BLISTER BEETLE (*EPICAUTA* SP.) POISONING IN HORSES

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IN HORSES

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PREFACE

This study is a review of natural and experimental blister beetle poisoning in 26 horses. Clinical, laboratory, and gross pathologic findings were compiled from case records; tissue sections from 14 post-mortem cases were examined for microscopic changes. The objective is to provide a descriptive account of pathologic features in relation to clinical signs and laboratory data.

Problems were encountered, as would be expected in a retrospective study. Some materials were more than ten years old, and many of the tissue sections were faded. Autolysis made many tissues useless, or made examination for subtle changes futile. Slides and embedded tissues from two cases had been lost. Inconsistent recording of data from case to case was probably the most significant difficulty encountered. Several questions concerning pathogenesis and laboratory findings were raised, which await future studies.

I am indebted to Dr. Roger Panciera, my major adviser, for assistance in many aspects of this study and for access to his material from experimentally poisoned horses. Dr. David Dodd provided valuable editorial suggestions, as did Dr. Talmage Brown. I am grateful to Dr. David McCarroll for help in locating certain case records and to Mr. Don C. Arnold for identification of blister beetles from some of the cases. Mrs. Linda Rogers deserves special thanks for typing the manuscript. Mrs. Katherine MacNeil procured copies of numerous references.

My wife, Sandra, and son, Zack, are offered apologies for my preoccupation during this study.

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CHAPTER I

INTRODUCTION

Poisoning of horses from ingestion of blister beetles was first reported in 1963 (25) and was recognized in Oklahoma in early 1964. At present, only striped blister beetles of the genus Epicauta have been known to cause equine toxicosis (7, 25, 30). The adult beetles feed on a number of crops, including potatoes and alfalfa (3), and it is thought that their habit of feeding in swarms and the modern method of harvesting alfalfa by simultaneously cutting and crimping lead to incorporation of beetles in baled alfalfa hay, which may then be fed to horses (30).

The hemolymph of beetles of the family Meloidae, the blister beetles, contains the poisonous substance cantharidin; defensive behavior of these beetles includes reflexive bleeding, usually from the limb joints (35). Applied topically, cantharidin causes acantholysis and formation of intraepidermal vesicles which appear within a few hours after contact with the beetle (9, 14, 15, 18-20, 22, 37-39). It is an extremely potent irritant, and is absorbed from the gastrointestinal tract and skin (38). The fatal oral dose in man can be as little as 10 mg (17, 38). Toxicosis can also result from cutaneous absorption (8, 11).

Cantharidin is the active component of cantharides, a preparation of the dried insects Cantharis (Lytta) vesicatoria (Spanish fly) (29). It is excreted by the kidney and is very irritating to the urogenital

tract, causing frequent and painful urination (38). Urethral irritation may cause priapism, which has resulted in the undeserved reputation of Spanish fly as an aphrodisiac (38). Although very dangerous, cantharidin was recommended as a sexual stimulant as recently as 1921 (43). The substance was once used in plasters as a counter-irritant in veterinary (11) and human medicine (38) and a method for treating warts with a weak solution of cantharidin was developed, but presently its only uses are experimental (9, 18).

Cantharidin is hexa-hydro-3a,7a-dimethyl-4,7-epoxyisobenzofuran-1, 3-dione, an anhydride of cantharidic acid (29, 36). It is a very stable (37) white crystalline substance only slightly soluble in water (29, 38) but soluble in acetone, ethyl acetate, chloroform, and oils, and less so in ether (36). Information regarding the concentration of cantharidin in Epicauta beetles is not available; however, cantharides contains not less than 0.6% cantharidin (36). Perhaps Epicauta beetles contain higher concentrations, since experience of some (7, 30) indicates that the fatal oral dose of dried Epicauta beetles may be less than the 15 g supposedly required of cantharides (11). It is actually an assumption that cantharidin is the agent responsible for equine toxicosis, since experimental poisoning of horses with purified cantharidin has not been done, but it is a reasonable one because other toxic substances in blister beetles have not been reported, and in man similar disease results from ingestion of either crystalline cantharidin or the crude preparation. Hereafter, the term cantharidin is used to include any form of the dead insects.

Since equine poisoning by these beetles is not rare in Oklahoma, and few reports exist, a description of the disease is warranted.

CHAPTER II

LITERATURE REVIEW

Poisoning in Man

The reputation of cantharidin as an aphrodisiac has on several occasions induced ignorant persons to take it or give it to others, often resulting in fatal poisoning (13, 26, 27). In some cases, exposure was accidental (21, 23). Children have been poisoned by contact with (8), or ingestion of (41), live blister beetles. Cantharidin has also been taken as an abortifacient (42), out of misguided curiosity (1), and as a prank (33). One patient denied knowingly taking the substance and no source was discovered (10). Fatal poisoning has resulted from ingestion of less than 1 grain, 65 mg, of crystalline cantharidin (21, 26), but persons have survived doses of 75 and 175 mg (27). It apparently has no noticeable taste (1, 13).

Clinical signs of cantharidin poisoning in man, aside from shock, have reflected gastrointestinal and urogenital tract damage. Burning oral and throat pain, often very severe, or emesis followed ingestion of cantharidin by 10 to 30 minutes (13, 21, 26, 27). Vesicles or excoriations in the mouth and pharynx appeared in many but not all victims (13, 26, 27). Emesis was nearly constant and while the vomitus sometimes did not contain visible blood (1, 21, 23), vomition of dark blood or blood-stained mucus was frequent (13, 26, 27, 33, 41). Diarrhea occurred occasionally (13, 21, 33), as did cramping abdominal pain

(10, 27, 33). In cases not fatal within a few hours, there was often lumbar pain over the kidneys (1, 10, 21, 23, 27) and suprapubic pain (10, 27), indications of injury to the kidneys and urinary bladder. Gross hematuria was a constant finding regardless of the duration of illness (1, 8, 10, 13, 21, 23, 33, 41, 42). Difficult or painful urination (1, 8, 27, 33) and frequent voiding of small amounts of urine (1, 8, 33, 41) were common observations. Oliguria (10, 23) and anuria (13, 21), manifestations of either severe nephrosis or reduced blood pressure, or both, were sometimes seen. Clinical shock evidenced by decreased blood pressure and rapid, thready pulse was a manifestation of severe, often fatal, toxicosis (13, 21, 26, 27). In only one of the case reports reviewed was priapism recorded (23). Vesiculation of the genital skin was also a singular observation (33). The recorded duration of illness in fatal cases was 17 hours (26) to three days (21). Survivors were ill for one week (27) to three weeks (10).

The laboratory finding of hemoconcentration (13, 21, 27) reflects clinical shock. One report attributed increased blood hemoglobin and erythrocyte count to bone marrow stimulation by cantharidin (13), but did not give corroborating evidence; other authors believed that this was more likely the result of hemoconcentration (21, 27). Leukocytosis was a frequent finding (8, 13, 23, 33, 41); it was usually moderate but in one patient the leukocyte count reached 104,000/mm^3 (14). Neutrophils represented a large proportion of the cells in cases in which differentials were recorded (8, 13, 41). Several reports cite laboratory evidence of renal injury. A man who died of cantharidin poisoning after a three-day illness had a blood urea nitrogen (BUN) of 160 mg/dl (21). Evidence that this elevation may not be solely the result of poor

renal perfusion is a BUN of 137 mg/dl in a non-fatally poisoned man without clinical shock or hemoconcentration (10). Other surviving patients had mild elevations of 50 and 32 mg/dl (27) and 30 mg/dl (41). Urinalysis findings have included hematuria (1, 10, 27, 33, 41) and albuminuria (1, 8, 10, 27, 33, 41) but in no instance was it determined whether the amount of protein could be accounted for by hemorrhage. Urine specific gravity of 1.008 (8) and 1.010 (41) was recorded, but not measurements or estimates of urine output. Glucosuria was reported twice (27, 41). The glomerular filtration rate of two patients was decreased (27). One male patient received an intravenous pyelogram which showed in the bladder irregularities thought to be mucosal vesicles (33). The interpretation of cystoscopic findings in another patient's bladder was "cystitis" (10). Serum sodium, potassium, chloride and calcium were measured in three cases (27, 41); these were normal except that one patient was hypocalcemic for a short time, the serum calcium being 7.3 mg/dl on the third day of illness (27). This patient also had electrocardiographic changes suggestive of myocardial injury, which were thought to be the result of the direct action of cantharidin rather than electrolyte imbalance since they persisted for several days (27).

