimens, a suppurative exudate flowed forth. Similar procedures were carried out on the opposite side; and as soon as individual injection sites were removed, they were identified and placed into an alcohol-formalin solution (10 parts formaldehyde solution, U.S.P., 37 percent, to 90 parts 95 percent ethyl alcohol) following which they were refrigerated to minimize decomposition. This solution in the ratio indicated was used to prevent shattering of the muscle during the sectioning procedure as is often encountered when 10 percent formalin or mercury fixatives such as Zenker's solution are used.

At the time of dissection, no attempt was made to cut the lesions into smaller pieces because they were much too suppurative, except for those obtained from the eight-day animals in which the inflammatory reaction was subdued. As a consequence, relatively large sections of muscle (approximately 2.5 cm. by 3.0 cm.) were taken so as to be sure to include any lesion present at a particular seat. Though this appeared to be haphazard, in actuality it worked out accurately, because when a lesion was present, it was most generally readily palpable. Only with the controls was there some doubt as to the exact location because there was little or no gross damage and inflammation.

Fixation in alcohol-formalin was carried out for a minimum of 72 to 96 hours; each identified muscle strip was then further incised to locate the central areas of the lesion and appropriate blocks taken for processing. In many instances, especially in the 24-hour and 96-hour intervals,

blocks had to be taken at the periphery of the roughly circumscribed lesions because the necrotic muscle would not hold together. Oftentimes the necrotic center would simply fall away, leaving an empty area in the block.

The blocks so obtained were placed in 70 percent ethyl alcohol and held for further processing. This consisted of dehydrating in 95 percent and absolute ethyl alcohol, clearing in chloroform and embedding in Altman's paraffin-stearin-beeswax mixture. The embedded tissues were cut at a thickness of six microns and stained using Gomori's one-step-trichrome stain (2). This stain provided better differentiation of the healthy and necrotic muscle fibers and collagen than the ordinary hematoxylin and eosin method. Healthy muscle fibers were stained red, necrotic muscle fibers blue to purple, collagen green and nuclei blue to black. The following procedure was used:

1.	Xylol, two changes	5 minutes each
2.	Absolute ethyl alcohol, two changes	5 minutes each
3.	95 percent ethyl alcohol	5 minutes
4.	Rinse in tap water	
5.	Place in Bouin's solution in oven at 56°C	1 hour
6.	Wash well in running water	5 minutes
7.	Stain nuclei with Weigert's iron hematoxylin	8 minutes
8.	Wash in running water	5 minutes
9.	Trichrome stain	18 to 20 minutes
10.	Acetic water, 0.5 percent	2 minutes
11.	Rinse in distilled water	
12.	95 percent ethyl alcohol	5 minutes
13.	Absolute ethyl alcohol, three changes	5 minutes each
14.	Xylol, three changes	5 minutes each
15.	Mount in Permount	

Thorough rinsing of the sections after being placed in Bouin's solution and Weigert's iron hematoxylin were found to be very important

steps. The staining with Gomori's trichrome gave best results when the time was not less than 18 minutes nor more than 20; under this time, the healthy muscle fibers could not be easily differentiated from the necrotic on the basis of staining alone because the former tended to retain a bluish tinge not unlike that of the necrotic muscle.

The following information concerning each of the lesions was obtained and recorded for each dog:

1. The presence and severity of hyperemia.
2. The presence and amount of hemorrhage.
3. The presence of hemosiderin.
4. The presence and amount of fibrin.
5. The presence and number of thrombi. Arbitrary figures were taken to indicate the latter: few, one to five; moderate, six to ten; numerous, 11 or more. Numerous hyaline thrombi were noted in the capillaries throughout the experiment in both control and drug sites. Hemolysis of the erythrocytes was observed because alcohol was used for fixation.
6. The presence of cloudy swelling.
7. The type and severity of the inflammatory reaction.
8. The amount and character of the exudate with especial reference to neutrophils, macrophages, lymphocytes and eosinophils.
9. The presence, type and extent of necrosis.
10. The presence of fibroblastic proliferation, early to indicate few to moderate numbers of fibroblasts with small amounts of immature collagen and late to imply many fibroblasts with considerable collagen.

11. The presence of endothelial cell hyperplasia.

12. The presence of phagocytosis.

13. The presence of muscle atrophy.

In addition, the effect of variations in the volume of diluent and of drug concentration were observed, and comparisons of the four tranquilizers were made at similar stages. The gross aspects of the myopathy were also considered, and finally, differences drawn between the two experimental parts.

RESULTS

Gross Observations

Twenty-four hours after the administration of the test drugs the subjects were still tranquilized, but palpation of the injection sites nevertheless revealed evidence of discomfort and pain. After euthanasia, the skin was reflected over the sites of injection. There was observed in several cases, a diffuse hemorrhagic and sometimes sero-hemorrhagic inflammation of the subcuticular layers. These were obviously the instances in which the drugs were partially or wholly administered subcutaneously. The apparent ease of infiltration in this layer was especially noteworthy. Similar observations were made at 96 hours and at eight days, but the exudate became progressively scantier.

In the great majority of cases where the injections were made exclusively into the muscle, the subcutis was free of inflammatory changes. The heavy fascial sheaths acted as an effective barrier to the tranquilizers as was borne out by subsequent microscopic studies.

After the skin, subcuticular layers and fascia were dissected free, small, tense swellings (approximately 1.2 cm. to 2.5 cm. in diameter) could often be palpated and/or observed along the back corresponding to the injection sites. Upon incision of these swollen sites there poured forth a suppurative exudate, usually of fluid consistency which was often tinged with blood. With perphenazine, crystalline deposits were found in the necrotic muscle as mentioned earlier. At 96 hours this acute focal suppurative myositis was still present, but the exudate was generally more copious and of thicker consistency and sometimes tenacious. On

the eighth day there was little or no suppuration; the inflammatory reaction had progressed to a subacute serous nature with connective tissue elements prominent. Where incision was previously difficult and resulted in the loss of central necrotic foci, it was now a simple matter, the lesion being relatively firm and of a drier consistency.

Since the injection sites were relatively close to each other, it was not surprising to find infiltration from one area into an adjacent one. Careful inspection revealed that infiltration was most prone to occur through the connective tissue, this being evidenced by the rapid spread into adjacent areas when the injection was made largely just beneath the epimysium. On the other hand, where the administration was deeply intramuscular, spreading was not extensive and the lesion confined to a roughly spherical area.

