THE PATHOLOGY OF EXPERIMENTAL COYOTILLO (KARWINSKIA HUMBOLDTIANA) POISONING IN GOATS

A Thesis

By

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INTRODUCTION

Coyotillo (Karwinskia humboldtiana) is a toxic shrub which grows in a large area of South and Southwest Texas. Death losses occur in domestic animals following consumption of fruits and the leaves of the plant. It assumes economic importance in the livestock raising areas of Texas where it grows. Marsh's (20) brief description of the gross and microscopic change in experimentally poisoned sheep, cattle, guinea pigs and chickens published in 1928, is the only known investigation on the pathology of coyotillo. Little progress has been made in the control of this disease to date. The objectives of this study were to determine certain clinical, gross and microscopic changes which may aid in understanding the pathogenesis of the disease.

HISTORY AND LITERATURE REVIEW

Clavigero (6) in 1789 first mentioned the poisonous properties of Karwinskia humboldtiana when he noticed Indian children poisoned with the fruits. Havard (11) in 1885 wrote that the poisonous principle lies in the seed, the pulp being innocuous. The signs were described as a paralysis of the spinal cord primarily affecting locomotion. Sosa (33) in 1890 stated that the paralysis could be easily cured. He reported that the sickness did not appear immediately, but after continued consumption of the plant for several days. Rose (31) in 1899 stated that the coyotillo leaves were crushed and soaked in water, and that this cold infusion was used in cases of fever in Mexico. Standley (35) stated that the seeds were oily and contained some principle which paralyzes the motor nerves. They were employed in Mexico as anticonvulsants, particularly in the case of tetanus. Marsh et al. (20) indicated that one Dr. Palmer stated in 1901 that several children were brought to him (Palmer) suffering from coyotillo poisoning, and that one girl lost the use of her limbs. Pamel (27) in 1911 and Marsh et al. (20) in 1928 observed that seeds and leaves were toxic to cattle, sheep, goats, guinea pigs, horses, swine and chicken. Vandersal (36) and Muenschner (23) reported that children have been fatally poisoned by berries. Marsh et al. (20) concluded that fruits were more than twice as toxic as the leaves. Aguilera (2), 1945, confirmed the presence of a glucoside as the principle toxicant. She found that none of the extract from the fruit pulp was poisonous either orally or parenterally. The watery extract of endocarp and the seed contained tannin, direct reducing sugar, an indirect reducing sugar which appeared to be a glucoside, colored material, vegetable acid, starch or dextrin

but no alkaloid. Rivero (30) in 1948 in her studies on the pharmaceutical aspects of coyotillo suspected that the poison in coyotillo attacked the cerebral trunk and that its effect should not be considered as poisonous or destructive as polioneuritis or poliomyelitis. She further stated that the toxic part of coyotillo was found in the tegument of the seed and that the toxicant was soluble in chloroform, ether, acetone, methyl alcohol, and benzene. In her studies on the effect of coyotillo on doves, she stated that the effect of poison began as soon as it was absorbed. The rapidity with which the clinical syndrome appeared depended on the dosage of the poison. She described the signs as diarrhea which appeared 24 hours after the first dose, malfunction of the eyes and eyelids, loss of balance, tremors in the extremities, and, finally, death by asphyxiation.

It is known that the serum glutamic oxalacetic transaminse (SGOT) level increases in muscular and hepatic degeneration. Cornelius (7, 8) stated that the elevation of SGOT in certain specific diseases of domestic animals have been observed to be diagnostic. He observed significant SGOT activity in myocardial infarction and hepatic necrosis in the dog, muscular dystrophy in chickens, sheep and cattle and azoturia and "tying up" in horses. Since considerable SGOT activities were found in almost all the tissues of the horse, cow, pig, dog and chicken, he further stated that elevation of this enzyme in serum could be expected to occur due to necrosis of many different tissues. He indicated that SGOT elevation was not organ specific and thus should be correlated with clinical signs and other pertinant laboratory findings.

Serum glutamic pyruvic transaminase (SGPT) becomes elevated in hepatocellular destruction (7). It, therefore, assumes importance as a hepatic

function test in both animals and man.

Alkaline phosphatase is found in appreciable amounts and is probably produced in osteoblasts, which apparently release the enzyme into the blood. As a result, derangements of bone resulting in increased osteoblastic activity will produce increased serum alkaline phosphatase values. It is normally excreted in the bile, so that serum alkaline phosphatase activity will also increase as a result of biliary obstruction (18, 34).

Balincoe and Marble (4) observed that in lambs with experimentally induced white muscle disease a highly significant linear correlation existed between serum lactic dehydrogenase and SGOT. Although no correlation was drawn between serum alkaline phosphatase and SGOT, alkaline phosphatase levels were reduced to half in severely affected lambs which also had high SGOT levels. The authors have shown the blood enzyme interrelationships in lambs as follows:

Constituent	Diseased Lambs	Normal Lambs
SGOT	1,290*	56*
Lactic dehydrogenase	22,310	1,152
Alkaline phosphatase	5.4 $\pm$ 1.9	10.5 $\pm$ 3.7

* Expressed as units

Kuttler and Marble (15) described the relationship of serum transaminase to naturally occurring and artificially induced white muscle disease in calves and lambs. They found greatly increased SGOT levels in 14 lambs with artificially induced white muscle disease and 17 lambs with the naturally occurring disease. The normal value of SGOT was less than 100 units per ml. in both calves and lambs. Signs of disease usually occurred in lambs with 2000 to 3000 units per ml. and in calves

with 296 to 890 units per ml. The SGOT values in normal control lambs were given in three different groups as 57 $\pm$ 18, 56 $\pm$ 12 and 53 $\pm$ 18.

Young and Keeler (37) stated that muscular exercise was essential for development of symmetrical muscle lesions associated with increase of SGOT level. They studied the effect of mechanical restraint applied to one foreleg on the distribution of lesions of nutritional muscular dystrophy in both forelegs of lambs born to ewes fed a dystrophy producing diet. The lesions were distributed in the muscles of the two forelegs without bilateral symmetry, characteristic of the disease. Lesions either did not occur in muscles of the restrained limb or involved them to much lesser extent than muscles of the unstrained limb.

Goresya and McCarty (10) studied serum proteins of 24 to 30-month-old goats by chemical and electrophoratic methods to elucidate reasons for the wide range of normal values encountered in goats. They found significantly increased total serum protein in older goats. Significant variation based on location and breed were found in total serum protein and serum protein components of the animals of the older group.

MATERIALS AND METHODS

Treatment Groups

Sixteen goats including six males and ten females whose ages ranged from one and one-half to four years were used in these studies. Twelve were of Spanish and four of Angora breeding.

The goats were maintained on pasture until the experiments were begun. During the course of the experiments some of the goats in one group (A) were kept in metabolism cages while all other groups were kept in a pen 20 feet square. All goats were given free access to water and prairie hay and were given alfalfa hay daily.

The animals were divided into five groups as given in Table I. Each group except group A consisted of two treated animals and one non-treated control. Group A had 3 treated animals and one control. All groups except A were exercised as described below.

Group A was given 0.15 percent of body weight of ground coyotillo fruit daily by mouth until the animals became recumbent. Groups B and D were treated at a rate of 0.075 percent of body weight daily until clinical signs occurred. The initial signs were mild incoordination of movements. When the latter signs occurred, the animals were given no more plant material. Groups C and E were given plant material at a dosage rate of 0.025 percent body weight. They were dosed until clinical signs occurred as described above.