Descriptions of gross postmortem findings are not detailed. Two girls that died of acute poisoning had very little mucosa remaining in the mouth, pharynx, and esophagus (26). In one, the most severe damage was in the pyriform fossae and lower part of the esophagus. In the other, the esophageal mucosa was in bloody, easily detached shreds and the lumen contained a blood clot. Bloody fluid was in the stomach. A man who lived for three days after ingestion of cantharidin had normal

oral and esophageal mucosa but hyperemia and petechial hemorrhage of the gastric mucosa (21). The remainder of the gastrointestinal tract was normal. Changes in the urogenital tracts of the two girls were intense hyperemia of the entire tract, with blood in the renal pelves, ureters, bladder, and ovaries, and hemorrhages in the surrounding tissues (26). The only finding mentioned in another report was bloody urine (42). The aforementioned man had a small amount of bloody urine in the bladder, and the bladder mucosa was petechiated, but the remainder of the tract was normal (21). The only other recorded change was pulmonary edema in one person (26).

Descriptions of microscopic findings are similarly incomplete. In one of the girls mentioned above, only "hemorrhage into the tubules with damage to the tubular epithelium" were recorded as microscopic renal changes (26). "Acute tubular necrosis," probably the result of the action of cantharidin but possibly aggravated by poor renal perfusion, was the only lesion reported in the man; no description was given (21). A more detailed characterization of changes found in renal biopsies of two patients was included in one report (27). The biopsies were done after 14 and 24 days of illness; lesions were the same in each. In the glomeruli, there was swelling of the endothelial and epithelial cells, which had granular cytoplasm containing a few small vacuoles. The glomerular basement membranes were swollen also, so that the capillary lumina were reduced. There were focal adhesions of the glomerular tufts to Bowman's capsule, and many Bowman's spaces contained red blood cells and protein. Much of the proximal tubular epithelium had lost its brush border; there was loss of identity, cloudy swelling, and hydropic degeneration of tubular epithelial cells, and occasional occlusion of tubular

lumina by swollen cells. In some tubules there was sloughing of dead cells into the lumen. A few tubules were represented by amorphous or granular eosinophilic casts with no cellular architecture remaining. The interstitium was edematous but without fibrosis or inflammation.

A biopsy of the liver of one patient was also examined and occasional degenerated cells were found (27). "Early fatty change" was a microscopic postmortem finding in a young woman (26).

Cantharidin was recovered from the vomitus of one patient (26) and from the urine of two (10, 42). In the former case, identification was by demonstration of blistering action of extracts of vomitus and by x-ray diffraction (26). Infra-red spectrophotometry has also been used (27).

Experimental Poisoning

Experimental study of cantharidin poisoning has been limited. In one experiment, 275 g male albino rats were killed at intervals of up to 30 minutes after intraperitoneal injection of 10 mg of cantharidin (5). Light microscopic changes were not well described, but severe damage to the epithelia of the esophagus, stomach, small and large intestines, urinary bladder, and ureters was reported. Degenerative changes of renal tubular epithelium were evident by 10 minutes after injection and were very severe at 30 minutes. Hepatic congestion and degeneration and separation of hepatocytes were also recorded.

In an earlier experiment, dogs were given 13 to 26 mg of cantharidin by intramuscular injection (28); this dose was usually fatal. Gross lesions were congested, turgid livers, gall bladder edema, prominent hepatic lymphatics and lymph nodes, and pale, swollen, moist kidneys.

A reduction of thoracic duct lymph flow after injection was demonstrated by thoracic duct cannulation; the fluid increased in protein content and was often bloody. Microscopic findings included "profound alterations in the convoluted tubules," edema of the liver, sometimes with red blood cells in the edema fluid, and dilated hepatic capsular lymphatics which contained fibrin and blood. The only laboratory findings reported were albuminuria and glucosuria in one dog.

Another early report briefly relates clinical findings in a dog given 13 mg cantharidin subcutaneously (31). The animal was polyuric on the second day but oliguric thereafter, with increasing urine specific gravity, and with albumin, blood, epithelial cells and casts in the urine. On the fifth day the dog was anuric and died. Also recorded were microscopic changes in the kidneys of experimentally poisoned dogs and rabbits. These consisted of granular degeneration of the cells of the convoluted tubules and Henle's loops, and desquamation of dead epithelial cells, most severe in Henle's loops. Evidence of regeneration was seen in some kidneys after three to five days; mitoses when present were most numerous in the convoluted tubules. There were a few in Henle's loops and none in the collecting ducts. A few tubules contained casts of unspecified composition. The glomeruli were congested but otherwise normal.

Poisoning in Horses

The first published report of naturally occurring cantharidin toxicosis in a horse was from Tennessee and appeared in late 1963 (25). An eight-year-old mare when first examined had a high fever, a pulse rate of 120/minute, and labored respirations at 40/minute. The mucous

membranes were cyanotic and congested. As the mare was led toward shade she staggered and fell, having generalized muscular rigidity. She was able to rise after therapy which included intravenous calcium. By then she was sweating profusely. She voided her bladder and drank water from the trough, but about 10 minutes later another episode of falling and muscular rigidity occurred, which was relieved by a repeat of the previous treatment. The mare voided a large amount of urine and drank more water. After cool water was poured over the mare her temperature fell somewhat. The next morning her condition was similar; although she was standing there was still some muscular stiffness which decreased with treatment as on the previous day. At that time, extensive ulcers of the gingival, buccal, and lingual mucosa were noted. Scanty feces covered with bloody mucus were passed. It was later learned that the mare had eaten some spillings from a mill wherein alfalfa hay containing dead striped blister beetles, identified as Epicauta vittata, had been ground. The mare recovered gradually within two weeks.

A second case report was published in 1968 (7). A nine-year-old stallion was found down with abdominal pain one afternoon. He became progressively ill into the night, when the conjunctivae were reddened, the temperature rising, and the packed cell volume 60% in spite of continuous fluid therapy. A small amount of thin bloody fluid was passed from the anus. The animal died in the early morning and was later necropsied. Gross findings were a ruptured stomach and fluid-filled small and large intestines. Three days later a mule died of a similar illness on the same farm; postmortem findings, if any, were not mentioned. During this time several animals did not eat all of the alfalfa hay given to them; after careful examination beetles identified as

Epicauta vittata were found in a bale of the hay. A Shetland pony was given 400 mg of ground beetles from the hay by stomach tube, with no clinical effect, but two days later the pony was given 1 g of the same insects and was found dead the next morning. Gross postmortem findings included reddening, hemorrhage and swelling of the mucosa of the ureters and bladder and reddening of the gastric mucosa. The proximal duodenal mucosa was sloughed and the jejunum was full of pink fluid. Other organs were grossly normal.

A third, unpublished, case was related to the author (12). The patient, a five-year-old stallion, was examined by an Oklahoma veterinarian in early 1975. The owner said that one day the animal ate a usual amount of alfalfa hay but a mare refused to eat from the same bale. The next morning the stallion was depressed and would not eat. The following day the horse was more depressed and had congested mucous membranes, elevated heart rate, periodic jerking of the diaphragm synchronous with the heart beat (synchronous diaphragmatic flutter), and soft stools. Treatment was ineffective. That evening these signs persisted and muscular tremors appeared. The mouth had a foul odor and the mucosa of the tongue was gray and sloughing. A profuse watery diarrhea began. The packed cell volume (PCV) was 60% and the serum calcium was 6.1 mg/dl. The diaphragmatic flutter ceased after intravenous calcium therapy. On the third day of illness the horse urinated frequently; abnormal urinalysis results were moderate proteinuria and glucosuria and a slight occult blood reaction. The specific gravity was 1.005; a large amount of intravenous fluid had been given. The PCV was elevated, to 68%, with severe mucous membrane congestion. The PCV continued to rise for a time despite intravenous fluids, but eventually fell to 58%. The

horse was very depressed, and splashed the muzzle in the water container. On the fourth day the animal's condition was improved; he ate a little feed and drank some water and the oral ulcers were healing. By the eighth day the horse was clinically normal. The serum calcium was 11.1 mg/dl and the mild elevation of serum glutamic oxalacetic transaminase (SGOT) found during the first four days had disappeared. When the owner returned, he brought a sample of hay fed the stallion; in it were striped blister beetles.