Microscopic Observations

Part I: the tranquilizers in physiological saline

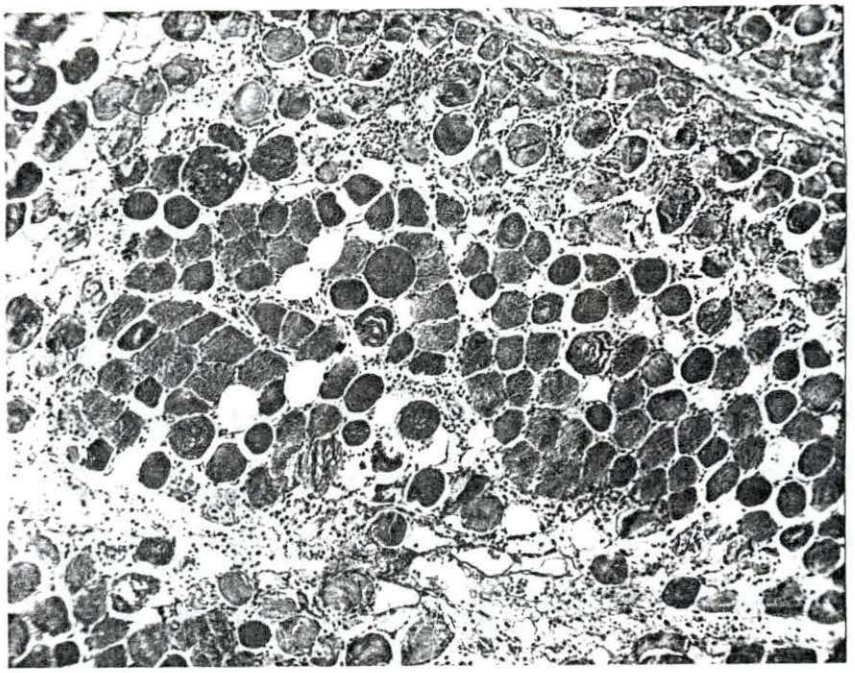
At twenty-four hours

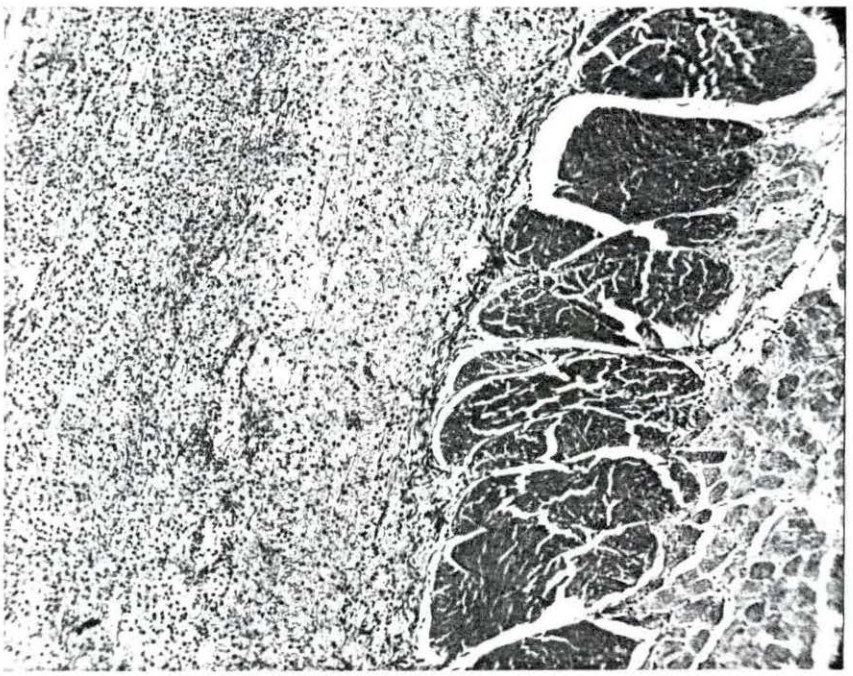
Chlorpromazine hydrochloride, Dogs No. 5, 6 These sections were characterized by an acute focal suppurative inflammation that varied from slight to severe. Neutrophils were the predominating cell type in the exudate, yet they were not extremely numerous (Figure 2). A few lymphocytes, macrophages and eosinophils were also present. Hemorrhage was slight to moderate and hyperemia moderate to severe. Moderate amounts of fibrin were observed, and there were a few blood vascular thrombi present.

Cloudy swelling was mild to moderate. Considerable muscle necrosis

Figure 2. Acute focal suppurative myositis with relatively few neutrophils. Note the marked difference in staining characteristics between the coagulated muscle (blue) and the muscle fibers that have only undergone cloudy swelling (red). X 96.

Figure 3. Heavy fascial sheath, indicated by area stained deep blue, preventing penetration of the drug into the underlying muscle which lies to the right. The inflammatory reaction is limited to the subcutaneous tissue. X 96.





was present with the coagulative type predominant over the liquefactive.

In five sites the tranquilizer was apparently injected subcutaneously, for lesions were confined to these areas, the heavy fascial sheath preventing penetration into the underlying muscle (Figure 3). The subcutaneous fat was infiltrated with neutrophils almost to the point of obliteration, yet adjacent adipose tissue revealed no degenerative changes.

The extent of damage for the most part corresponded with the variations in concentration of the drug administered, but the variations in the volume of diluent did not appear to affect the results appreciably. Controls using physiological saline as well as the hypodermic needle alone showed no inflammatory changes, but there was slight cloudy swelling on occasion.

Ethyl isobutrazine-10 phenothiazine. Dogs No. 7, 8 Acute focal suppurative inflammation which appeared to be more severe than with chlorpromazine hydrochloride was characteristic of these sections. Yet, neutrophils were not as plentiful, the exudate being scanty in several instances. Numerous eosinophils were observed in sections from Dog No. 7, but they were not seen in Dog No. 8. Where present, they seemed to congregate at the periphery of necrotic areas. A few lymphocytes, macrophages and plasma cells were also observed in the exudate. Phagocytosis of the necrotic muscle was moderate for the most part.

Cloudy swelling and necrosis were moderate to severe in degree, the latter being mostly of the coagulative type (Figure 2). Hyperemia was moderate to severe, and hemorrhage was slight to moderate. The presence

of fibrin varied from slight to copious, and blood vascular thrombi were few to moderate in number.

The degree of inflammation was approximately in positive correlation with the drug concentration administered, but differences in volume of diluent seemed to be without effect. Control sections revealed slight cloudy swelling, and in one instance with the hypodermic needle alone, a few neutrophils were present.

Perphenazine. Dogs No. 13, 14 As with the preceding drugs, perphenazine produced an acute focal suppurative myositis, but its reaction was distinguished by an extremely heavy concentration of neutrophils. This zone of cellular defense was so conspicuous that it could easily be observed with the unaided eye by holding the slide up to the light (Figure 4). Despite the many neutrophils, muscle necrosis did not appear to be as severe as with chlorpromazine hydrochloride, and here the liquefactive type was more prominent. Cloudy swelling was moderate to severe.

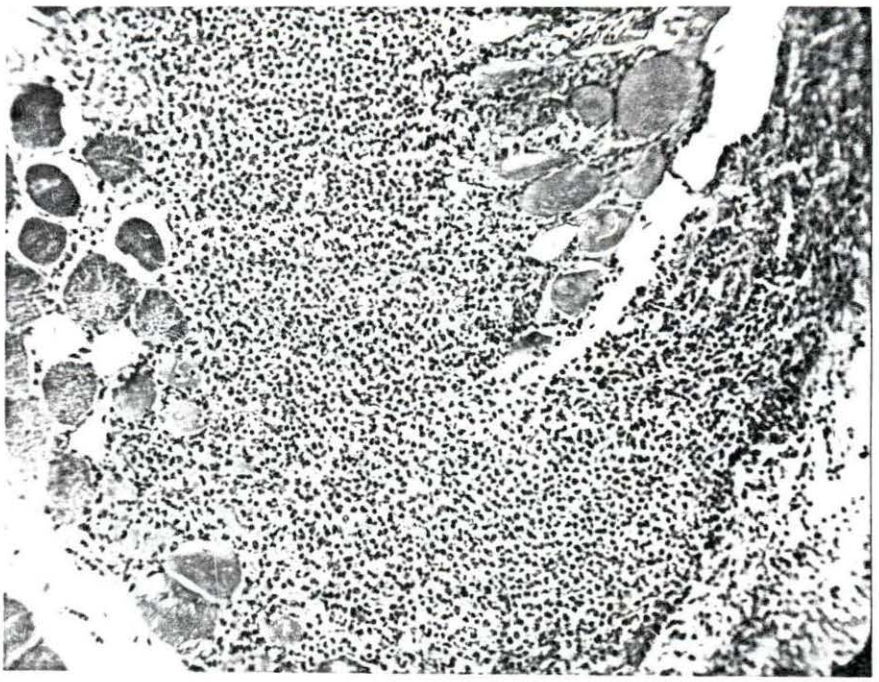
Hyperemia was moderate to severe and hemorrhage was slight to moderate. Fibrin strands were moderate to numerous, and the near absence of thrombi other than the hyaline variety was conspicuous.

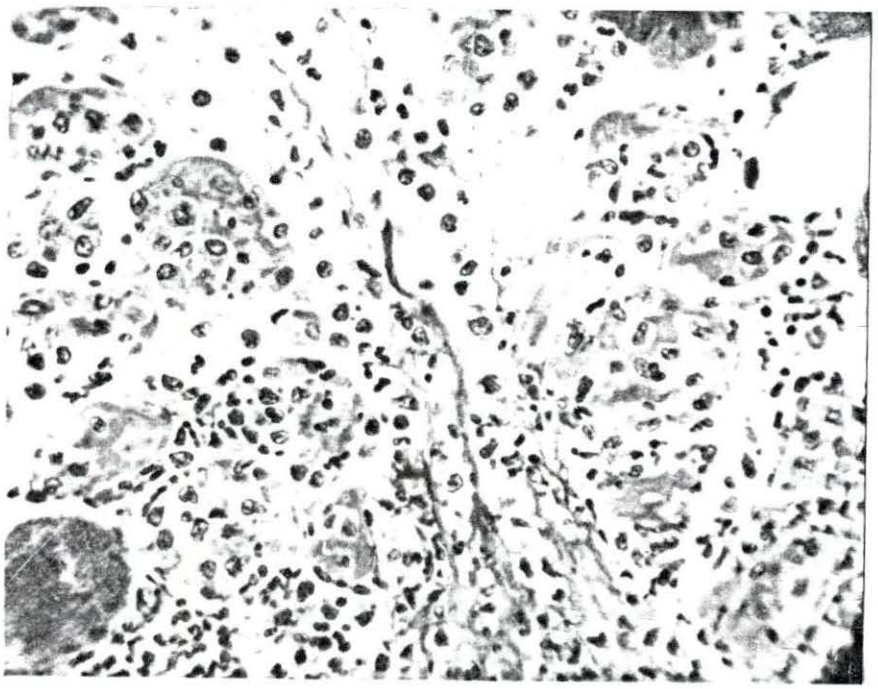
The exudate was of high cellular content and consisted mostly of masses of neutrophils with a few macrophages, lymphocytes and eosinophils, the latter being regularly observed in sections taken from both animals. Phagocytosis of necrotic and fragmented muscle was especially active (Figure 5).