All animals except those in Group A were exercised twice daily for a minimum of 5 minutes and a maximum of 10 minutes. The goats were driven around a large pen. The animal's gait varied from a fast walk to

a run. Three animals in group A (24563, 24584 and N-1) were kept in metabolism cages and were not exercised or removed from the cages until recumbent. One animal (24322) of this group was placed in a large pen and allowed to move at will but was not forced to exercise.

All goats were observed 3 times daily for any clinical signs. Abnormal postures, movements and other evidence of clinical illness were recorded.

Plant Material

Mature fruits of the coyotillo plant (Karwinskia humboldtiana) were gathered directly from the plant or from the ground under the plant after they had fallen. The fruits were ground in a Wiley mill and the ground plant material was kept in sealed glass jars at 5°C until used.

Immediately prior to administration, sufficient water was added to the weighed coyotillo material to produce a stiff paste which was molded to fit a balling gun for oral administration.

Blood and Serum

The goats were placed in the treatment pen for a week prior to the initial administration of the coyotillo. Blood was collected from the jugular vein of each animal on each of 3 days before initial treatment. Blood for total and differential leukocyte counts, hemoglobin and hematocrits was collected in tubes containing disodium ethylenediaminetetracetate (EDTA) every day or on alternate days at approximately 1:30 p.m. Another blood aliquot was placed in a tube and allowed to clot. Serum was removed for use in the various procedures described below. The

serum was divided into 3 aliquots and maintained frozen at -21°C until used.

Total and differential leukocyte counts were conducted according to standard procedures. Packed cell volumes were determined by the use of the microhematocrit technique using an International microhematocrit centrifuge.* All counts and packed cell volume determinations were carried out within an hour after blood collection. Hemoglobin determinations were carried out by the oxyhemoglobin method (22) in a "Spectronic 20" colorimeter at a wave length of 545 u . Urea nitrogen was determined by the use of diacetyl monoxime after deproteinization of serum with trichloracetic acid (5, 26).

Serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) determinations were conducted by the modified Reitman-Frankel method (17, 30). Reagents used in these tests were purchased from the Dade Reagents, Inc., Miami, Florida. Enza-trol purchased from the same company was used as a control for these procedures. Serum alkaline phosphatase levels were determined by a modified Bodansky method (18). A Bausch and Lomb "Spectronic 20" colorimeter was used in all these studies.

Tissue Collection and Preparation

All animals were examined by necropsy within 2-3 hours after death and most were examined within an hour. Four goats were killed *in extremis* by intravenous injection of a concentrated solution of pentobarbital sodium. Routine necropsy procedures were used and representative

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* International Equipment Co., Boston, Mass.

tissue samples were taken from all organs and systems. The tissues were fixed in isotonic neutral ten percent formalin.

Representative portions of various skeletal muscles in the body were removed, wrapped in gauze and labeled as to their origin. This label was maintained throughout the embedding and sectioning procedures.

Multiple areas of the nervous system were examined. These included a transverse section through the cerebrum and brain stem at the level of the thalamus and the mammilary body; transverse section through the cerebellum at the level of the middle peduncle to also include the brachium pontis and pons; medulla oblongata approximately one-half inch posterior to the cerebellum; transverse sections of the spinal cord at the levels of C_4, T_{12} and L_3; and longitudinal sections of the sciatic nerves and brachial plexuses. Portions of intercostal muscles were fixed in a solution of equal parts of filtered lemon juice and distilled water prior to staining for nerve endings (38).

All tissues were embedded in paraffin and sectioned at 6 microns. They were then stained with hemotoxylin and eosin for routine examination. Cardiac muscle was also frozen, sectioned in a cryostat and stained for fat by oil red O (19).

The portions of the nervous tissue described above were also stained by several additional methods. A modified Glees' silver impregnation method (21) was used to demonstrate various phases of axonal and preterminal degeneration. Luxol fast blue was used to stain myelin (13). Nucleic acids of neurons were stained by a modified galocyanin method (28). Nerve endings in intercostal muscles were stained with gold chloride after fixation in lemon juice (38).

RESULTS

Clinical Course and Signs

The period of time between initial ingestion of the plant material and the occurrence of clinical signs and death varied and depended on the dose of the fruits fed and apparently on the resistance of individual animals. The goats which received large daily doses (Groups A, B, and D) showed the first clinical signs in 4 to 5 days and died 2 to 3 days later. The animals receiving the lowest doses (Groups C and E) showed some variation in the time between initial dosing and occurrence of signs and death. One goat (25911 of Group E) which was fed at the rate of 0.025 percent of body weight developed clinical signs on the 7th day, whereas another of the same group (25993) showed the first sign of the disease after 21 days. The latter animal had the longest period between treatment and occurrence of clinical signs. The time of occurrence of clinical signs was about the same for those animals receiving 0.025 percent and 0.075 percent. Goat No. 25993 was an exception as explained above. The most obvious difference in the treated groups was the length of time the animals lived following the occurrence of clinical signs. Those goats in the lower dosage groups (C and E) lived longer than those fed higher dosages. One animal in Group E (25993) was an exception since she died two days after signs occurred. These data are summarized in Table II.

In all cases the disease was first manifested by drooping of the ears, slight hyperresponsiveness to sound, occasional leg "buckling" when weight was placed on them and slight muscular tremors and

incoordination. More severe incoordination of movements then occurred, especially when the animal attempted to walk a certain path or step over low objects. "Knuckling" of the fetlocks often occurred when the goats were forced to move rapidly. In some there was a crossing of the hind legs when the animals attempted to run. The gait progressively became more "stilted" with the legs adducted to maintain balance. Late in the clinical course of the disease the legs were moved forward in a jerky fashion without the usual flexing of some of the joints. Late in the disease the goats would stand with their legs wide, with muscular tremors and occasionally one limb would tend to collapse or relax. The latter seemed to occur if the leg was flexed to any degree. The apparent weakness became progressively worse until the animal could no longer stand. The muscles never appeared to be hard or rigid, but during the course of the disease they became flabby and seemed to have little tone.

After the goats became recumbent they would remain on their sternum, eat, drink and ruminate until respiratory distress occurred. They continued to be alert and would attempt to move to feed when called. Although they lost the complete use of their legs, the animals did not lose reflex responses. This was evidenced by the animal's ability to jerk the legs away when pricked with a pin. The animals appeared to have difficulty closing their eyes, especially late in the course of the disease. Respiratory distress invariably appeared with the progressive deterioration of the animal. The sign first noticed would be a watery discharge from the nostrils accompanied by slight dyspnea. Dyspnea accompanied by rales appeared when the animals were recumbent.

Sometimes the oral cavity would be filled with froth which appeared to come from the trachea. Respiration progressively became more difficult until death occurred.

Gross Pathology

No gross lesions were found in the brains of any of these animals. The sciatic nerves of the poisoned goats in Group C fed 0.025 percent of body weight appeared slightly edematous but the changes were questionable.

The skeletal and cardiac muscles did not have uniformly detectable gross changes. One goat in Group A (24322) and one in Group D (25637) had bilaterally involved supraspinus muscles which were mottled in appearance due to scattered portions of muscle which were paler than normal, soft and slightly gelatinous in appearance. The remainder of the muscle appeared normal. One goat (25525) in Group C fed 0.075 percent of body weight had similar but unilateral lesions in the left gastrocnemius muscle. No other muscle of these or other goats had evident gross lesions, except that most of the muscles of the limbs were slightly pale and flabby.