Mechanism of Action

Investigations of the biochemical basis of acantholysis, and of tissue damage in systemic poisoning, have not been very extensive or fruitful. The conclusion of a 1959 study was that cantharidin causes the release of an acantholytic enzyme, possibly sulfhydryl-dependent, in the skin, by an unknown mechanism (37). Publications in 1961 suggested, similarly, that an "acantholytic factor" is induced by cantharidin, in some way mediated through effects on oxidative carbohydrate metabolism (39), or that cantharidin is metabolized to an active principle, the acantholytic effect of which is somehow dependent on adenosine triphosphate (ATP) (9). A 1963 report also suggested the requirement of energy supplied by ATP for the action of cantharidin (15). Another author concluded in 1965 that cantharidin reacts chemically with the membranes of cell organelles (16); uncoupling of oxidative phosphorylation in liver mitochondria of poisoned rats was found in another experiment (4). The following year, the uncoupling of oxidative phosphorylation was stated to be an effect of cantharidin but not a cause of acantholysis, and that cantharidin acantholysis requires ATP (14). Another work in

that year recorded finding cantharidin only in the "light mitochondrial" fraction of rat liver, a fraction which contains lysosomes, although cantharidin was not found in purified lysosomal preparations (6). These authors described methods of quantitative and qualitative determination of cantharidin in tissues which could be useful in future studies (6). An electron microscopic study in 1968 of cantharidin acantholysis showed that the desmosomes of prickle cells do not separate at their junctions, but detach from the cytoplasm of the cells (20). Investigators in 1969 found no difference in content of phospholipid and cholesterol in subcellular particles of poisoned and control rat livers (5).

CHAPTER III

MATERIALS AND METHODS

Records from the files of the Departments of Pathology and Large Animal Medicine and Surgery were searched for records of horses poisoned by blister beetles. Twenty-one cases in which clinical and pathologic findings were supported by the finding of beetles in hay fed to affected animals were selected, although presumptive diagnoses of cantharidin poisoning were made on the basis of postmortem findings in several others. Males and females were equally represented; ages ranged from 1 to 10 years. Most were quarter horses. Routinely prepared microslides were available from 14 of 15 fatal cases. In addition, five experimentally poisoned horses were included. These were given ground beetles collected from baled hay, suspended in saline and administered by stomach tube. Histologic preparations were available from two experimental horses, and laboratory data from four. All available clinical, laboratory, and gross postmortem findings were compiled from these records. Tissue sections were compared to those from two normal horses and from several other horses that died of various causes during the course of this study, and morphologic changes recorded.

CHAPTER IV

OBSERVATIONS

Natural Disease

Circumstances of Poisoning

In all naturally occurring cases, striped blister beetles were found in alfalfa hay fed to affected horses or in feed boxes or mangers where the hay had been eaten. Twice, beetles were found in only one flake of baled hay. The hay in one incident had been stored for two years. In one case, some horses wouldn't eat hay from a particular bale; a horse that did was poisoned. Beetles collected from hay in four of these instances were identified as Epicauta occidentalis and E. lemniscata; both were found in each sample (Figure 1, Appendix B).

The 21 horses were poisoned in 16 incidents; in nine cases, two or more horses were affected. On one farm 8 of 11 mares in one pen were poisoned; four died. On another, six of six were affected and two died. On a third farm seven horses were ill and two died. In two other incidents, 2 of 12 and 2 of 10 horses were affected; in each case both animals died. Where the distribution of the horses was specified, most or all affected were in the same pen or pasture.

Clinical Findings

The most consistent clinical signs recorded in animals ill for less

than two days were, in order of decreasing frequency, abdominal pain, fever, depression, increased pulse and breathing rates, and congested mucous membranes with slow capillary refill times. Sweating, intestinal hypomotility, and soft stools, but not actual diarrhea, were occasionally noted. Frequently, if only one animal was affected, it was thought to have "colic." Several horses were seen repeatedly splashing their muzzles in water without drinking. "Ataxia" was sometimes recorded, but no neurologic examinations were done. Frequent urination was observed once. The more severe of these manifestations were as a rule observed in those animals that died; of those that recovered only one was clinically ill for less than two days. In one incident, a group of horses were fed alfalfa hay one day and two were found dead the next. Horses affected two days or more usually had evidence of urinary tract irritation, most commonly frequent voiding of small volumes of urine. Apparent pain on urination was recorded in a few cases and one horse had gross hematuria so severe that clots of blood were passed. Priapism was not recorded. The condition of horses that died after several days sometimes seemed to improve slightly during the clinical course, then to worsen; death when it came was rapid and accompanied by muscular spasms. Clinical signs of non-fatal illness were similar to those of fatal illness but milder; frequent urination was more often noted than abdominal pain. Two animals had a synchronous diaphragmatic flutter. Only three fatally poisoned horses lived longer than two days.

Laboratory Findings

Laboratory data were recorded for nine horses, three of which died. The PCV of each was determined at least once and one or more complete

hemograms were done for eight of the nine horses. The PCV of affected horses was up to 59% and was highest in the most severely ill horses, which also had an increased serum protein concentration. Fluid therapy and clinical improvement were accompanied by falling PCV and serum protein concentration. Most horses had, at the time of admission, leukocytosis of up to 17,000/mm^3, but one surviving horse was mildly leukopenic on the first day. Increases in nonsegmented neutrophils occurred in three severely ill animals; two of these died, one on the fourth day, at that time having a mild neutropenia. Monocytosis of 1200 to 1400/mm^3 occurred in four horses. Leukocyte counts returned to normal with clinical recovery.

Serum chemical analyses were done for six of the nine horses. Increased BUN, up to 50 mg/dl, generally accompanied early illness. However, the BUN of one horse was 87 mg/dl at admission and at the animal's death three days later was 77 mg/dl. This was the only horse that had increased serum creatinine, which rose to 6.6 mg/dl on the second day and fell to 3.9 mg/dl before death, and was the only one with an increased serum phosphorus, which was 6.5 mg/dl and 6.7 mg/dl on the third and fourth days. No abnormal serum concentrations of sodium, potassium, or chloride were found, but significant decreases in serum calcium were found in four of six horses. The most severe hypocalcemia was on the first or second day in each patient. The serum calcium of the horse that died on the fourth day was the lowest recorded at 5.9 mg/dl on the first day; it did not rise above 8.2 mg/dl. In the others it returned to normal in three or four days. The two animals with normal serum calcium were only mildly affected. All horses during the first two days had increased serum glucose up to 185 mg/dl. One

survivor had a slightly increased SGOT of 290 IU/1, but the horse that died on the fourth day had an SGOT of 1355 IU/1.

One or more urinalyses were done for seven horses. The specific gravity of urine samples collected before fluid therapy from four horses ranged from 1.003 to 1.006. The specific gravity determined on the first day in the other three was also very low but these animals were given large volumes of intravenous fluids. In two surviving horses, the urine specific gravity returned to normal by the second and third days. Erythrocytes, up to 20/high power field, were seen in the urine of five animals; these samples also had mild to moderate occult blood test reactions. Epithelial cells were seen in the urine of five horses but casts were not reported. Mild proteinuria was recorded in one surviving horse.