In the 0.5 ml., 35 mg. section, muscle atrophy was encountered for

Figure 4. Acute focal suppurative myositis with an extremely heavy concentration of neutrophils produced by perphenazine. X 190.

Figure 5. Active phagocytosis of necrotic muscle. X 495.





the first time in the experiment (Figures 6, 7). As described by Robbins (9, p. 1204) and Smith and Jones (10, p. 75), this was marked by the diminution in the diameter of individual myocytes, resorption of the sarcoplasm and with the presence of sarcolemmal or muscle nuclei which appeared to be increased in number, size and staining intensity.

What effect the insolubility of this drug in physiological saline had in eliciting this more intense cellular response could not be determined, but the reaction was quite unlike that obtained where the commercial product was used.

Control sections appeared essentially normal, and variations in the volume of diluent did not influence the results. However, unlike the previous two drugs, increasing concentrations of drug did not show a definite trend toward creating more extensive lesions.

Gamma dimethylamino-n-propyl phenothiazine. Dogs No. 19,20

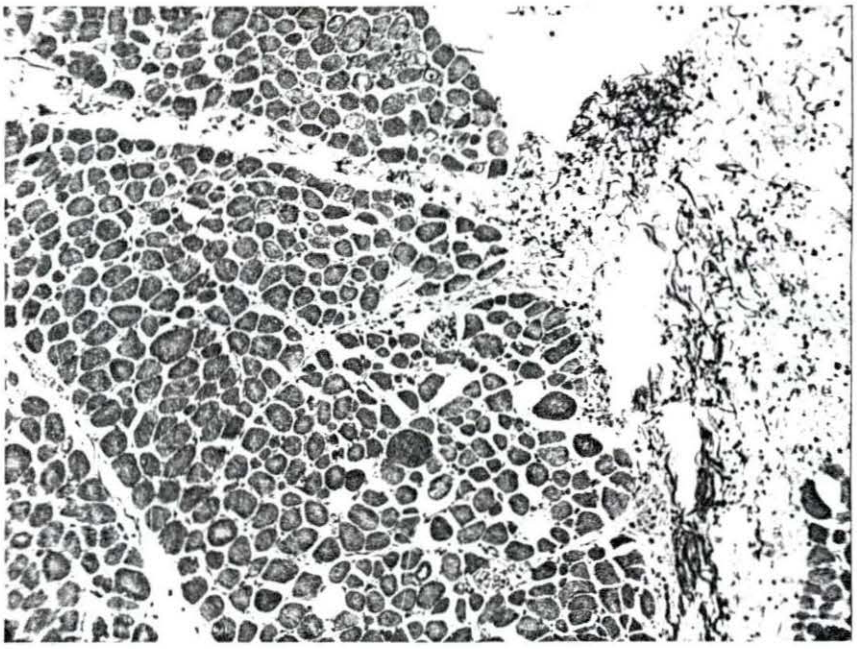
The inflammation was again acute focal suppurative in nature with extensive coagulative muscle necrosis and cloudy swelling but with relatively few cellular elements present in the exudate. Neutrophils tended to be sparse, and eosinophils were numerous but present only in Dog No. 20. Phagocytosis of necrotic muscle was slight to moderate.

Hyperemia varied from mild to severe, hemorrhage from slight to severe; and there were slight to large amounts of fibrin and a few blood vascular thrombi (Figure 8).

Eosinophils beyond their usual numbers were observed in the exudate with all four drugs in this part of the experiment at the 24-hour interval, but only in sections obtained from one drug (ethyl isobutrazine-10 phenothiazine) when the commercial products were tested. Their signifi-

Figure 6. Early muscle atrophy. Note the variation in the diameter of individual myocytes. X 96.

Figure 7. Higher magnification of the section shown in Figure 6. Sarcolemmal nuclei appear to be increased in number and size. X 495.



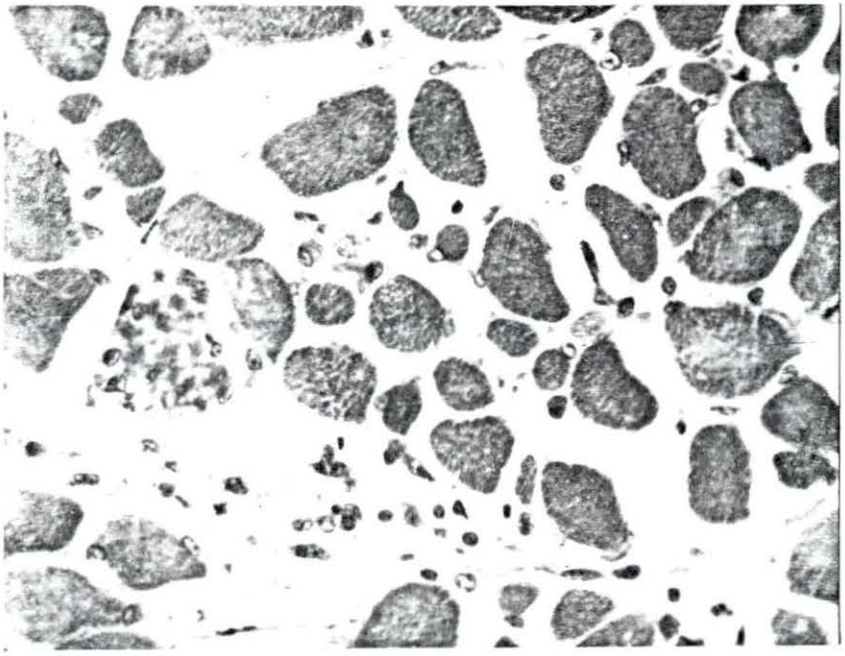
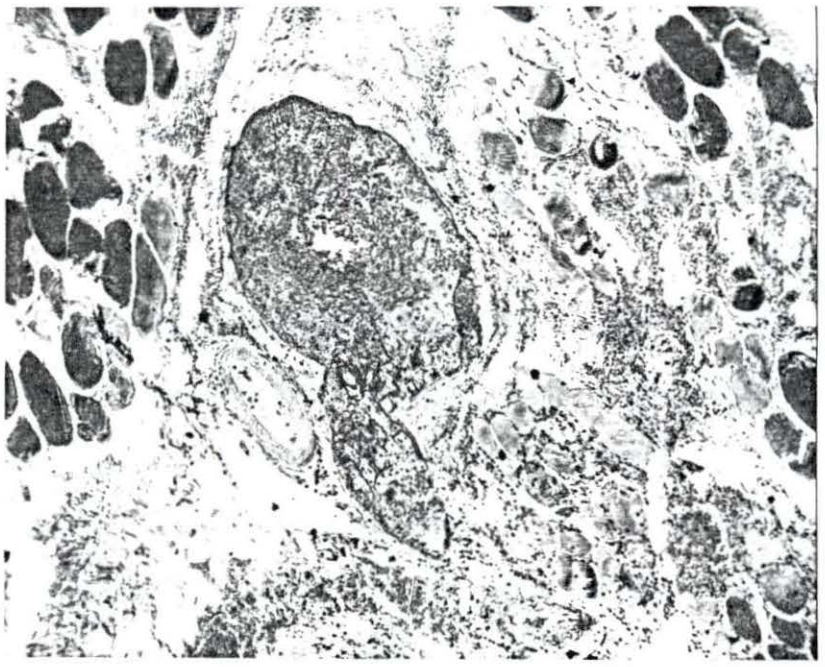
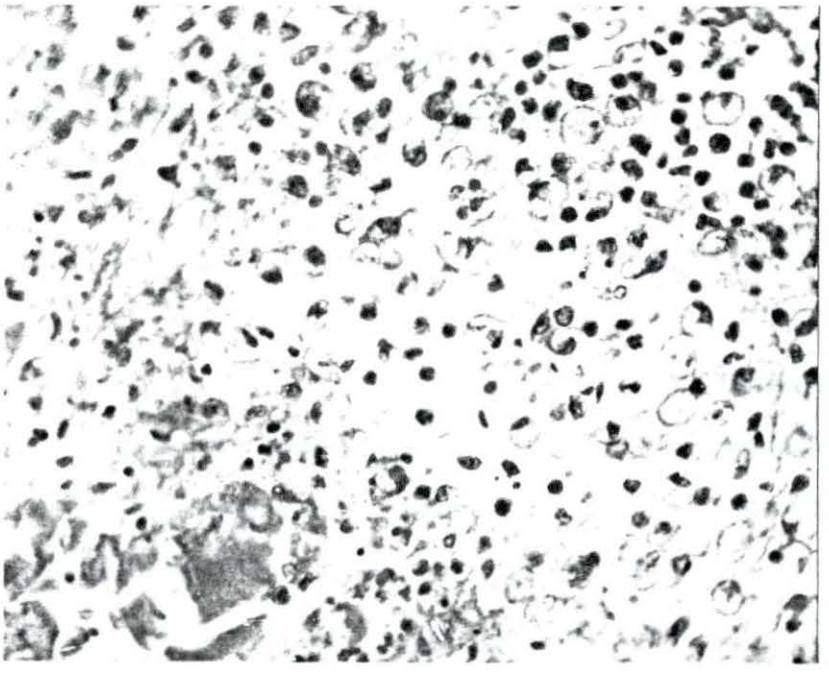