Hearts of none of the goats showed any appreciable gross change. The livers appeared light brown to light yellow in color with a slightly accentuated lobular architecture. This was especially evident in the treated goats of Group C which survived for 12 and 18 days. The liver parenchyma did not appear swollen.

In the lungs of all the animals there was generalized congestion and edema but the lesions were more pronounced in apical lobes.

There were scattered areas of emphysema, also. The tracheas contained varying amounts of clear to slightly red froth and fluid exuded from the cut surface of the pulmonary parenchyma.

There were no gross lesions in any of the other organs.

Histopathology

Heart. The lesions in the hearts of the goats fed 0.075 and 0.15 percent of body weight of coyotillo fruits varied somewhat in appearance. Some of the animals had fatty degeneration involving all of the cardiac fibers (Figure 1 and 2). These fibers had a diffuse granular appearance throughout which tended to obscure both cross and longitudinal striations.

Other hearts had irregular focal granular areas. Some of the fibers in these areas were also necrotic as shown by more eosinophilic staining cytoplasm which often was fragmented and had pyknotic muscle nuclei (Figure 3). The control goats had none of the above described changes.

The microscopic lesions in groups C and E fed 0.025 percent body weight were disseminated, small focal areas of sarcolemmal proliferation in the form of an irregular loose collection of cells. The nuclei of the latter often were larger and more plump than normal with slightly folded nuclear membranes and a more than normal vesicular appearance. In some instances the sarcolemmal tube persisted with complete loss of sarcoplasm or a small amount of eosinophilic debris. In other instances the sarcoplasm persisted but had a granular appearance. In no instance were the lesions very extensive (Figure 4).

Skeletal Muscles. All the poisoned animals in this study had lesions in the skeletal muscles although the amount and degree of

involvement varied both within a given group as well as between groups.

The skeletal muscle lesions in the group fed 0.15 percent body weight (Group A) were not extensive except for the one animal (24322) allowed to exercise at will. The lesions in the latter were as severe as those described for the other high dosage groups. The two non-exercised goats in this group had involvement of a part of a single or several muscle fibers with a random distribution in a given muscle. In this group of animals as well as the others there was never complete involvement of a single fiber. The changes consisted either of a fiber or fibers with a granular appearance and proliferation and migration of some of the sarcolemmal nuclei to the center of the fiber or proliferation of sarcolemmal nuclei with the latter randomly distributed in sarcoplasm which was fragmented.

In the other high dosage groups (B and D), the distribution of lesions was generalized as in group A and included the ocular muscles, diaphragm, and the other skeletal muscles. The pattern was not uniform with occasional groups of involved fibers interspersed between which were normal appearing fibers. Again, the entire length of the fibers was not involved. The changes were characterized by the development of an eosinophilic hyaline or granular appearance and loss of both longitudinal and cross striations. Many fibers were fragmented with the formation of retraction caps, clots or curdy masses of muscle protoplasm with interspersed empty clefts (Figures 5 and 6). In the more severely affected fibers, the sarcolemmal nuclei appeared to be necrotic as evidenced by pyknosis and their folded, distorted appearance. In the less severely affected fibers or portions of fibers the sarcolemmal nuclei persisted

and became rounded, vesicular, began proliferating, and some moved to the centers of the fibers.

In the lower dosage groups (C and E) the predominant change in the skeletal muscle was that of activation of the sarcolemmal nuclei, but fibers were present which were hyaline, granular or fragmented. The involved areas, which were randomly distributed in the muscles, varied in their severity. Occasional fibers had vacuolar degeneration. Others were fragmented with marked proliferation of sarcolemmal nuclei at the extremity of the normal portion of the fiber. Other fibers had many proliferated sarcolemmal nuclei within the sarcolemmal tube. In the more severely affected portions of muscle, especially where many fibers were involved, there was a proliferation of sarcolemmal nuclei, macrophages and scattered lymphocytes (Figure 7). These cells often formed irregular sheets or bands of cells between normal and/or regenerating fibers. The latter appeared as narrow fibers with numerous elongated, rather vesicular sarcolemmal nuclei in a thin column of sarcoplasm which often appeared slightly basophilic. There was no evidence of fibrous connective tissue replacement. In severely affected fibers removal of muscle debris by macrophages was seen. There were a few mitotic figures at the center of some fibers, but whether these were of sarcolemmal cells or of macrophages could not be determined.

Nervous System. It was not possible to demonstrate any changes in the nervous tissue which could be attributed to coyotillo in the poisoned goats when they were compared to the controls. No interpretations could be made on changes in the neuromuscular junctions or nerve end plates because of failures in making satisfactory preparation by the techniques

used.

Liver. The hepatic changes essentially the same in all poisoned goats although the degree of involvement varied. The lesions were more severe in groups A, B, and D but again differed from groups C and E only in degree. There was mild fatty degeneration accompanied by necrosis of a small number of hepatic cells centrally and mild congestion. The cytoplasm of the latter cells was eosinophilic with karyorrhexis and pyknosis of nuclei. The necrotic cells were scattered in the central portion of the lobule with no detectable relationship to one another or to any other anatomic structure.

Lungs. The lesions in the pulmonary system varied between groups fed different coyotillo levels. The lesions in the high dosage groups (A, B, and D) consisted primarily of congestion and edema with occasional small areas of emphysema. The lesions in the groups fed lower doses of the plant (C and E) had other changes present. Those in group E had purulent bronchopneumonia superimposed on the congestion and edema. This was particularly severe in animal 25911. The poisoned goats in group C had a different type of change. The vessels were widely dilated and the endothelium was hyperplastic. There was an adenomatoid appearance to the parenchyma due to proliferation of the lining cells along the alveolar walls. The two poisoned goats in this group were the only animals with this type of lesion.

Kidneys. The kidneys of the poisoned goats in group D were the only ones which had detectable lesions. These kidneys from these animals had marked cytoplasmic vacuolation more severe in the proximal tubules but involving the loops of Henle to a lesser extent. No evidence of other

changes were present.

Serum Enzymes and Blood Values

The final SGOT levels of the poisoned goats were elevated when compared to the pre-treatment levels and to the levels found in the control animals. The degree of elevation in the animals fed 0.075 percent of body weight (groups B and D) was about ten times the pre-treatment levels. The animals receiving less coyotillo (groups C and E), however, did not have this magnitude of elevation. The SGOT elevation in these two groups was approximately 4 times the pre-treatment levels. These values are given in Table VI.

The initial rise in SGOT levels is compared in Figures 8 and 9 and Table II to the initial occurrence of clinical signs. It can be seen from these figures that the goats fed 0.075 percent of body weight (Figure 8 and Table II) had initial SGOT elevations one to 2 days before clinical signs occurred. The initial SGOT elevation in the lower dosage groups coincided, however, with the initial observation of clinical signs or one to 2 days afterward (Figure 9 and Table II).

In all the poisoned goats the SGOT level continued to rise until death occurred or the animals were killed *in extremis*. The rise in SGOT was extended over a longer period of time in the low dosage groups as compared to the high dosage groups (Figures 8 and 9).

The final SGPT values for all the poisoned goats were slightly elevated (Table VI). There was no significant difference between the animals fed the different dosage levels. The above is consistent with the histopathological observation of minimal hepatocellular damage in all

poisoned goats.

The alkaline phosphatase values were consistently lower in the treated animals although the degree of decrease was not marked (Table VI).

The pre-treatment range and final values for the packed cell volume and hemoglobin are summarized in Table III. There were no changes present which were considered to be of significance.

The white blood cell counts were not significantly altered except in one animal (25911) in which there was a marked neutrophilia (Tables III and V). This animal also had a purulent bronchopneumonia.