Gross Pathology

Esophagus and Nonglandular Stomach. The esophagus was described in 13 of 15 postmortem records; it was normal in 11. Ulceration and erosion of the mucosa of the distal esophagus occurred in the other two. In one, the exposed surface was red and granular; in the other, there were linear areas of detachment of the epithelium.

A description of the esophageal part of the stomach was included in 14 of 15 descriptions; in seven horses it was normal. The major lesion in the others was epithelial sloughing. In most of these, areas of the epithelium were missing, and the remainder was easily pulled away in sheets; in some it lay in ragged, loosely attached tags but in others it was more intact, being visibly detached at the edges of denuded areas (Figure 2, Appendix B). Reddening or exudation of

exposed surfaces was not recorded. One horse that was ill for five days had extensive, irregular, linear, fissure-like ulcers of the squamous gastric mucosa. The submucosa was edematous in three stomachs.

Glandular Stomach and Intestines. The glandular gastric mucosa was moderately or intensely hyperemic in six of the eight cases wherein it was described. In one it was normal; in another, the stomach was ruptured along the greater curvature in the glandular part and the animal had an acute diffuse peritonitis.

Thirteen descriptions included the small intestine; changes were noted in eight. These ranged from increased mucus through patchy reddening of the mucosa and watery, flocculent content to patchy pseudomembranous inflammation (Figure 3, Appendix B). The latter was in the duodenum of one horse, the proximal one-half in another, and irregularly distributed throughout in a third. The small intestine of a fourth animal contained large flakes and strands of fibrin loosely adherent to the mucosa. The submucosa was edematous in two severely affected intestines. Patches of squamous gastric mucosa were found in the contents of one grossly normal small intestine.

The large intestine had abnormally watery contents in 9 of the 15 animals; it was not described in one. On the mucosa of one were numerous 2 to 3 cm white plaques of exudate, and in two others the submucosa was more than 1 cm thick with straw-colored fluid. Mucosal hyperemia was recorded once. Abnormalities in the cecum were not noted.

Kidney. Gross features of the kidneys of all 15 animals were recorded, although in three they were too autolyzed for critical examination. In six horses, ill for one to five days, the kidneys were

slightly enlarged and pale, with a slightly bulging, pale, moist cut surface. Single or multiple acute infarcts were recorded in the kidneys of two horses and there were small, sparsely scattered cortical hemorrhages in those of one. The kidneys of the others were normal.

Renal Pelvis, Ureters, and Bladder. The renal pelves of four horses contained more than usual mucus. In another horse, the mucosa of the pelves contained petechial hemorrhages. In other reports they were not characterized. Where the ureters were described, they were normal (three animals) or petechiated or diffusely reddened (four animals). The bladders of all 15 were abnormal; in three the mucosa was merely patchily or diffusely hyperemic, but in the others it contained petechiae or multiple, confluent, 1 to 2 cm hemorrhages. The bladder of the horse with hematuria was one of the most severely hemorrhagic and contained several small blood clots. The urethrae of one male and one female horse had mucosal petechiae; those of three others were normal.

Myocardium. Five horses, ill for seven hours to five days, had gross myocardial lesions. Discrete, gray-red or brown-red to pale tan or yellow patches were in the ventricular myocardium and were visible on the cut as well as internal and external surfaces. They were irregular but often more or less linear with the longer dimension oriented as the cardiac muscle fibers, and up to 5 cm in greatest dimension. The more red and presumably more acute lesions were in horses affected for two days or less. Those in a horse that died after five days were gray-yellow and soft and surrounded by a narrow red zone.

Other Organs and Tissues. Petechial and ecchymotic hemorrhages of the pleura, epicardium, and endocardium, and various degrees of adrenal cortical hemorrhage were seen in nearly all horses. One horse had a large hemorrhage under the hepatic capsule. Another had about 200 ml of serosanguinous fluid in the pericardial sac and reddened vulvar and vaginal mucosae. Lesions in other organs and tissues, if any, were not recorded.

Microscopic Lesions

Esophagus and Squamous Gastric Mucosa. Sections of esophagus were available from one of the two horses with gross esophageal lesions. The salient microscopic change was patchy but locally extensive necrosis of the deep layers of the epithelium, resulting in focal separation of the epithelium from the basement membrane. In the spaces created by this separation were erythrocytes, neutrophils, and cell debris. Neutrophils were also within the separated epithelium, the underlying lamina propria and submucosa, and the submucosal lymphatics. The endothelium of superficial vessels was swollen. Another horse without gross esophageal changes had an area of epithelium separated from its deep layers but still attached at the edge of the area of separation and leaving three or four cell layers on the basement membrane (Figure 8, Appendix B). The most superficial of the remaining layers was flattened and eosinophilic; the basal cells had large nuclei and prominent nucleoli. There were few mitoses. A few tags of amorphous proteinic and cellular debris adhered to the surface of this thin area of epithelium. In the separated epithelium, and to a lesser extent in other areas, there was necrosis of many individual cells of the basal and deeper prickle cell

layers. The lamina propria and submucosa were not inflamed. Sections of esophagus of two other animals were unremarkable.

There were sections of nonglandular stomach of three of the seven animals with gross lesions. In one of the horses with esophageal lesions, there was degeneration and necrosis of many individual basal and parabasal epithelial cells, which were shrunken and eosinophilic and had pyknotic or fragmented nuclei. Mitoses were numerous in the basal cell layer. There were several focal superficial accumulations of pale, rhomboid epithelial cells, similar to those seen in sections from one control horse, except that the cells were swollen and very loosely packed, with numerous small intercellular spaces. A few of the larger of these spaces contained pink wispy material, cell debris, and erythrocytes. The lamina propria and submucosa were normal. In the second animal, there were extensive and severe degenerative changes in all layers of the squamous epithelium, including necrosis of basal and parabasal cells. Changes in prickle cells ranged from finely granular cytoplasmic swelling through vacuolar degeneration to frank necrosis of individual cells, or small groups of cells. In some areas, there was extensive focal necrosis of prickle cells, leaving numerous small spaces within which were neutrophils and cell debris. The lamina propria beneath contained a few neutrophils. In one section, there were a few larger intraepithelial spaces, empty except for bits of debris. Another section had an area of very thin epithelium, about five flattened layers thick, adjacent to which the superficial epithelial layers were missing and the exposed prickle cells were loosely arranged and in various stages of degeneration (Figures 9, 10 and 11, Appendix B). One section of the nonglandular stomach of the third horse also had attenuated

epithelium but without underlying inflammation. Gross gastric lesions were not recorded in another animal in which the squamous epithelium was completely missing in one section. There was superficial necrosis of the exposed lamina propria and an intense neutrophilic response which extended to the muscular tunic. This horse had died after less than 24 hours of illness. The stomach from a fifth horse was normal.

Glandular Stomach and Intestines. Sections of glandular stomach of five horses were examined. In two stomachs there was mild superficial hemorrhage and a few neutrophils were in the lamina propria. The others were normal except for vascular engorgement. The stomach and intestines were difficult to evaluate in some cases because of autolytic changes.

Although the small intestines of three horses were too autolyzed for interpretation, there were lesions in all eight others from which sections were available. These ranged from mild dilation of crypts with mucus in one animal to a pseudomembrane of fibrin and cellular debris that merged with necrotic villous tips in four (Figure 13, Appendix B); in one horse, necrosis extended into the submucosa in small areas. Lesions were most severe in the proximal small intestine and gradually decreased in severity or disappeared altogether in the distal jejunum and ileum. In some areas the villi were short and had flat epithelium; occasional crypts contained cellular debris. Four intestines had moderate to severe edema of the lamina propria and submucosa, with mild to severe focal hemorrhage, but in only one of these was there also severe epithelial damage. The lamina propria and submucosa of one contained scattered neutrophils; many were also in the lymphatics.

In the colon, the mildest change seen was slight dilation of crypts with excess mucus. In one, occasional crypts also contained a few

fragmented cells. The submucosa of two colons was extremely edematous; however, these were not from animals with small intestine edema. Mild focal hemorrhage in the submucosa and mild neutrophilic inflammation of the lamina propria were also seen. Sections of five colons were examined; one was severely autolyzed.