Figure 8. Blood vascular thrombi. X 96.

Figure 9. Section showing the appearance of macrophages in the exudate at 96 hours. X 495.





cance was difficult to evaluate at best. Increased numbers of them may have been the result of the presence of internal parasites or pollen allergy since the experimental phases were conducted during the summer. A third possibility is that the drugs themselves produced this eosinophilic response; Goodman and Gilman (4, p. 1067) indicated that an allergic reaction may be found when chlorpromazine hydrochloride is administered.

The extent of damage was directly proportional to the dosage, and the effect of changes in the volume of diluent appeared negligible. One saline control section showed slight hyperemia, edema and cloudy swelling and two small foci of coagulative necrosis with a few neutrophils.

As compared with the other tranquilizers, this drug produced the most extensive necrosis and cloudy swelling, but the exudative elements were least numerous. Ethyl isobutrazine-10 phenothiazine's neutrophilic response was also a weak one. Perphenazine definitely produced the greatest numbers of leucocytes with distinct zones clearly evident with the unaided eye. Chlorpromazine hydrochloride also produced a heavy concentration of neutrophils, but it ran a poor second to perphenazine in this respect.

At ninety-six hours

Chlorpromazine hydrochloride. Dogs No. 1, 2 The inflammatory reaction was still of the acute focal suppurative nature, but cell types were beginning to change. In Dog No. 1, the neutrophils were many and still the predominating cell type, but the macrophages were also abundant (Figure 9). In Dog No. 2, the macrophages appeared more numerous and perhaps equaled the numbers of neutrophils. At any rate, the condition

was now one primarily of liquefactive necrosis with the coagulative type taking a secondary role. The exception was the 0.5 ml., 65 mg. section in which coagulative necrosis was definitely the more conspicuous. Phagocytosis of the necrotic muscle was moderate to extensive.

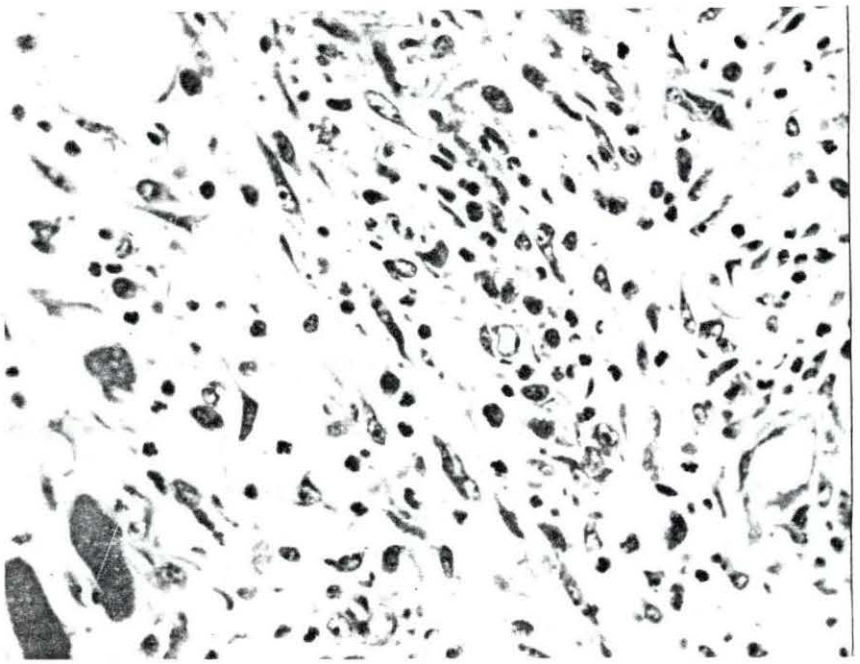
In addition to the phagocytic elements, the area of inflammation now also contained numerous fibroblasts with a small amount of immature collagen (Figure 10). Moderate endothelial cell hyperplasia was also encountered at this stage, and this was in keeping with Boyd's observations (3, p. 110-111). While Boyd further stated that fibroblastic and endothelial cell proliferation occurs by the end of 12 hours, this was not found to be true here. It was not until 96 hours that these two phenomena were observed in this experiment.

Hyperemia was moderate to severe, hemorrhage slight to mild, and fibrin strands few to numerous. Phagocytized hemosiderin was observed only in one section, and blood vascular thrombi were more numerous here than at 24 hours, ranging from few to many. Cloudy swelling, as with necrosis, was moderate to extensive. Slight to mild atrophy was present in all drug sections.

The size of the lesions varied in direct proportion according to the concentration of drug administered, while changes in the volume of the diluent at a given level exerted no influence. Controls revealed no pathologic changes with one exception in which the 1.0 ml. saline section showed cloudy swelling and slight coagulative necrosis with small amounts of exudative elements.

Ethyl isobutrazine-10 phenothiazine. Dogs No. 9, 10 Acute focal suppurative inflammation was featured with slight to extensive

Figure 10. Fibroblasts and collagen making their entrance at 96 hours. X 495.



cloudy swelling and necrosis, the latter approximately equally liquefactive and coagulative. The neutrophil was the principal constituent of the exudate, but just as with chlorpromazine hydrochloride, macrophages were quite numerous in an occasional section. Phagocytosis was extensive; and the exudate, judging from the intense eosin staining, had a high protein background.

Hemosiderin was present in the majority of sections and for the most part was contained within the macrophages. Hyperemia was moderate to severe, hemorrhage slight to moderate and fibrin slight to abundant. Blood vascular thrombi were numerous to many, and there was slight to moderate muscle atrophy. Fibroblastic and endothelial cell proliferation were also evident.

Although there was a positive trend, the extent of inflammation deviated widely at the various concentrations; and increasing volumes of diluent seemed not to weaken the response at any level. Again, one of the saline controls revealed a small area of cloudy swelling and accumulation of neutrophils.

Perphenazine. Dogs No. 17, 18 These sections were characterized by mild to severe acute suppurative myositis with the neutrophils numerous and the predominant cell type. In comparison with chlorpromazine hydrochloride and ethyl isobutrazine-10 phenothiazine at the same stage, perphenazine did not appear to stimulate as early a macrophagic response; the macrophages definitely took a secondary role in the exudate. A moderate number of fibroblasts and immature collagen were observed making their appearance at the periphery of the necrotic area.

Necrosis was mainly liquefactive, but there were smaller coagulative areas as well as moderate to extensive regions of cloudy swelling. There was slight to moderate muscle atrophy, this being more advanced than that previously described at 24 hours. While the reduction in size of the muscle fasciculi at 24 hours was due primarily to a decrease in size of the individual myocytes, here the atrophy was also the result of a decrease in the number of structural units or myocytes, many of them having shrunk to a hollow tube with preservation of only the nuclei (1, p. 62; 9, p. 1204).