None of the experimental or control goats showed any change in the level of total serum protein (Table IV).

There was an elevation of the BUN in the two poisoned animals in group C (Table IV). These two animals were also the only ones with renal damage as stated previously.

DISCUSSION

The clinical signs and course of experimental coyotillo poisoning in the goat suggest a syndrome of progressive muscular weakness, both skeletal and cardiac. Although the animals did not lose their ability to move their legs, there seemed to be insufficient strength to support their body weight so that every animal poisoned finally was unable to stand. The cardiac asthenia was more apparent as a terminal event since all of the goats developed respiratory distress. The respiratory distress could have been due to cardiac involvement resulting in pulmonary congestion and edema and also due to degenerative changes in the muscles of the diaphragm.

The occurrence of clinical signs closely parallel the initial rise in SGOT. The continued rise of SGOT until death occurred, although further ingestion of the plant material ceased, suggests continuing degenerative muscular changes. Exercise may have influenced the continued SGOT rise to a certain degree. The animals were exercised (except group A) daily until the goats could no longer stand. The relationship between initial SGOT rise, occurrence of clinical signs, and lack of nervous depression as evidenced by the persistence of the reflex arc, the general alertness and only slightly altered appetite of the poisoned goats suggests that the primary cause of symptomatology and death in coyotillo poisoning is a generalized myodegeneration. The above is further substantiated by the lack of lesions in the central and peripheral nervous system. It is realized that the techniques used in the evaluation of the nervous system were limited and that all

portions of the brain were not examined. A more exhaustive search of the nervous system by different techniques might reveal changes.

The lesion in the cardiac muscle appeared to be more generalized than those of the skeletal muscle. The granular appearance of the myocardial fibers was often generalized with poorly delineated areas of more severe damage. The generalized cardiac involvement may be accentuated due to the continued work load placed on this organ.

The skeletal muscle lesions were less generalized than those of the heart but were more severe in their nature. The involved fibers were not completely affected and more normal appearing portions were interspersed between degenerated portions of the same fiber.

Exercise seemed to play an important role in determining the severity of the skeletal muscle lesions. This is indicated by the following observations. The skeletal lesions in group A except for animal 24322 were less severe than those in groups B and D although all had degenerative changes. The latter two groups were exercised while the former group was not. Moreover, animal 24322 of group A which was allowed to exercise developed more severe lesions than the non-exercised animals in the same group.

The histologic lesions in the skeletal muscles of coyotillo poisoned goats resemble those described for plasmocid (1) and for white muscle disease (12) and differ from those described for denervation (1).

Plasmocid, a protoplasmic toxin, apparently has an affinity for striated muscle. It produces granular and fragmented degeneration of a focal nature in skeletal and cardiac muscle (9).

Similar histologic changes occur in white muscle disease (12) and

coyotillo but the two seem to differ in several points. Large areas of muscle are often involved in white muscle disease and this is usually accompanied by calcification. The result is gross evidence of discoloration of the involved portion of muscle. Calcification did not occur in coyotillo poisoning. In addition, the areas of involvement in coyotillo were not as large as in white muscle disease. It appears also that the cardiac lesions in white muscle disease are more severe than coyotillo and that calcification occurs in the heart also in the former but not the latter.

The initial change in denervation is in the sarcolemmal nuclei followed later by decrease in fiber size. The nuclei become rounded and move toward the center of the fibers (1). There is none of the severe necrotizing myodegeneration as seen in coyotillo poisoning.

The final SGPT levels of the control goats ranged from 20 to 33 units. The SGPT level increased to a certain extent in all animals poisoned with coyotillo, the range being 34 to 94 units (Table VI). All affected animals exhibited a certain degree of liver damage but the damage was not extensive which probably explains these increases in serum enzyme activity.

In all animals poisoned with coyotillo a decrease in alkaline phosphatase levels was found. In some cases (Table VI) the final values decreased to less than half of the pre-treatment value. The cause of reduced levels is unknown but substances in coyotillo may depress osteoblastic activity.

The cause of death in coyotillo poisoning probably results from the interaction of several factors. There is an omnipresent toxic myocarditis which would contribute to the development of pulmonary congestion and

diaphragm also would decrease respiratory efficiency. As a result there is decreased pulmonary function evidenced by dyspnea, rales and other clinical manifestations. The changes in the respiratory tree offer a fertile field for bacterial growth and secondary pneumonia. Anoxia develops and would tend to become more severe as the changes progressed. All of these factors probably contribute to the ultimate death of the animals.

CONCLUSION

The absence of nervous lesions and presence of skeletal muscular and myocardial degenerations accompanied with increases in SGOT levels suggest that coyotillo fruits contains a substance that has an almost specific affinity for striated muscle. Because of its specific action on muscles and rapidity of its action, the effects could probably be ascribed to a selective interference with the metabolism of the muscle fiber, such as the blockage of muscle enzyme systems.

These interferences in the vital systems of muscle metabolism may well be the only dysfunction resulting in so-called "limber leg" in sheep and goats. A more thorough investigation of the actions of coyotillo in muscles may provide a means of obtaining valuable information about the metabolic function of the muscle fiber as well as define the exact mechanisms in coyotillo poisoning.

Toxic myocarditis and the degeneration of diaphragmatic musculature were thought to be the cause of the pulmonary congestion and edema resulting in eventual death.

TABLE I

THE EXPERIMENTAL GROUPS, ANIMAL NUMBERS AND DOSAGE OF GROUND COYOTILLO FRUITS IN THE TOXICITY STUDIES

GROUP NO.	ANIMAL NO.	DOSAGE (% BODY WT)	BREED	SEX	AGE (YEARS)
A	24322	0.15	Spanish	Male	2
	24563	0.15	Spanish	Male	2
	24584	0.15	Angora	Male	2
	N-1	Control	Spanish	Male	2
B	25162	0.075	Spanish	Female	2
	25175	0.075	Spanish	Female	2
	25184	Control	Spanish	Female	2
C	25483	0.025	Angora	Female	3
	25525	0.025	Spanish	Female	3
	25618	Control	Spanish	Male	3
D	25637	0.075	Angora	Male	1½
	25628	0.075	Spanish	Female	1½
	25641	Control	Angora	Female	1½
E	25911	0.025	Spanish	Female	4
	25993	0.025	Spanish	Female	4
	26074	Control	Spanish	Female	4

TABLE II

A SUMMARY OF THE AMOUNT OF COYOTILLO FED, TIME OF APPEARANCE OF CLINICAL SIGNS AND DEATH AND THE TIME OF OCCURRENCE OF SGOT ELEVATIONS

Goat No.	Weight (lbs)	Daily dosage (% body wt)	Total amount of coyotillo fed (gms)	Total amount of body wt (percent)	Days after initial feeding first sign appeared	Days after initial feeding SGOT first increased	Days after initial feeding animal died or sacrificed
24322	64	.15	173.2	.59	4	---	5
24563	64	.15	130.5	.45	3	---	4
24584	62	.15	168.0	.50	5	---	7
N-1	65	Control	---	---	---	---	---
25162	81	.075	186.0	.50	6	5	7
25175	64	.075	126.0	.43	6	5	8
25184	67	Control	---	---	---	---	---
25483	61	.025	54.0	.17	7	7	12
25525	67	.025	57.0	.18	7	9	18
25618	65	Control	---	---	---	---	---
25637	47	.075	86.0	.40	5	5	7
25628	47	.075	86.0	.40	5	4	6
25641	53	Control	---	---	---	---	---
25911	75	.025	51.0	.15	7	8	14
25993	92	.025	180.0	.43	20	21	23
26074	72	Control	---	---	---	---	---