Kidney. Microslides of kidneys of 12 horses were examined; in five the tissues were too poorly preserved to interpret. In three of the remainder, there was slight to moderate swelling of the glomerular endothelium and epithelium, with occasional focal adhesions of the tuft to Bowman's capsule. In these and two others, there were degenerative changes of the tubules of the medullary rays and medulla. The mildest change was slight dilation of the medullary ray tubules, a few of which contained protein or small amounts of cellular debris, though no necrosis of tubular epithelium could be seen. In some kidneys, however, individual cells or small groups of cells of the medullary ray tubules were hyalinized and had pyknotic or fragmented nuclei, and luminal cellular debris was more common, although it was never abundant. It seemed that the collecting ducts were more severely affected, but in some cases there was sufficient tubular dilation, epithelial flattening, and nuclear hyperchromatism and irregularity to make positive identification impossible. Only occasional proximal tubules had recognizable changes; some individual degenerate cells were seen, but epithelial necrosis and cellular casts were quite rare. Changes in the medulla resembled those in the medullary rays. Lesions were mild in all kidneys but those of one horse, in which changes differed only in degree from those in the others (Figure 15, Appendix B). No relationship between clinical findings and renal lesions was found; even in the horse with laboratory

evidence of renal disease, microscopic changes were mild. The interstitium and vasculature of all kidneys were normal.

Renal Pelvis, Ureters, Urinary Bladder, and Urethra. Sections of renal pelvis and ureters were prepared in only two cases. In both, the mucous glands were dilated with mucus, and a thick layer of mucus overlay the mucosa. The ureteral mucosa of one horse contained small focal hemorrhages and there were neutrophils in the lumina of many of the mucous glands. The urinary bladders of six horses were examined microscopically; two were autolyzed beyond interpretation. Changes in the others were similar (Figure 22, Appendix B). The epithelium was focally or diffusely denuded and the exposed areas were acutely inflamed. Tags of proteinic debris clung to the surface below which were numerous neutrophils. Lymphatics contained neutrophils and occasional fibrin plugs. The lamina propria in two cases was severely hemorrhagic. One section of urethra was available; there was necrosis of a few epithelial cells.

Myocardium. There were microslides of the hearts of 12 horses. Four, whose durations of illness were two, four and five days and one unknown, had myocardial lesions (Figures 23 and 24, Appendix B); microscopic changes were not seen in the heart of the horse ill for only seven hours. There was discrete or confluent focal necrosis, or in some sections, extensive patches of necrosis. Muscle fibers were swollen, eosinophilic, and granular, without striations, and were usually severely fragmented. In one, almost every focus was heavily mineralized but in the others mineralization was minimal. Degenerated muscle fibers were separated by edema, and there was more or less extensive swelling and proliferation of satellite cells, particularly at the

periphery of affected areas. A few neutrophils and extravascular red cells were scattered between degenerated muscle fibers. Mild lesions were found in the heart of one horse in which gross myocardial changes were not described. Gross atrial lesions were not seen, but microscopic foci of necrosis were in the atrial myocardium in one animal with severe ventricular lesions. Of the eight horses without myocardial lesions, none had survived more than two days.

Other Tissues. The lungs of seven horses were moderately to severely hyperemic and edematous. The liver of one horse with extensive myocardial necrosis had severe centrilobular congestion, mild acute necrosis of centrilobular hepatocytes, and edema. In five horses the adrenal cortex was moderately to severely hemorrhagic.

Experimental Disease

Clinical Signs

Horses 1 and 2, of 182 kg (estimated) and 409 kg, were given 5 g ground dried beetles each; death followed in 4 and 19 hours respectively. Horse 3, 329 kg, was given oral doses of 1.5, 3 and 6 g beetles at five-day intervals. Mild transient illness of about one day resulted from the first dose, more severe signs for four days followed the second, and death occurred after six days of severe illness after the third dose. Three g ground beetles produced nonfatal illness in horse 4, weight 166 kg. Horse 5, 138 kg, survived less than 18 hours after administration of 6 g of beetles and was found dead the following morning. Clinical signs were much the same as in the natural disease and included severe abdominal pain and depression, rapid thready pulse, and frequent

voiding of small amounts of urine (Table I, Appendix A). Horse 2 had an erection; this was not observed in the other two male experimental horses. Hematuria occurred in horse 3 after the 3 g dose. Horses 3, 4, and 5 splashed their muzzles in their water.

Laboratory Findings

Laboratory examinations of daily, or more frequent, blood samples of horses 2, 3, 4, and 5 were done (Tables II and III, Appendix A). Increases in PCV of 5 to 15% followed each dose of beetles within 24 hours. The PCV of horses 2 and 5 rose to 50%; that of horse 3 increased to the same figure after the second and third doses. It returned to near the original figure by the third day in the former instance, but fell only slightly in the latter. The serum protein concentration increased moderately with the PCV in horse 3, but not in horse 2; the serum protein concentration of horses 4 and 5 was not determined. The leukocyte counts of horses 2 and 5 dropped sharply after dosing, from 8000/mm^3 to 4600/mm^3 and from 11000/mm^3 to 7400/mm^3 respectively, as a result of decreases in both neutrophils and lymphocytes. Nonsegmented neutrophils increased in horse 5 but decreased in horse 2; toxic changes in neutrophils were not seen. However, white counts rose within one day to as high as 27000/mm^3 in horse 3 after the 6 g dose and to 18000/mm^3 in horse 4. In each instance there was an increase in neutrophils, to as many as 18000/mm^3, a pronounced left shift of up to 6000 nonsegmented neutrophils/mm^3, and a slight to moderate decrease in lymphocytes. Monocytes and eosinophils varied inconsistently. The BUN increased after each dose, but did not exceed 30 mg/dl except in horse 3 after the third dose, when a gradual increase to a terminal

concentration of 163 mg/dl occurred, accompanied by rises of serum creatinine to 9.8 mg/dl and of serum phosphorus to 22 mg/dl. The serum phosphorus of horse 4 decreased to 1.2 mg/dl on the second day. Significant changes in the serum phosphorus of horses 2 and 5 did not occur.

Decreases in serum calcium of 2.0 to 6.0 mg/dl from the original concentration occurred after each dose in horses 3 and 4; the maximum decrease was on the second day in each. The lowest concentration recorded was 4.2 mg/dl in horse 3 after the third dose; it gradually rose to 8.6 mg/dl shortly before death, not returning to the predose figure of 10.2 mg/dl. The serum calcium of horse 3, after the first and second doses, and of horse 4, gradually returned to predose concentrations. Although horses 2 and 5 died within 24 hours, decreases of 2.6 and 1.8 mg/dl from the predose figure occurred before death. Serum sodium, potassium, and chloride did not change significantly.

Slight increases in SGOT occurred after each dose of beetles. Serum creatine phosphokinase (CPK) rose slightly in horse 4; only one determination of CPK was done in horse 5 after dosing and none in the others.

The only urine sample obtained was during the necropsy of horse 3; it contained a moderate amount of protein, 5 to 10 erythrocytes/high power field, and gave a moderate occult blood test reaction.