Except for the hyaline type, thrombi were conspicuous by their absence. Hyperemia was moderate to severe, hemorrhage slight to mild, and there was considerable phagocytized hemosiderin. Moderate amounts of fibrin were still present.

Variations in volume of diluent and drug concentration gave similar results to those of chlorpromazine hydrochloride. The physiological saline and hypodermic needle insertion controls produced no pathological reaction.

Gamma dimethylamino-n-propyl phenothiazine. Dogs No. 21, 22

Mild to severe acute focal suppurative inflammation was characteristic here, but in contrast to the others, the fibroblast was very nearly the most prominent cell type. As with the 24-hour series, neutrophils were sparse throughout the area of inflammation, and there were few macrophages present. In addition, the fibroblasts and the cellular elements of the exudate seemed to be arranged as though to contain each muscle fasciculus in its original architectural outline; they were not diffusely scattered as with other drugs. Fibrosis was more advanced but still early with

only moderate amounts of collagen.

Hyperemia again was moderate to severe and hemorrhage slight to severe; there were slight to copious amounts of fibrin. No hemosiderin was observed in sections obtained from either dog. There were few to moderate numbers of blood vascular thrombi, and muscle atrophy was slight to mild. Cloudy swelling was slight to extensive, phagocytosis moderate to extensive and necrosis chiefly of the liquefactive type.

Results regarding different volume and concentration levels were essentially similar to those of chlorpromazine hydrochloride. All control sections revealed no pathologic changes.

At eight days

Chlorpromazine hydrochloride. Dogs No. 3, 4 Subacute focal serous inflammation which ranged from mild to severe was typical of this group. There were considerable amounts of fibroblasts and collagen, some of which exhibited a tendency toward maturation. For the most part, however, the connective tissue elements were immature. Neutrophils and macrophages were present, but in much fewer numbers than before (Figure 11). A few lymphocytes and plasma cells were present, and phagocytosis of the necrotic muscle was slight.

Muscle necrosis, primarily coagulative, was slight to mild with one exception where it was extensive. Cloudy swelling varied only from slight to mild, and there was considerable muscle atrophy which was further advanced from that described at 96 hours. Fibroblasts and collagen surrounded the individual myocytes, and hence, pressure appeared to be a factor resulting in the atrophy observed at this stage (Figures 12, 13).

Figure 11. Subacute focal serous myositis at eight days with only a few inflammatory cells present. X 96.

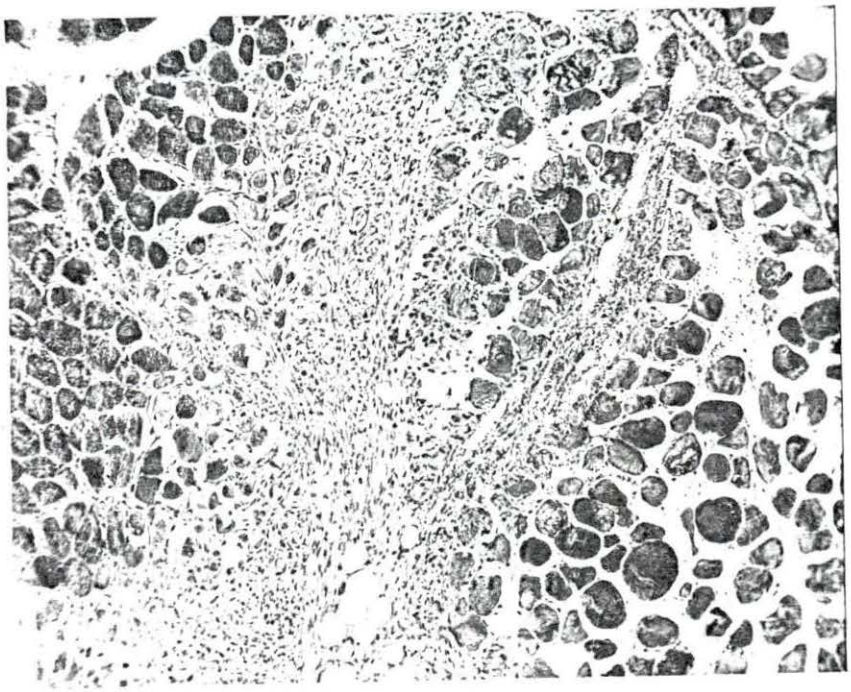
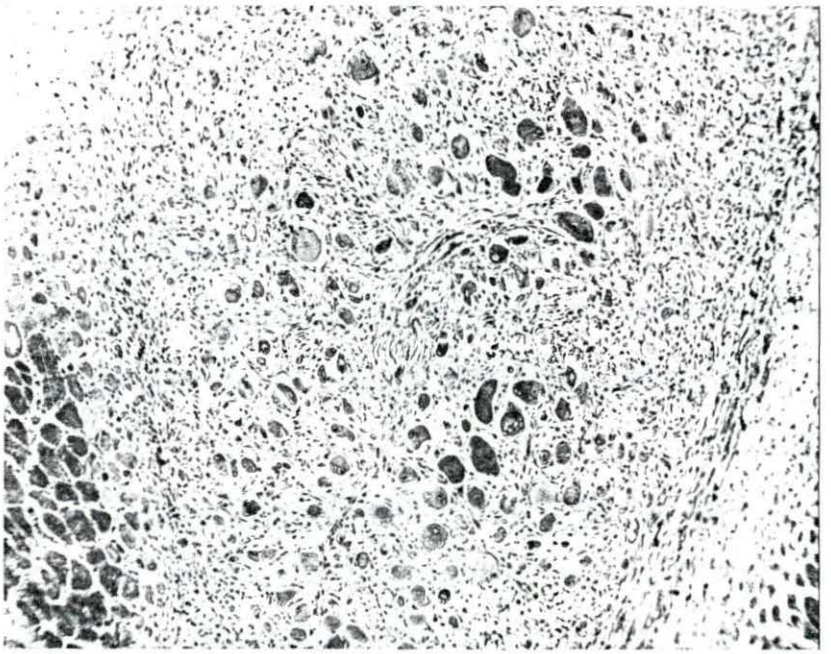
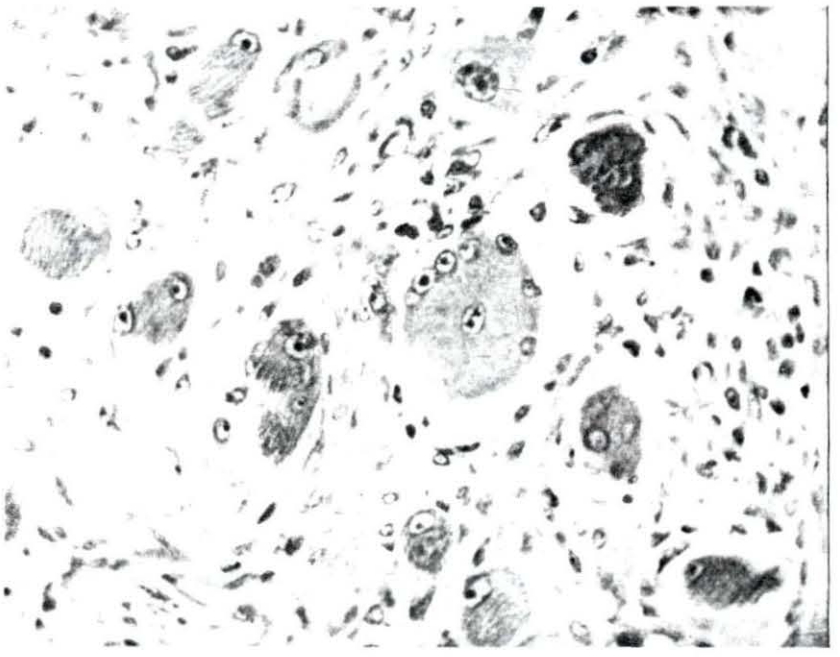


Figure 12. Advanced muscle atrophy. Note that fibroblasts and collagen surround the individual myocytes and appear to have replaced those that are no longer present. X 96.

Figure 13. Higher magnification of the section shown in Figure 12. The sarcolemmal nuclei are numerous and prominent. X 495.