TABLE III

THE PACKED CELL VOLUME. HEMOGLOBIN AND TOTAL LEUKOCYTE COUNTS OF GOATS POISONED WITH COYOTILLO FRUITS

Goat No.	Dosage (% body wt.)	PACKED CELL VOLUME Pre-treatment range	Final value	HEMOGLOBIN* Pre-treatment range	Final value	TOTAL LEUKOCYTE COUNT Pre-treatment range	Final value
25162	0.075	27-34	31	9.3-13.3	13.7	8,500-10,500	8,725
25175	0.075	28-32	31	9.6-11.7	15.0	10,000-11,500	9,600
25184	---	29-34	29	9.3-12.0	10.7	11,000-12,600	9,200
25483	0.025	20-25	26	7.3-07.8	7.6	6,750-08,500	7,300
25525	0.025	28-29	28	11.3-11.7	11.6	9,550-10,750	13,200
25518	---	18-20	20	5.6-05.8	5.7	6,000-08,200	8,100
25637	0.075	38-40	35	13.8-15.3	13.9	12,250-17,200	11,600
25628	0.075	40-42	32	12.6-14.1	12.9	15,600-19,300	18,000
25641	---	37-41	37	12.8-15.7	12.9	12,100-14,200	14,200
25911	0.025	30-34	31	12.0-12.5	12.0	16,200-17,020	35,775
25995	0.025	30-34	35	10.7-12.0	12.2	6,250-07,500	16,600
26074	---	34-35	35	12.9-13.0	12.2	11,500-15,200	14,650

* gms/100 ml.

TABLE IV

THE TOTAL PROTEIN AND BLOOD UREA NITROGEN VALUES IN GOATS POISONED WITH COYOTILLO FRUITS

Goat No.	TOTAL PROTEIN		B. U. N.	
	Pre-treatment range (gm %)	Final value (gm %)	Pre-treatment range (mg %)	Final value (mg %)
24322	6.0-8.0	8.0	15.8-24.2	18.0
24563	6.9-8.6	7.6	10.8-20.8	21.6
24584	5.0-7.1	6.0	12.0-24.0	12.8
N-1	6.9-8.3	7.9	12.0-17.0	14.6
25162	7.1.7.6	6.9	14.0-19.0	18.5
25175	4.6-4.8	6.2	15.2-20.0	19.8
25184	5.0-5.8	5.0	15.0-19.5	20.0
25483	6.4-8.2	8.3	12.6-29.0	13.8
25525	5.8-6.8	7.1	16.8-20.0	18.0
25618	6.4-7.5	8.3	17.6-26.2	28.0
25637	5.8-8.2	8.1	18.0-22.8	40.5
25628	5.6-8.9	6.9	20.0-22.8	40.5
25641	5.9-8.8	7.5	22.8-30.9	22.2
25911	5.8-8.0	7.6	16.0-18.8	18.9
25993	5.0-8.6	7.0	17.5-25.0	26.8
26074	6.0-8.0	7.5	25.0-28.0	28.6

TABLE V

DIFFERENTIAL LEUKOCYTE COUNTS BEFORE AND AFTER FEEDING COYOTILLO FRUITS TO GOATS

Goat No.	Basophils	Eosinophils	Neutrophils stab	Neutrophils segmented	Lymphocytes	Monocytes
25162	0-2*	3-5	1-3	32-38	51-62	1-4
	0-1**	0-2	2-4	48-61	35-49	2-3
25175	0-1	1-5	1-2	33-44	57-64	0-2
	---	0-6	3	38-69	26.55	0-1
25184	0-1	1-5	0-2	37-44	46-52	1-2
	---	0-2	---	43-49	38-55	1-4
25483	1-4	9-11	0-1	26-43	43-59	0-3
	0-1	0-1	2-5	41-48	48-49	0-1
25525	---	2-5	2	41-48	47-56	1-4
	---	---	4-5	61-70	26-31	0-3
25618	0-1	10-14	1-2	48-56	31-40	0-1
	0-1	10-13	3	47-50	36-40	1-2
25637	0-1	0-5	---	24-36	56-75	0-2
	0-1	0-1	3	40-45	55-60	---
25628	1-2	5-8	0-1	31-40	47-64	0-2
	0-1	0-2	3-5	45-50	50-55	---
25641	0-1	1-4	0-1	41-46	52-55	0-1
	0-1	0-4	---	42-51	46-52	1-3
25911	0-1	3-7	0-1	54-69	29-40	1-2
	---	---	5-6	72-84	15-28	0-1

TABLE V - Continued

Goat No.	Basophils	Eosinophils	Neutrophils stab	Neutrophils segmented	Lymphocytes	Monocytes
25995	0-1	2-6	1-2	53-63	29-46	0-1
	---	---	4-6	83-93	7-17	0-2
26074	0-1	4-6	---	44-48	46-48	1-3
	---	1-3	0-1	37-48	50-63	0-1

*The range of three average values determined from three daily blood samples, collected before the animal was poisoned.

**The range of three counts determined from one blood sample collected just before animal was dead or sacrificed.

TABLE VI

THE PRE-TREATMENT RANGE AND FINAL SGOT, SGPT AND ALKALINE PHOSPHATASE VALUES IN GOATS POISONED WITH COYOTILLO

Goat No.	Dosage (% body wt)	S. G. O. T.* Pre-treatment range	S. G. O. T.* Final value	S. G. P. T.** Pre-treatment range	S. G. P. T.** Final value	ALKALINE PHOSPHATASE*** Pre-treatment range	ALKALINE PHOSPHATASE*** Final value
25162	0.075	120-132	2,000	22-31	40	3.4-3.8	1.3
25175	0.075	132-144	1,440	22-24	34	3.2-3.7	1.1
25184	---	120-144	150	18-28	28	3.2-4.7	5.0
25483	0.025	130-144	440	22-36	50	4.2-5.3	2.7
25525	0.025	144-164	650	19-28	94	4.1-5.3	1.5
25618	---	108-144	130	19-24	24	2.2-3.7	2.0
25628	0.075	128-130	1,620	30-33	86	4.2-4.3	2.6
25637	0.075	130-140	1,300	24-33	76	3.9-4.5	2.6
25641	---	102-140	122	30-43	33	4.8-5.0	4.9
25911	0.025	130-144	500	16-26	50	4.3-5.0	3.7
25993	0.025	122-140	450	24-30	45	4.1-5.0	2.0
26074	---	112-120	112	24-26	20	2.0-3.0	2.4

*Sigma Frankel (S.F.) units **Sigma Frankel units *** Bondansky unit

Figure 1 Left ventricular myocardium of a goat fed 0.075 percent body weight of coyotillo fruits, showing numerous dark fat globules. Oil Red O X 850.