Gross and Microscopic Pathology

Horses 1, 2 and 3 were necropsied; findings were similar to those in naturally poisoned horses. The esophagus was normal in horses 1 and 3, but in horse 2 there was extensive mucosal sloughing and submucosal edema. None had gross lesions in the squamous gastric mucosa; sections

were available only of the stomach of horse 3, wherein no changes were seen. The glandular gastric mucosa of all three horses was reddened, and in horse 3, in addition to hyperemia, there was mucus and debris in the glandular crypts, and severe submucosal edema with occasional focal hemorrhage and mild acute inflammation (Figure 12, Appendix B). The small intestines of horses 1 and 3 were grossly and microscopically normal, but a gray pseudomembrane covered the mucosa of the entire small intestine of horse 2. The underlying mucosa was intensely reddened and the mesentery was mildly edematous near its attachment to the intestine. Colonic lesions were limited to horse 3 in which there were numerous pinpoint to 3 cm indurated ulcers covered with yellow granular exudate; these tended to occur in groups (Figures 4 and 14, Appendix B). The kidneys of horses 1 and 2 were grossly normal, but those of horse 3 were slightly swollen and pale, with a moist cut surface. Microscopic changes in the tubules of the medulla and medullary rays in horse 3 were as those in natural cases but more severe (Figure 16, Appendix B), and many of the convoluted tubules had flattened epithelium with large hyperchromatic nuclei and occasional mitoses and cellular debris in the lumen. The lower urinary tract was grossly normal in horse 1, but the renal pelves of horse 3 contained excess mucus (Figure 17, Appendix B) and the ureteral mucosa of horse 2 was stippled red. The bladders of horses 2 and 3 were intensely red and had dull, granular, hemorrhagic mucosal surfaces (Figure 5, Appendix B). The urethra of each was normal. In the one section of bladder from horse 3 in which the epithelium remained, there was extensive necrosis of cells, singly or in small groups, usually in association with a few intraepithelial neutrophils (Figures 20 and 21, Appendix B). The transitional epithelium of the

renal crest of this animal contained numerous small discrete spaces. Many of these contained cell debris and neutrophils but some were apparently empty (Figures 18 and 19, Appendix B).

The myocardium of horse 1 was grossly normal but contained microscopic foci in which myocardial fibers were slightly swollen and more eosinophilic than usual, although striations were visible. The hearts of horses 2 and 3 contained gross lesions resembling those seen in several of the naturally poisoned horses. In horse 2 they were dark red. Those on the external surface in horse 3 were fine pale striate areas while those on the internal and cut surfaces were discrete yellow-white patches several centimeters in greatest dimension (Figures 6 and 7, Appendix B). Microscopic changes were as in the natural disease (Figure 25, Appendix B). Other organs and tissues were unremarkable except for serosal and adrenal hemorrhage in horses 2 and 3; horse 3 also had a slight excess of clear pericardial fluid.

CHAPTER V

DISCUSSION

Under the current system of classification of the genus Epicauta, E. vittata does not occur in Oklahoma or Texas, but is found from Iowa east to Maine and New Jersey (40). E. occidentalis occurs in Nebraska, Kansas, Colorado, Oklahoma, Texas, and Louisiana, and E. lemniscata in these states and east to New Jersey and Florida (2, 40). However, these three species are very similar and may not actually be separate species (3, 40). In no instance of natural poisoning were beetles found in any feedstuff other than alfalfa hay. These beetles have a particular tendency to feed in swarms on alfalfa (3), and it has been suggested that this habit, and the modern method of harvesting alfalfa by simultaneous cutting and crimping, result in incorporation of large numbers of beetles in individual bales or even single flakes of a bale (30). One 2.4 kg flake of alfalfa hay yielded 145 g of dried beetles (3), so it is obvious from the doses given experimentally that ingestion of only a small amount of contaminated hay could be fatal. Refusal of horses to eat hay containing beetles was reported by the owner in one case in this study and has been noted by others (7, 12); the reason for this is unknown. Storage of contaminated hay for two years did not affect its ability to cause fatal disease in another instance. It seems odd, in view of the range of striped blister beetles and the common growing of alfalfa, that poisonings have been recognized only in Tennessee (25),

Texas (7), and Oklahoma (12, 30).

It is not surprising that some acutely poisoned horses were at first thought to have a gastrointestinal catastrophe such as infarction or displacement, since many had apparent abdominal pain with depression, rapid breathing, sweating, and rolling, and sometimes decreased sounds of intestinal movement. Signs were vague in some horses, however, and in several cases, two or more horses were affected. Congested mucous membranes with poor capillary refill time, a rapid thready pulse, and increased PCV and serum protein concentration were taken to indicate shock, whatever the cause.

No other signs were noted in most animals ill for less than a day or so, although frequent attempts to urinate were recorded in one acutely ill horse. Generally, indications of urinary tract irritation were not seen in animals affected for less than two days, but it is quite possible that such signs were obscured by more noticeable manifestations. Gross hematuria was not noted as often as might be expected from comparison to cases of human poisoning and from the urinary bladder lesions, although microscopic hematuria was usually found when urinalyses were done. The previously reported synchronous diaphragmatic flutter (12) was recorded in two horses in this study and was probably related to the hypocalcemia usually found when serum electrolyte studies were done (24). The mechanism of hypocalcemia is unknown; it should be investigated in future studies. The repeated immersion of the muzzle in water might indicate oral irritation but should not be regarded as a specific sign since horses with other illnesses, particularly gastrointestinal, may also do this. Oral lesions were not seen in horses in this study, but oral mucosal sloughing has been reported in two other

cases (12, 25). Irritative lesions were not seen in areas contacted by urine except in one mare; however, since cantharidin is excreted in the urine (38), such might be found by careful examination.

The neutrophilic leukocytosis with a left shift recorded in some horses is readily accounted for by the inflammatory lesions. The occasional early decrease in lymphocytes is likely a corticosteroid effect, which could also contribute to neutrophilia though not to a left shift (34). Two experimental horses that died acutely, and one naturally poisoned horse shortly before death, had left shifts but were leukopenic. The latter horse also had toxic changes in neutrophils. These changes could result from endotoxemia or septicemia following severe intestinal injury (34). The mild elevations of BUN frequently recorded were probably the result of poor renal perfusion. Although the gross appearance of some kidneys suggested nephrosis, most animals, including the single naturally poisoned horse with laboratory evidence of renal dysfunction, had only mild tubular damage no different from that which might be found in animals dying from various causes and similar to that seen in one of the controls. Horse 3 had fairly extensive tubular necrosis which included the convoluted tubules. This lesion was not seen in any of the other horses, but horse 3 had been given three doses of beetles, a total of 10.5 g. Therefore, it is believed that although cantharidin is nephrotoxic, nephrosis seldom is responsible for the death of a poisoned horse, and lesions found in natural cases are likely to be nonspecific. Furthermore, urinalyses did not indicate tubular necrosis, although most of those done were on samples from horses with mild clinical illness. Four horses had a low initial specific gravity of 1.003 to 1.006, which occurred before fluid therapy when there was

hemoconcentration. From current knowledge of the mechanism of urine dilution, it would seem that a transient decrease in the permeability of the collecting ducts to water occurred, given that these figures are correct (32). Whether this is a result of effects on the epithelium or of some interference with antidiuretic hormone requires investigation.

The mild proteinuria in one horse could have resulted from urinary tract hemorrhage. This supposition is supported by the occult blood reactions and finding of erythrocytes in most urine samples. However, a few tubules containing protein were seen in sections of kidneys of some other horses, so the origin of the urinary protein was not established.

In view of the known properties of cantharidin, one would expect extensive acantholysis in the stratified squamous epithelium of the upper digestive tract. Histologic evidence of this was only occasionally seen, the usual finding being that the epithelium was either unaffected or denuded. However, the areas of very thin epithelium probably represent areas of acantholysis and sloughing of the superficial layers. The intraepithelial spaces seen in one stomach are not likely to be artifactual, since they contain cell debris. Acantholytic separation of the superficial layers could be followed by digestion of the exposed deep layers, resulting in gastric ulceration. An alternate mechanism is suggested by the degeneration and focal necrosis of basal cells and prickle cells; if extensive enough, it could result in sloughing of the whole thickness of the epithelium. Whatever their pathogenesis, the fully developed ulcers were not microscopically distinctive, resembling those seen along the margo plicatus in many horses. Gross lesions in the stomachs of cantharidin-poisoned horses were much more characteristic. The squamous epithelium was sloughed in patches

and the areas still attached were easily peeled away. In contrast, the squamous gastric epithelium of other horses is very difficult to remove, even several hours after death. The lack of sloughing in the stomachs of the experimentally poisoned horses is unexplained; however, it occurred in the esophagus of one, and was not recorded consistently in the natural disease. The occurrence of focal accumulations of pale, rhomboid cells on the surface of the squamous gastric epithelium of one horse is unexplained. These accumulations themselves were probably not lesions of cantharidin poisoning since similar ones, without degenerative changes, were seen in sections of the stomach of a control animal that had salmonellosis.