As with the fibroblasts, there was endothelial cell proliferation (Figures 14, 15). A few thrombi were observed in the blood vessels but not in all sections. Hyperemia and hemorrhage ranged from slight to moderate, and there were moderate amounts of fibrin.

There was positive correlation between drug concentration and the measure of the inflammatory reaction. As with all other sections of this series, the varying of the volume of diluent appeared to have no bearing on the extent of inflammation created. One hypodermic needle and one saline control exhibited slight cloudy swelling, hyperemia and hemorrhage.

Ethyl isobutrazine-10 phenothiazine. Dogs No. 11, 12 These sections were very similar to those of the preceding drug with perhaps more pronounced endothelial cell hyperplasia and greater amounts of phagocytized hemosiderin.

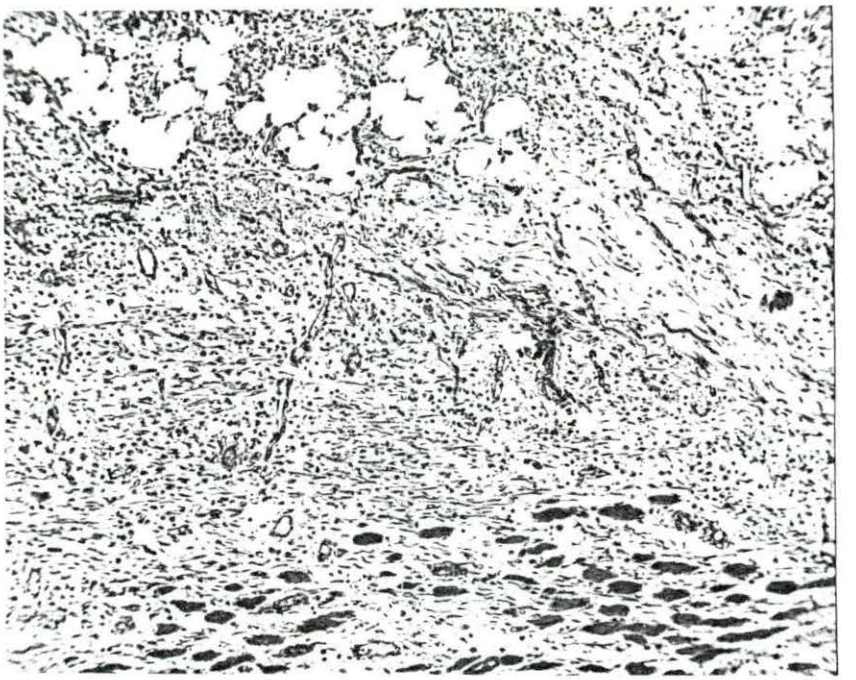
Both dogs were obese, and because the hypodermic needle was too short, four injections were made partially into the subcutis. The fat layers were undergoing phagocytosis and being invaded by fibroblasts. Neutrophils were most numerous in the adipose layer and sparse in the necrotic muscle.

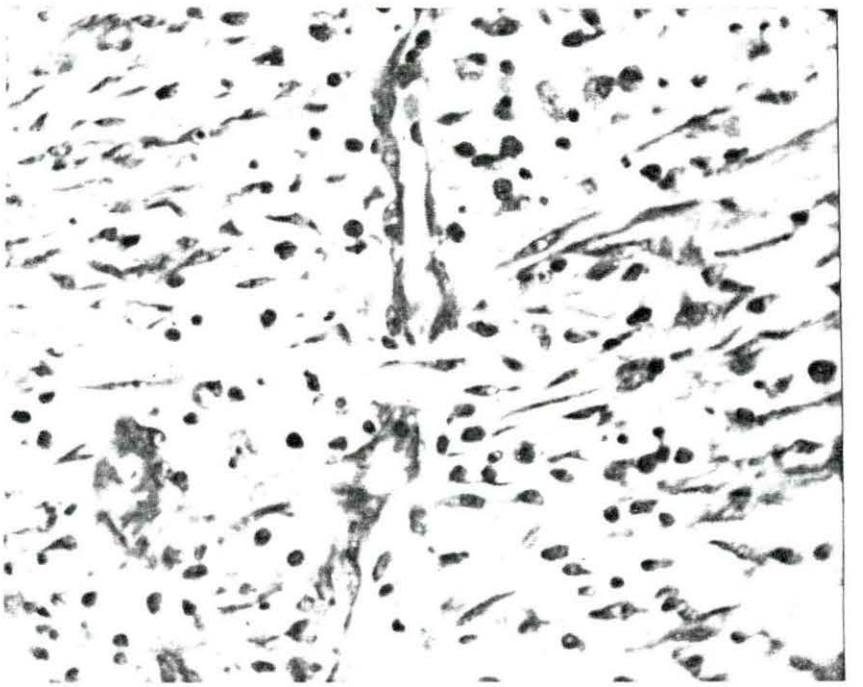
There was agreement between the severity of inflammation and the concentration of drug administered. Control injections produced no inflammatory changes.

Perphenazine. Dogs No. 15, 16 The type of inflammation observed in these sections was not uniform in all cases, but the majority were of a subacute focal serous nature with fibroblastic elements dominant. In three sections, however, the condition was still suppurative with

Figure 14. Endothelial cell proliferation. Note that the proliferating capillaries run perpendicular to the fibroblasts. X 96.

Figure 15. Higher magnification of the section shown in Figure 14. X 495.





macrophages the main exudative element. In a few others, macrophages and fibroblasts appeared to be about equal in number. In this respect, perphenazine differed from the previous two drugs where the fibroblast was always the most numerous cell found at eight days. This observation was, however, in keeping with those made at 24 and 96 hours in which the neutrophilic response was greater and later sustained and in which the macrophages made a later appearance than the others.

Muscle atrophy, slight to moderate, also did not appear as extensive, and blood vascular thrombi were absent. There was mild to moderate cloudy swelling and slight to moderate necrosis, mostly of the liquefactive type. Phagocytosis was slight to extensive, hyperemia slight to moderate and hemorrhage slight to mild with only slight amounts of fibrin deposited. No hemosiderin was encountered.

The extent of damage as compared with concentrations administered varied widely here, more so than at any other time. Except for one instance in which cloudy swelling was present, the controls were otherwise free of pathologic changes.

Gamma dimethylamino-n-propyl phenothiazine. Dogs No. 23, 24

The inflammation was a subacute focal serous one which appeared to be more advanced than that found with the other drugs at eight days. Fibroblasts and collagen were present in abundance and tended toward maturity. Small foci of coagulative necrosis and cloudy swelling still remained, but at this time there were relatively few neutrophils and macrophages. This scarcity of neutrophils and macrophages was also characteristic in the 24-hour and 96-hour sections. Phagocytosis of necrotic muscle was only slight to moderate.

There was considerable atrophy in this group, this being another prominent trait common to all but not quite so extensive in the other drugs. Hyperemia and hemorrhage were slight to moderate, and the quantity of fibrin was slight to copious. Few blood vascular thrombi and no hemosiderin were observed.

There was good correlation between concentration of drug administered and the degree of inflammation produced, but volume changes in the diluent were without effect. Controls were void of pathologic changes.

Part II: the tranquilizers as commercially prepared

At twenty-four hours

Chlorpromazine hydrochloride. Dog No. 30 Acute focal suppurative inflammation of moderate degree was characteristic. Neutrophils were very numerous and the only readily noticeable cell type in the exudate. Phagocytosis was moderate to extensive, necrosis moderate and mostly coagulative and cloudy swelling moderate to extensive.

Hyperemia was moderate to severe, hemorrhage mild to severe and fibrin slight to copious. Thrombi were few to many. Muscle atrophy, fibroblastic and endothelial cell proliferation were absent.

The lesions produced in this part compared closely with the corresponding sections of the preceding experimental phase in which sterile physiological saline was used as the diluent except that neutrophils were more numerous here.

Ethyl isobutrazine-10 phenothiazine. Dog No. 30 These sections were characterized by moderate to extensive acute focal

suppurative inflammation in which the neutrophils were abundant and practically the only exudative cell type visible. In one section (15 mg.) however, neutrophils were scanty in number and eosinophils more noticeable than usual in the exudate. Necrosis was mostly liquefactive at this stage, but the coagulative type was also present. Cloudy swelling and phagocytosis were moderate to extensive.