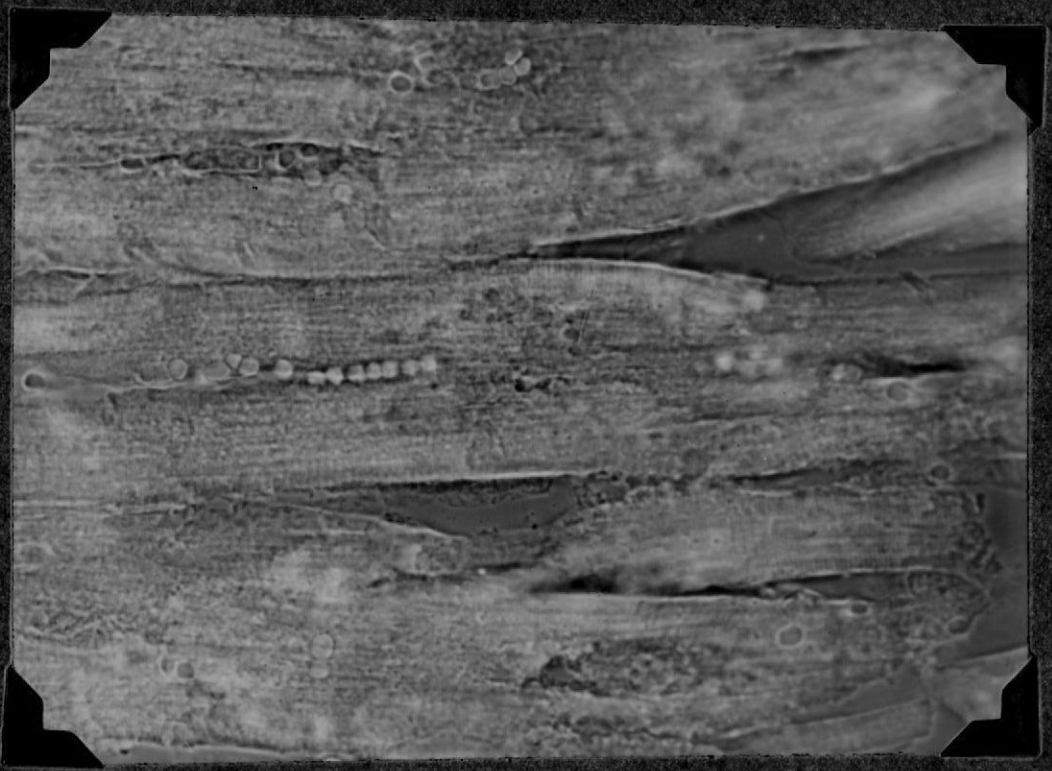

Figure 2 Left ventricular myocardium of a control goat. Oil Red O X 850.

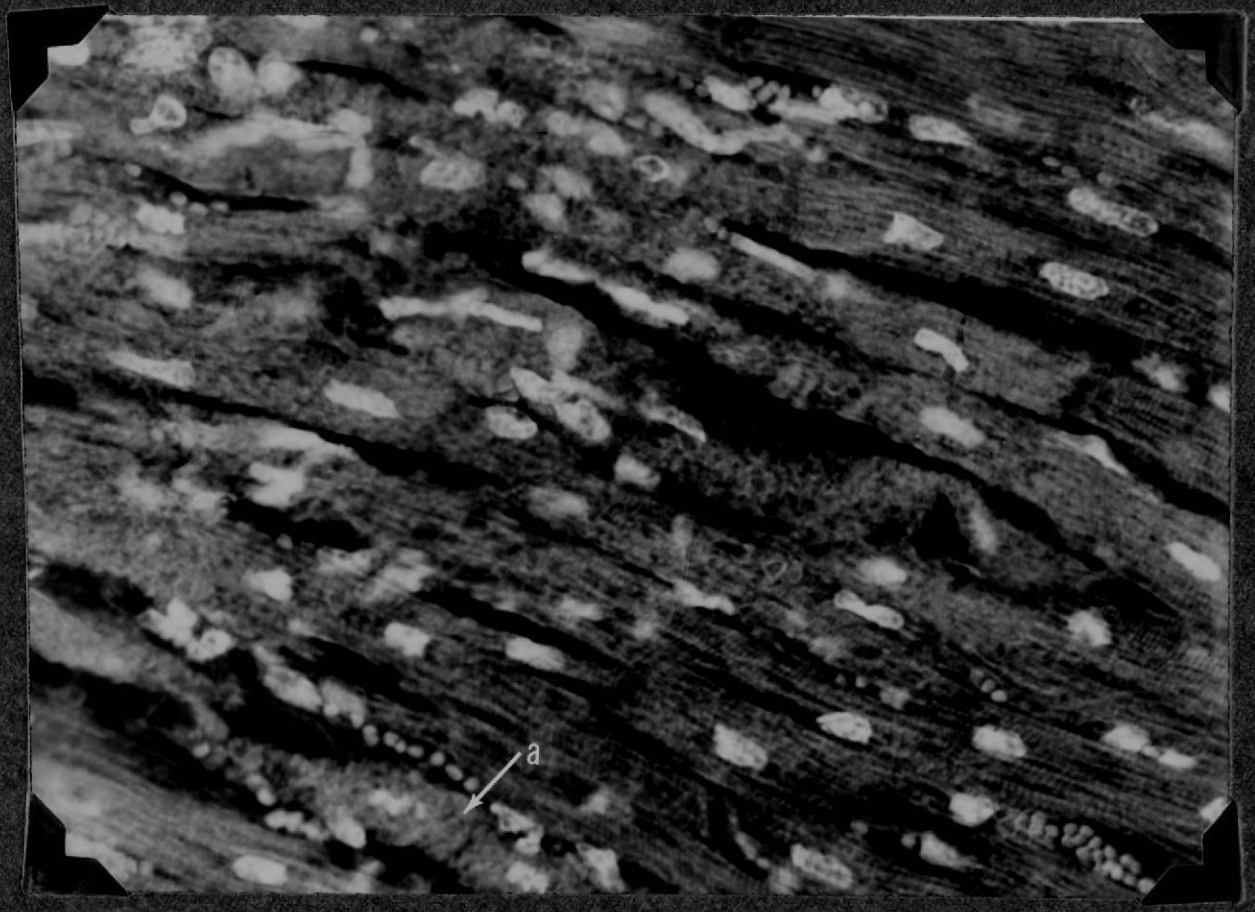

Figure 3. Myocardium of the left ventricle of a goat fed 0.075 percent of body weight of coyotillo fruits showing granulo-fatty degeneration. a. Degenerating muscle fiber and nuclei. H&E X 800.