Lesions in the remainder of the gastrointestinal tract reflect the potent toxicity of cantharidin. Apparently it is capable of causing necrosis of intestinal epithelium, and perhaps vascular damage as evidenced by the hemorrhage and edema seen in some of the gastrointestinal tracts, as well as the hemorrhages in most of the urinary bladders. Vascular lesions other than endothelial swelling were not seen, however. The focal necrosis of transitional epithelium in one experimentally poisoned horse suggests that bladder ulceration could result from confluence of these foci. Alternatively, extensive vesiculation, as suggested by the small spaces containing fragmented cells in the transitional epithelium of the renal crest of the same horse, could lead to ulceration. However, as in the stomachs, most sections had either normal epithelium or extensive loss, so the pathogenesis of bladder ulceration remains obscure. That there actually is ulceration rather than postmortem decomposition of the epithelium is assured by the inflammatory response of the denuded areas.

It is tempting to attribute death in animals ill for several days to heart failure as a result of myocardial damage, but supporting evidence of passive congestion was found in only one horse. It is believed that the myocardial necrosis is caused by the direct effect of cantharidin, because lesions of myocardial vasculature sufficient to cause infarction were not seen, and the pattern of fine, linear, diffusely distributed lesions seen in some hearts does not suggest a vascular origin. Significant hypokalemia was not recorded, and the literature does not indicate that hypocalcemia causes myocardial necrosis. Myocardial necrosis is not recorded in previous reports of cantharidin poisoning, although one human patient had electrocardiographic evidence of myocardial damage (27).

Findings such as serosal and adrenal hemorrhage and pulmonary congestion were regarded as nonspecific.

CHAPTER VI

SUMMARY

The hemolymph of beetles of the family Meloidae, commonly called blister beetles, contains the toxic substance cantharidin. It has a potent acantholytic effect when applied to stratified squamous epithelium, and is also toxic to other tissues. Poisoning of horses by ingestion of alfalfa hay containing dead striped blister beetles was first reported in 1963. Since then, several instances of such poisoning have been recognized in Oklahoma. In this study, cases with diagnoses of cantharidin or blister beetle poisoning in horses were collected from the necropsy and clinic case records of the Oklahoma State University College of Veterinary Medicine. Twenty-one cases, in which clinical and pathologic features were supported by the finding of striped blister beetles in hay fed affected horses, were selected for review. Beetles from four cases were identified as Epicauta lemniscata and E. occidentalis. Clinical illness was most often characterized by apparent abdominal pain, sweating, rolling, rapid breathing, and fever, although some horses were simply depressed. These signs were accompanied by manifestations of shock, including rapid pulse, congested mucous membranes with poor capillary refill times, and increased packed cell volumes and serum protein concentrations. Some horses were thought at first to have acute intestinal disorders such as displacement or infarction. Frequent voiding of small amounts of urine was commonly

noted in animals surviving for more than one day. Only three fatally poisoned horses survived for three or more days. Laboratory findings in one suggested renal disease, but microscopic kidney lesions were usually mild and indistinguishable from those found in horses that died of other causes. Other laboratory findings included neutrophilic leukocytosis with left shift; hypocalcemia usually maximal on the second day and probably responsible for synchronous diaphragmatic flutter in two animals; microscopic hematuria; and low urine specific gravity in four horses in spite of hemoconcentration and before fluid therapy. The causes of hypocalcemia and low urine specific gravity were not determined.

Postmortem findings included peeling and ulceration of the mucosa of the squamous part of the stomach and occasionally of the distal esophagus; mild to severe, superficially necrotizing or ulcerative enterocolitis, sometimes with submucosal edema and hemorrhage; reddening, hemorrhage, and ulceration of the urinary bladder mucosa; and discrete gray-red or yellow patches or striate areas of myocardial necrosis. The latter occurred in animals ill for two days or more. Evidence of heart failure was seen in only one horse with heart lesions. Tissue sections from most cases were compared to those from two normal horses and others necropsied during this study. The manifestations of experimental poisoning in five horses were similar to those of the natural disease.

From a diagnostic viewpoint, "colic" or acute vague illness in a horse fed alfalfa hay should prompt a search for beetles, especially if several animals are simultaneously affected or if frequent urination or hematuria are observed. Clinical manifestations of hypocalcemia can be

seen in cantharidin poisoning, and laboratory demonstration of hypocalcemia or hematuria would be helpful. The oral and genital mucous membranes should be examined for irritative lesions. Sloughing or easy manual removal of the squamous epithelium of the stomach, hemorrhagic and ulcerative cystitis, and evidence of alimentary tract damage were common gross postmortem findings and should be regarded as suggestive of cantharidin poisoning when they occur in combination, and particularly when accompanied by myocardial necrosis. However, since these findings were not constant from case to case, and since microscopic studies seldom provided additional information, access of the horse to hay containing blister beetles should be demonstrated if cantharidin poisoning is suspected.

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APPENDIX A

TABLES

TABLE I

CLINICAL SIGNS IN HORSES EXPERIMENTALLY POISONED WITH GROUND BLISTER BEETLES

Horse	Weight (kg)	Dose (g)	Clinical Signs	Duration	Result
1	182	5	abdominal pain frequent urination	4 hours	death
2	409	5	depression abdominal pain erection	19 hours	death
3	329	1.5	abdominal pain	1 day	survival
		3	depression abdominal pain hematuria	4 days	survival
		6	depression abdominal pain	6 days	death
4	166	3	depression abdominal pain	4 days	survival
5	138	6	depression	1 day	death

TABLE II

HEMATOLOGIC FINDINGS[1] IN HORSES BEFORE AND AFTER EXPERIMENTAL POISONING WITH GROUND BLISTER BEETLES

Horse	Packed Cell Volume, % before/after[2]	Total Leukocytes/mm^3 before/after[2]	Nonsegmented Neutrophils/mm^3 before/after[2]	Segmented Neutrophils/mm^3 before/after[2]	Lymphocytes/mm^3 before/after[2]
1	———	———	———	———	———
2	32/50	8000/4600	800/230	3000/3100	4300/1500
3[3] a	30/44	8600/15000	0/1600	4500/10500	2500/1800
b	35/48	8300/15000	160/2500	3300/6000	2400/1600
c	38/50	11000/27000	0/6000	4400/18000	5000/2700
4[4]	32-33/38	9300-10700/18000	0/3400	2900-4100/10700	3900-6000/2300
5[4]	35-41/50	8100-11200/7400	0-100/500	3200-5300/3900	3300-4500/2600

[1]Monocyte and eosinophil numbers are not tabulated because they varied widely before and after dosing with beetles.

[2]Obtained the day after dosing. In horse 3 after the first and second doses and in horse 4, counts gradually returned to near original figures.

[3]a, 1.5 g beetles; b, 3 g; c, 6 g.

[4]Preinoculation counts determined for four consecutive days.

TABLE III

SERUM CHEMICAL ANALYSES IN HORSES EXPERIMENTALLY POISONED WITH GROUND BLISTER BEETLES

Horse	BUN, mg/dl before/after[1]	Creatinine, mg/dl before/after[1]	Phosphorus, mg/dl before/after[1]	Calcium, mg/dl before/after[1]	SGOT, S-Fu/ml before/after[1]	CPK, Su/ml before/after[1]
1	———	———	———	———	———	———
2	14/24	2.5/5.7	4.4/4.2	10.6/8.0	———	———
3[2]a	14/24	1.5/2.3	3.2/2.5	10.5/7.2	78/158	———
b	12/26	1.4/2.5	3.9/3.5	11.0/8.6	83/124	———
c	12/163[3]	2.3/9.8[3]	4.5/22.0[3]	10.2/4.2	83/110	———
4[4]	9-18/24	0.7-1.1/1.6	3.9-4.9/1.2	10.9-11.3/6.0	230-360/475	28-32/56
5[4]	9-18/28	0.7-1.1/1.4	2.9-5.2/3.9	9.9-11.7/8.1	176-280/315	15-21/15

[1]Obtained 24 to 48 hours after administration of beetles, except as noted below.