Muscle atrophy, fibroblastic and endothelial cell proliferation were absent. There was moderate to severe hyperemia and slight to severe hemorrhage. Moderate to large quantities of fibrin and few to many blood vascular thrombi were present. In general, the areas of necrosis and cloudy swelling were slightly more extensive than with chlorpromazine hydrochloride.

Corresponding sections from Part I revealed a suppurative inflammation of less intensity as regards numbers of neutrophils; otherwise the myopathy was similar.

Perphenazine, Dog No. 25 As with chlorpromazine hydrochloride, these sections were characterized by slight to severe acute focal suppurative myositis with only moderate neutrophilic invasion of the necrotic areas. Necrosis was mainly of the coagulative type and ranged from slight to extensive. Phagocytosis of the necrotic and fragmented muscle was slight to moderate.

Blood vascular thrombi were again absent or fewer than observed in sections from chlorpromazine hydrochloride and ethyl isobutrazine-10 phenothiazine. Hyperemia and hemorrhage were slight to moderate, fibrin strands slight to plentiful and atrophy, endothelial and fibroblastic proliferation absent.

Drug penetration into the muscle fasciculi was not as deep as observed with the other tranquilizers. Peripheral portions of muscle bundles were coagulated while the central areas had only undergone cloudy swelling. On the other hand, this drug appeared to spread through the epimysium and perimysium more extensively than any other drug; and as a result, a greater number of fasciculi were affected but not as severely as with the others. This was not unexpected when one recalled that perphenazine was insoluble in physiological saline. It may have been that the body fluids rendered the commercial product less soluble and resulted in penetration along a line of lesser resistance, the connective tissue in deference to the more impervious muscle parenchyma.

In general, this drug's action most resembled that of chlorpromazine hydrochloride, but it did not agree with the results obtained in the previous part of the experiment where physiological saline was the diluent. The neutrophilic response in the latter was very prominent, here much less so; furthermore, no muscle atrophy was observed in this portion as it was in Part I.

Gamma dimethylamino-n-propyl phenothiazine, Dog No. 25

These sections were characterized by the absence of heavy concentrations of neutrophils as were found with the use of ethyl isobutrazine-10 phenothiazine and chlorpromazine hydrochloride. The myositis was nevertheless of an acute focal suppurative nature and varied from mild to severe. Phagocytosis of the primarily coagulated muscle was slight, this again differing from the above-mentioned drugs. Cloudy swelling was extensive.

Hyperemia was moderate to severe, hemorrhage slight to severe and fibrin slight to copious. Blood vascular thrombi were either absent or few in number.

Atrophy, endothelial cell and fibroblastic proliferation were absent. Compared with Part I, the extent of necrosis was not uniformly as great, but the lesser numbers of neutrophils was expected.

At ninety-six hours

Chlorpromazine hydrochloride. Dog No. 28 With the exception of the 15 mg. section, the inflammation was still of the acute focal suppurative type. Macrophages were numerous, and fibroblasts and collagen made their entrance in lesser quantities. Necrosis was an approximately equal combination of liquefaction and coagulation, and cloudy swelling was slight to extensive. Fibroblasts were the chief cell type in the 15 mg. section, and there was only a very small focus of muscle necrosis.

Atrophy of muscle was slight to mild, and fibroblastic and endothelial cell proliferation were in the early stages. Hyperemia was slight to severe, hemorrhage slight to moderate and fibrin slight to copious in amount. Blood vascular thrombi were present only at the 50 mg. and 65 mg. level, and no hemosiderin was observed at any time here or in the sections obtained from the other three drugs in this series.

The inflammatory reaction observed in these sections was similar to that of Part I with perhaps more macrophages and fibroblasts in the latter.

Ethyl isobutrazine-10 phenothiazine. Dog No. 28 There was

moderate to severe acute focal suppurative myositis with somewhat lesser numbers of macrophages encroaching upon the moderate to large areas of coagulative muscle necrosis. Small to extensive areas of cloudy swelling were present, and phagocytosis of the necrotic muscle was extensive. Fibroblastic and endothelial cell proliferation were in the very early stages.

A significant feature was the numerous blood vascular thrombi encountered; in one instance (65 mg.), it demonstrated the progressive infiltration of the tranquilizer. This was evidenced by the similar location of endothelial damage and thrombus formation in blood vessels found at different depths in the muscle (Figure 16).

Hyperemia was uniformly severe, hemorrhage moderate and fibrin copious for the most part. Muscle atrophy was slight and considerably less than found with chlorpromazine hydrochloride.

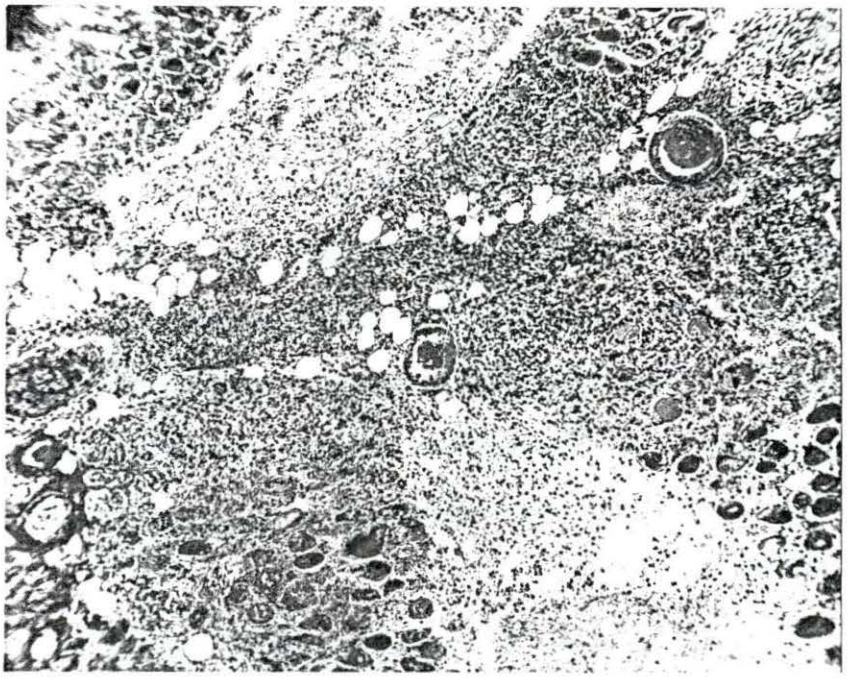
Neutrophils were much more numerous in these sections than in their correspondents of Part I, but muscle atrophy was not so pronounced in these.

Perphenazine. Dog No. 29 Except for the 15 mg. section, there were large numbers of neutrophils invading the necrotic areas. The myositis was acute focal suppurative, and phagocytosis moderate to extensive with macrophages taking a secondary role. Fibroblastic and endothelial cell proliferation were in the early stages.

There was severe hyperemia, slight to moderate hemorrhage and slight to copious amounts of fibrin. Blood vascular thrombi were absent or moderate in number, and there was slight to mild muscle atrophy.

The findings here were in agreement with those of Part I, especially

Figure 16. Blood vascular thrombi demonstrating progressive infiltration of the tranquilizer. Note similar location of endothelial damage and thrombus formation in the blood vessels found at different depths. X 96.



as concerns the extent and type of necrosis and the large numbers of neutrophils encountered. Thrombi were conspicuous by their absence previously, but here were moderate in number.

Gamma dimethylamino-n-propyl phenothiazine. Dog No. 29

The inflammation was of an acute focal suppurative type, and numerous macrophages were present in the exudate. The chief difference between this tranquilizer and perphenazine was that the neutrophilic zone of cellular defense surrounding the necrotic area was not as prominent.

Fibroblasts were not prominent, but in the 15 mg. section they were the most numerous cell type found. The latter condition was true whenever the focus of necrosis was small; healing was more rapid as evidenced by the earlier appearance of connective tissue elements.

Necrosis was mostly coagulative and was mild to moderate. Cloudy swelling was moderate to extensive, and there was slight muscle atrophy. Hyperemia was slight to severe, hemorrhage slight to mild and fibrin slight to copious in amount.

These sections differed from those of Part I in several respects. Fibroblastic components were least prominent, the neutrophils and macrophages were present in much greater numbers and coagulative necrosis was more extensive than liquefaction here; the opposite was true of Part I.

At eight days

Chlorpromazine hydrochloride. Dog No. 26 In four sections the myositis was moderate to severe and subacute focal serous in type, while in the remaining one (50 mg.) it was an acute focal suppurative reaction. In the former, fibroblasts were the predominant cell, but

there were also numerous neutrophils and macrophages and a few lymphocytes. In the 50 mg. section, macrophages and neutrophils exceeded the numbers of fibroblasts.