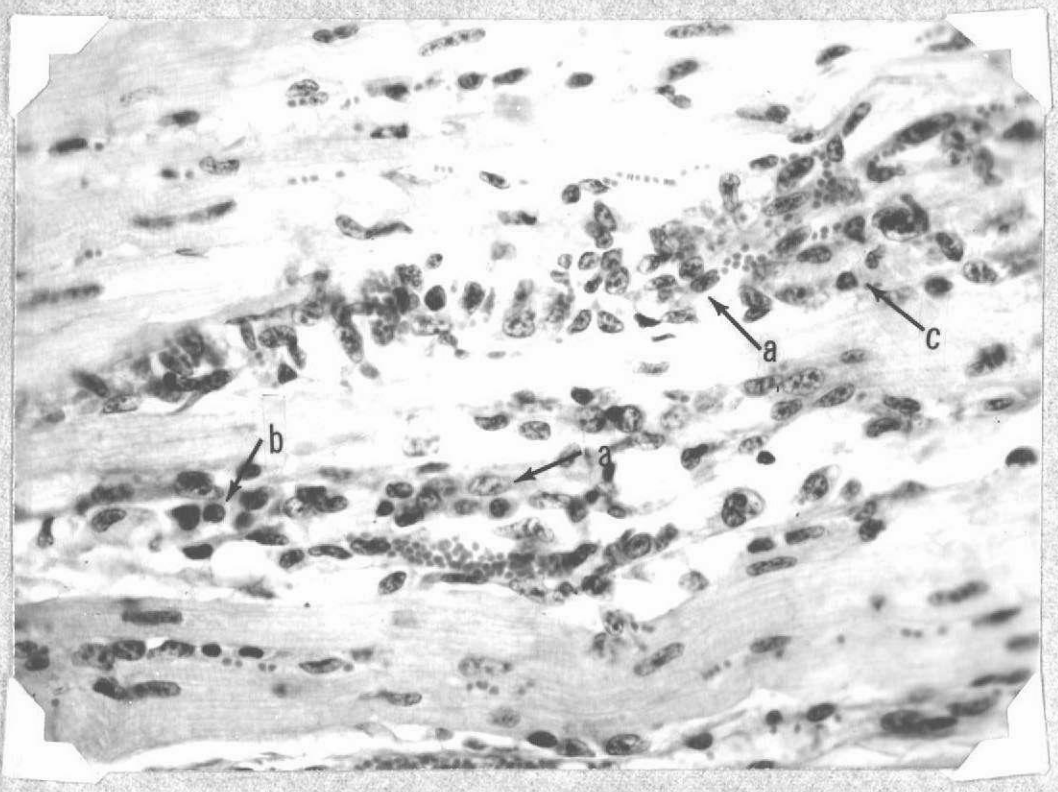

Figure 4. Left ventricular myocardium of a goat fed 0.025 percent body weight of coyotillo fruits, showing loss of fibers and increased sarcolemmal activity. a. Proliferation of sarcolemmal nuclei. b. Histocytes. c. Degenerating fiber. H&E X 600.

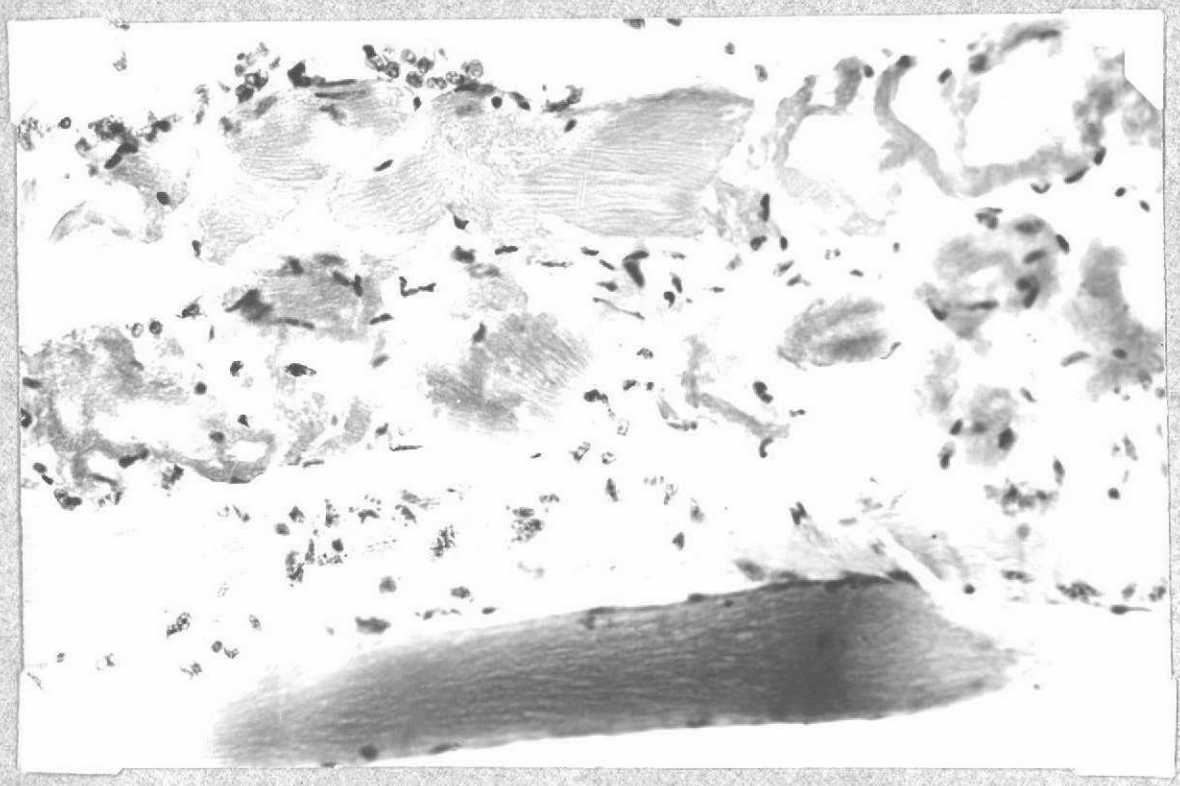

Figure 5. Supraspinatus muscle of a goat fed 0.15 percent body weight of coyotillo fruits, showing necrosis and fragmentations of muscle fibers and an eosinophilic fiber. H&E X 375.