There was slight to mild coagulative muscle necrosis and moderate to extensive cloudy swelling. Phagocytosis of necrotic muscle was slight to moderate, and there was considerable endothelial cell hyperplasia.

Muscle atrophy varied from slight to extensive, and there were few to many blood vascular thrombi. Hyperemia was moderate to severe, hemorrhage slight to moderate and fibrin moderate to plentiful. No hemosiderin, either phagocytized or extracellular, was observed in these sections or in those obtained from other drugs in this series.

There was close correlation of results found in both parts of the experiment testing this drug.

Ethyl isobutrazine-10 phenothiazine. Dog No. 26 In contrast to the other three tranquilizers, this drug was unusual in that the inflammation, with one exception (15 mg.), was still of the acute focal suppurative type. Macrophages and neutrophils were abundant, and a few lymphocytes and plasma cells were also in evidence. Fibroblasts and collagen were scanty.

The persistence of extensive areas of coagulative and liquefactive necrosis as well as cloudy swelling also was noteworthy. Phagocytic components barely penetrated the necrotic muscle fasciculi, the central areas of necrosis being relatively free of exudative material.

Endothelial cell proliferation was prominent, and, as with chlor-

promazine hydrochloride, numerous thrombi were still evident. Muscle atrophy was slight to extensive. There was slight to severe hyperemia, slight to mild hemorrhage and moderate to copious amounts of fibrin.

The presence of numerous blood vascular thrombi here and in sections from chlorpromazine hydrochloride was in contrast to the other two drugs in which few were observed at this stage. In Part I, none of the four drugs produced thrombi that were present at eight days. In addition, the myositis here appeared to be distinctly more acute and suppurative as compared with the same sections from Part I.

Perphenazine. Dog No. 27 With the exception of the 65 mg. section in which acute focal suppuration was manifest, these were similar to those of gamma dimethylamino-n-propyl phenothiazine. The reaction was thus a subacute focal serous inflammation of mild to severe intensity. Small areas of coagulative necrosis and cloudy swelling were found, but liquefactive necrosis of any appreciable extent appeared only in the 65 mg. section.

The exudate was composed mainly of macrophages and neutrophils. Fibroblastic and endothelial cell proliferation was pronounced, and atrophy of muscle was mild to extensive. The vascular changes were similar to those of the preceding drug, and a few blood vascular thrombi were observed.

Parts I and II involving perphenazine were essentially alike.

Gamma dimethylamino-n-propyl phenothiazine. Dog No. 27 As with the eight-day ethyl isobutrazine-10 phenothiazine sections, there still remained a few foci of coagulative necrosis. Here, however, macrophages and neutrophils were much fewer than the fibroblasts. Cloudy

swelling was moderate.

Endothelial cell hyperplasia was prominent, and muscle atrophy was mild to extensive. Hyperemia and hemorrhage were mild to moderate, fibrin moderate in amount and blood vascular thrombi few.

The relatively small numbers of neutrophils and macrophages at any stage was a consistent finding with this drug throughout the experiment in both Parts I and II. Widespread muscle atrophy also was characteristic in both parts beyond 96 hours.

DISCUSSION

As mentioned in the Introduction, dogs were selected as experimental animals because it was not economically feasible to use cattle. There is no reason to indicate that the muscle reaction to the phenothiazine-derived tranquilizers is different in the bovine muscle, and the discussion that follows assumes this to be true. The results indicate what probably occurs in the bovine species.

The significant myopathy produced by the intramuscular administration of the phenothiazine-derived tranquilizers is its necrotizing effect upon muscle followed by a suppurative inflammatory reaction. When the drugs have been administered to cattle just prior to shipment to market, the result is the destruction of several pounds of meat whichare found at slaughter. The loss to the meat packer is however, even greater than this, because in the process of trimming out the necrotic and inflammed muscle, the cut of meat into which the tranquilizer has been injected is destroyed. The portions left can be salvaged only as less expensive cuts, i.e., stew or ground meat. Since a common site of administration is in the round or gluteal muscles, the monetary losses thus sustained are considerable.

The use of tranquilizers is not limited to cattle being shipped to slaughter. Savings of up to 50 percent of the usual shrink in transit are claimed; and they are also advertised to reduce the stresses of weaning calves (castration, dehorning, branding), help heifers adapt to the stanchion quickly and assure the quick and easy adjustment of feedlot steers to new surroundings. In essence, they are purported to reduce

losses attributable to shipping and adaptation.

In these latter uses, the acute reaction shown to occur in the experiment at 24 and 96 hours goes unnoticed unless an injection is made subcutaneously, in which instance a grossly visible swelling is produced. At eight days, the myositis is subdued with the healing process well underway. Fibrosis and muscle atrophy are prominent at this stage, and in two to three weeks one could expect healing by substitution to be complete.

Since the deposition of connective tissue in the healing process is irreversible and since muscle does not regenerate to any great extent, it is reasonable to assume that there would be a focus of connective tissue (cicatrix) present thereafter. While this may go unnoticed at the packing house, the butcher or consumer would encounter it as a tough, inedible area within the muscle parenchyma. In all probability, the significance of the scar would not be apparent, but it is nonetheless an undesireable result.

SUMMARY AND CONCLUSIONS

1. An experiment was undertaken to determine the nature of the myopathy caused by the intramuscular injection of phenothiazine-derived tranquilizers. The project was divided into two parts, the first to test the drugs with sterile physiological saline as the diluent, and the second to test the drugs as obtained commercially. Several concentrations of drug and volumes of diluent were selected as a comparative measure.

2. The combined results indicate clearly that the phenothiazine-derived tranquilizers have a potent necrotizing effect upon skeletal muscle. This results in an acute focal suppurative myositis within 24 hours and gives way to an subacute focal serous reaction in eight days. Increasing concentrations of the tranquilizers were found generally to produce correspondingly larger areas of necrosis and inflammation, but variations in the volume of physiological saline produced no significant changes in the results.

3. The comparisons made of the four phenothiazine-derived tranquilizers studied indicate that, while minor differences do occur in the myopathy they produce, the end results are essentially the same.

4. Subcutaneous injections of the phenothiazine-derived tranquilizers produce a diffuse hemorrhagic to sero-hemorrhagic inflammation of the subcutis which is often manifested grossly by swelling. This possibly is the reason the manufacturers recommend deep intramuscular injection which masks the undesirable reaction.

5. The use of phenothiazine-derived tranquilizers intramuscularly in meat producing animals as a means of saving weight losses (shrink) in

transit, in the feedlot and for other purposes is a questionable practice because the resultant muscle losses sustained at slaughter or at the dinner table may very well off-set these purported gains.

6. The results suggest strongly that a less injurious and costly route of administering phenothiazine-derived tranquilizers be used or, at best, that only muscles containing the cheaper cuts of meat be the sites of injection.

LITERATURE CITED

1. Anderson, W. A. D. Pathology. 3rd ed. St. Louis, Mo., The C. V. Mosby Company. 1957.

2. Armed Forces Institute of Pathology. Manual of histologic and special staining technics. Washington, D.C., Author. 1957.

3. Boyd, William. A text-book of pathology. 6th ed. Philadelphia, Pa., Lea and Febiger. 1953.

4. Goodman, Louis S. and Gilman, Alfred. The pharmacological basis of therapeutics. 2nd ed. New York, N. Y., The Macmillan Company. 1955.

5. Hawaii. Bd. of Agr. and For. Div. of An. Ind. Annual report for fiscal year ended 1959. [1959.]

6. Jones, L. Meyer. Veterinary pharmacology and therapeutics. 2nd ed. Ames, Iowa., The Iowa State College Press. 1957.

7. Krantz, John C., Jr. and Carr, C. Jelleff. The pharmacologic principles of medical practice. 4th ed. Baltimore, Md., The Williams and Wilkins Company. 1958.

8. Paget, G. E. and Scott, H. McG. A comparison of the local effects of various intramuscular injections in the rat. British Journal of Pharmacology and Chemotherapy. 12: 427-433. 1957.

9. Robbins, Stanley L. Textbook of pathology. Philadelphia, Pa., W. B. Saunders Company. 1957.

10. Smith, Hilton Atmore and Jones, Thomas Carlyle. Veterinary pathology. Philadelphia, Pa., Lea and Febiger. 1957.

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