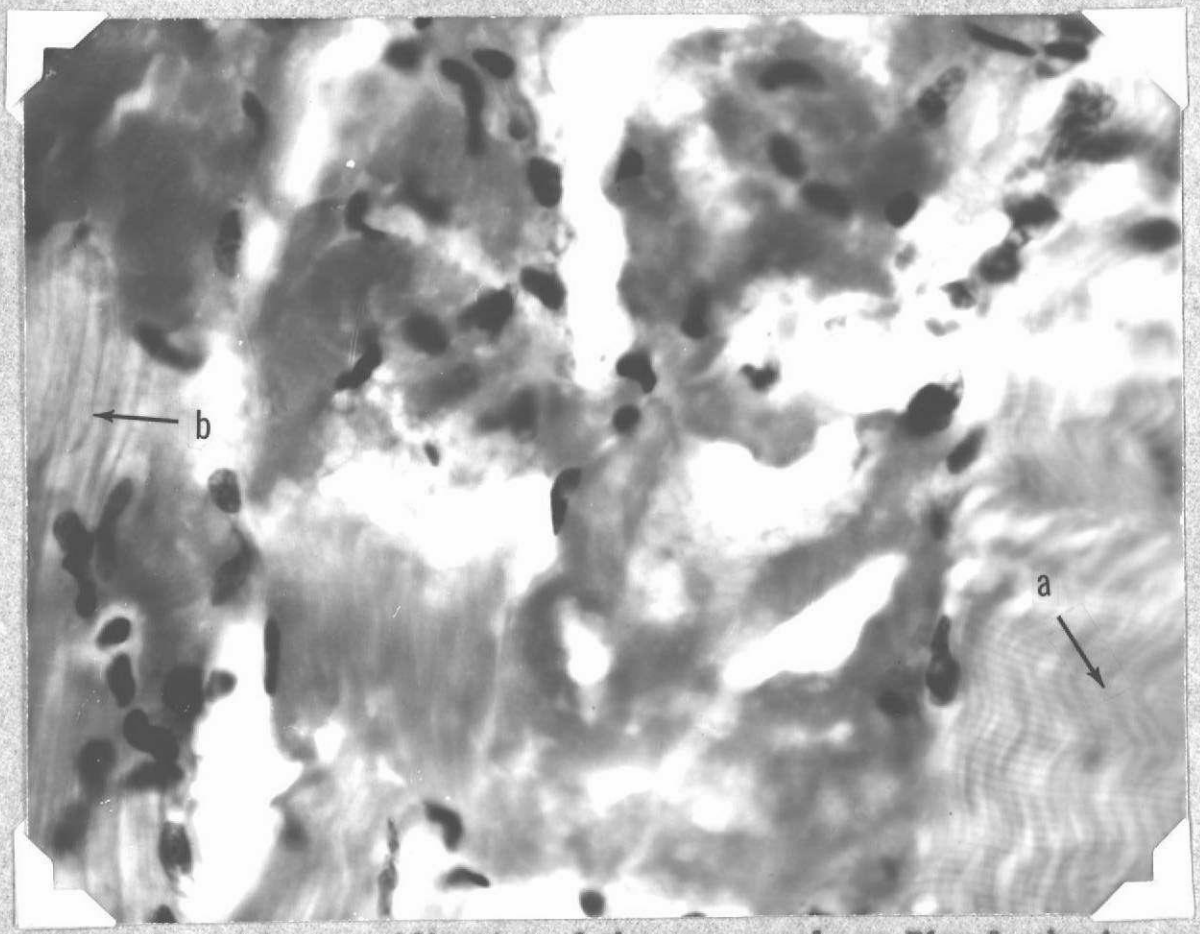

Figure 6. Higher magnification of the same muscle as Fig. 5, showing necrosis of a portion of muscle fibers. a. Normal appearing adjacent fiber. b. Less severely involved portion of a fiber. H&E X 700.

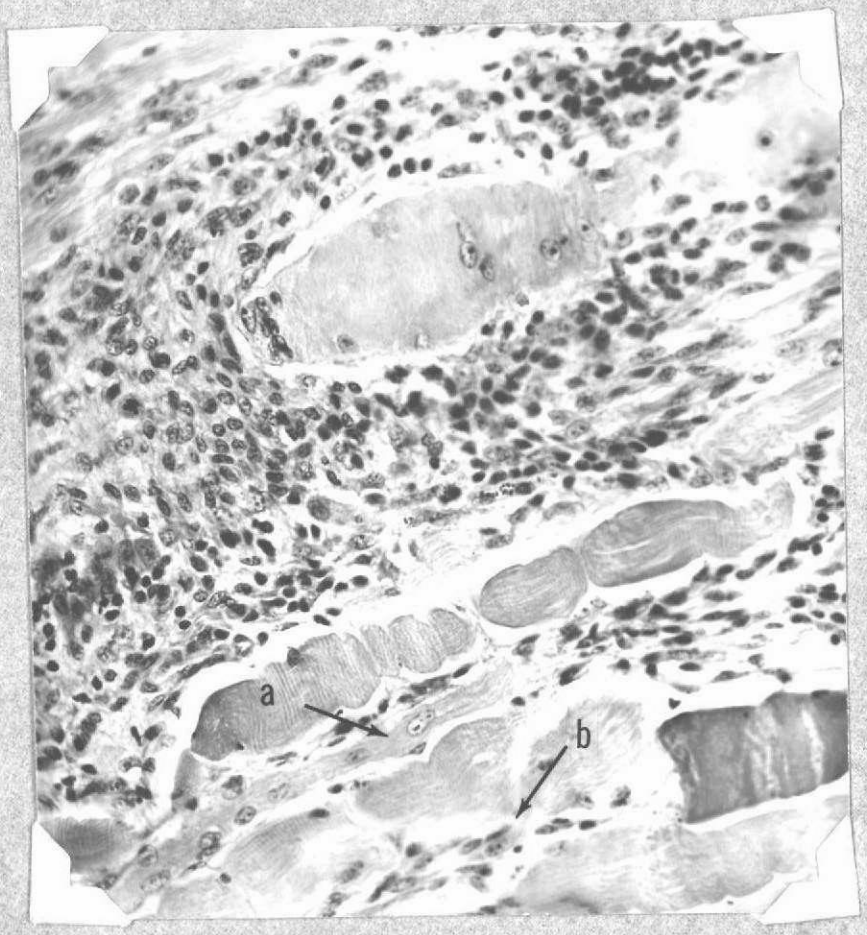

Figure 7. Gastrocnemius muscle of a goat fed 0.025 percent body weight of coyotillo fruits, showing marked sarcolemmal, histocytic and lymphocytic proliferation and remaining viable portion of muscle fibers. a. Small possibly regenerating fiber with prominent sarcolemmal nuclei. b. Proliferating spindle cells. H&E X 300.

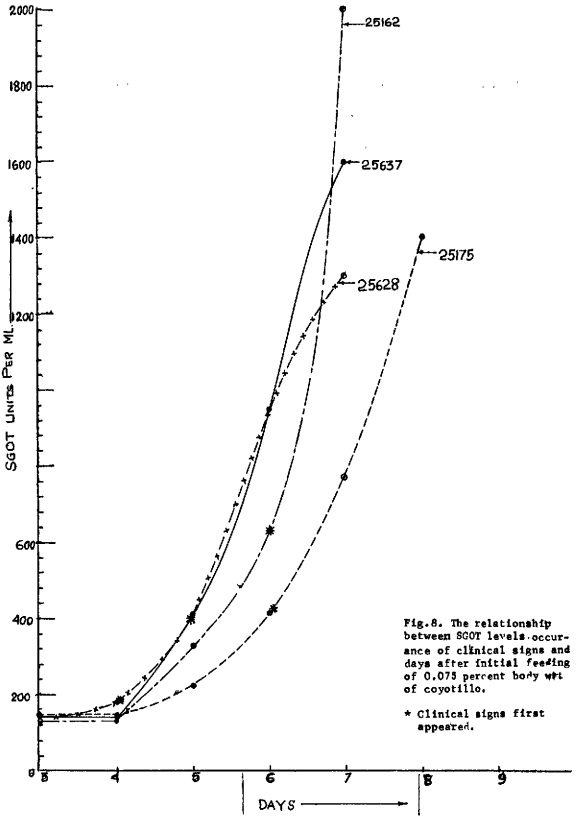

Fig. 8. The relationship between SGOT levels occurance of clinical signs and days after initial feeding of 0.075 percent body wt of coyotillo.

* Clinical signs first appeared.

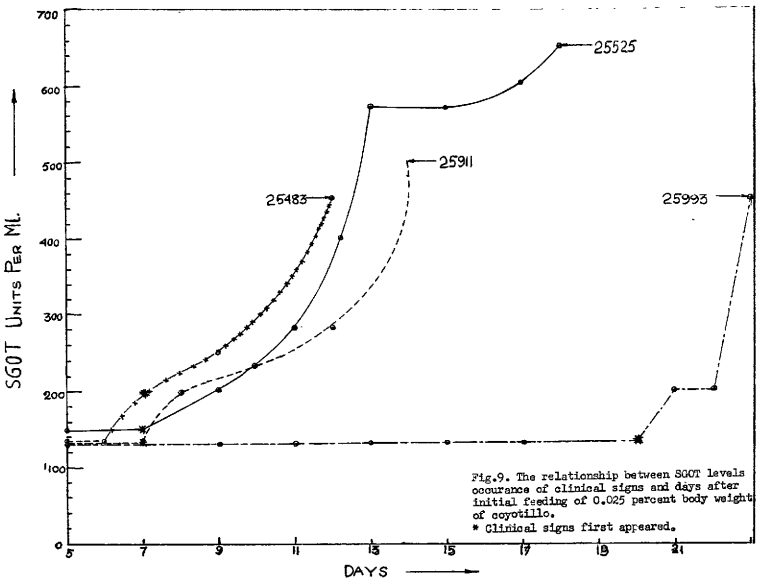

Fig.9. The relationship between SGOT levels occurance of clinical signs and days after initial feeding of 0.025 percent body weight of coyotillo.
* Clinical signs first appeared.

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