[2]a, 1.5 g beetles; b, 3 g; c, 6 g.

[3]Obtained on the fifth day after dosing, just before death.

[4]Preinoculation determinations done on four consecutive days.

APPENDIX B

ILLUSTRATIONS

Figure 1. Striped blister beetles from baled alfalfa.

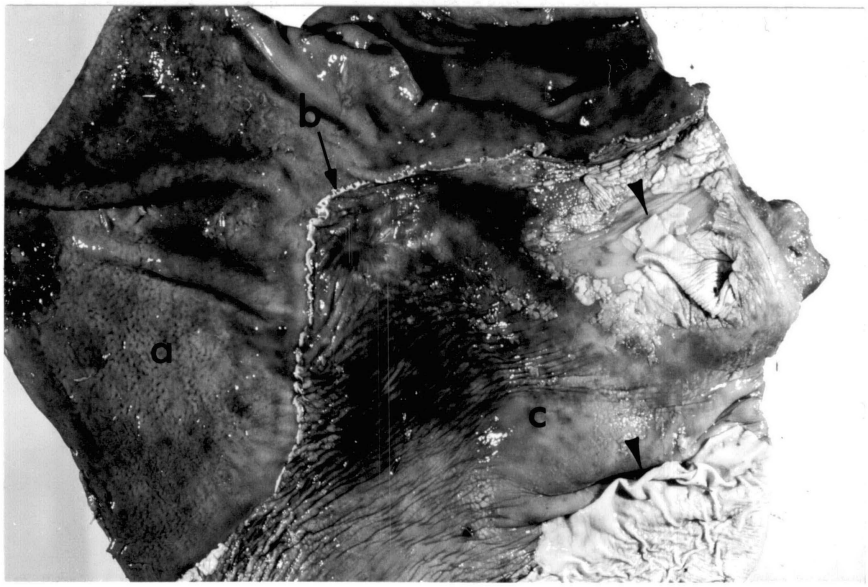

Figure 2. Stomach. Glandular mucosa (a), margo plicatus (b), nonglandular mucosa with large denuded areas (c). Partially attached epithelium (arrows).

Figure 3. Duodenum. Pseudomembranous inflammation.

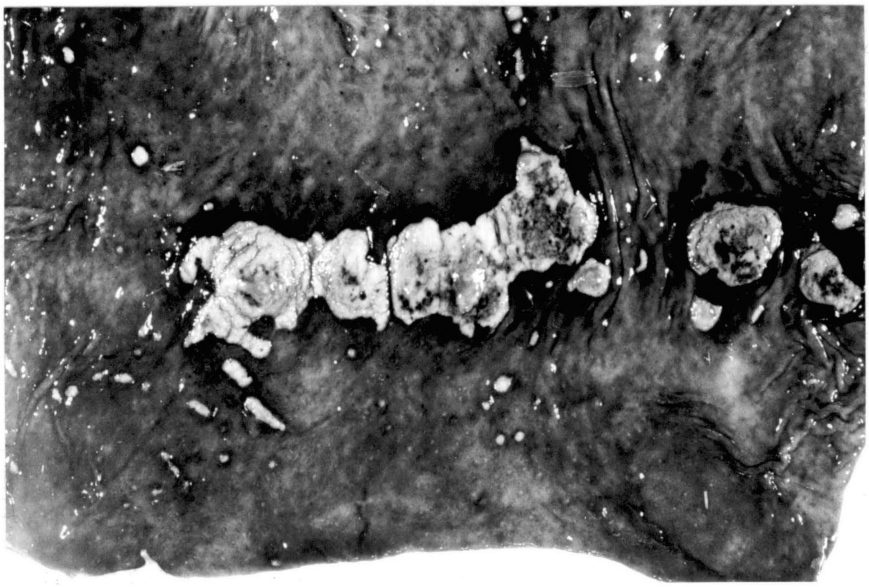

Figure 4. Colon. Exudate adherent to multiple ulcers.

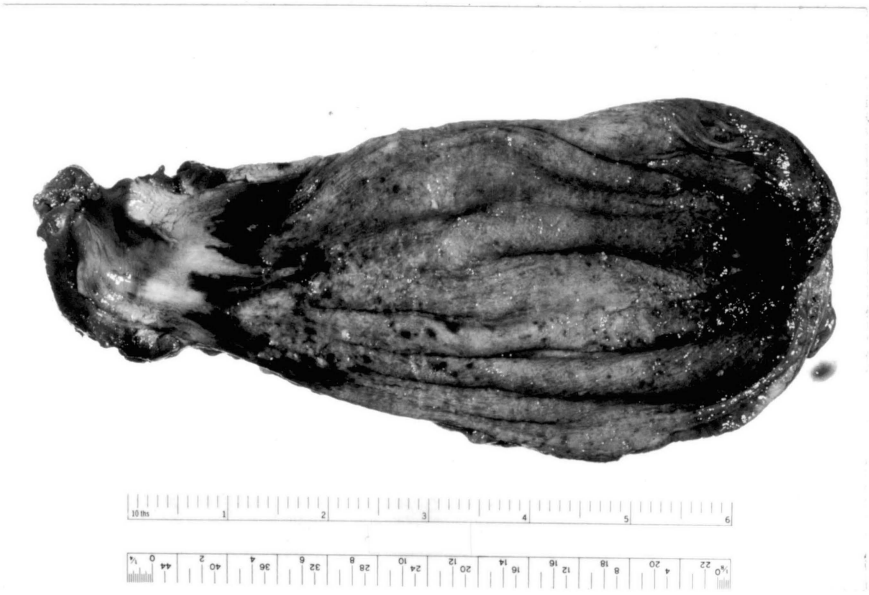

Figure 5. Urinary bladder. Hemorrhagic and ulcerative cystitis.

Figure 6. Heart. Pale linear areas of necrosis in ventricular myocardium.

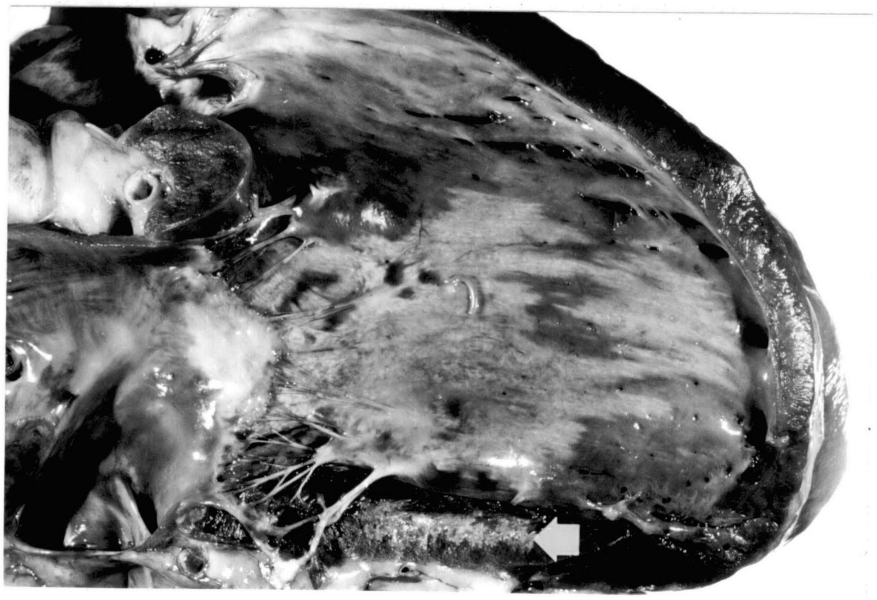

Figure 7. Heart. Pale areas of myocardial necrosis in interventricular septum and ventricular wall (arrow).

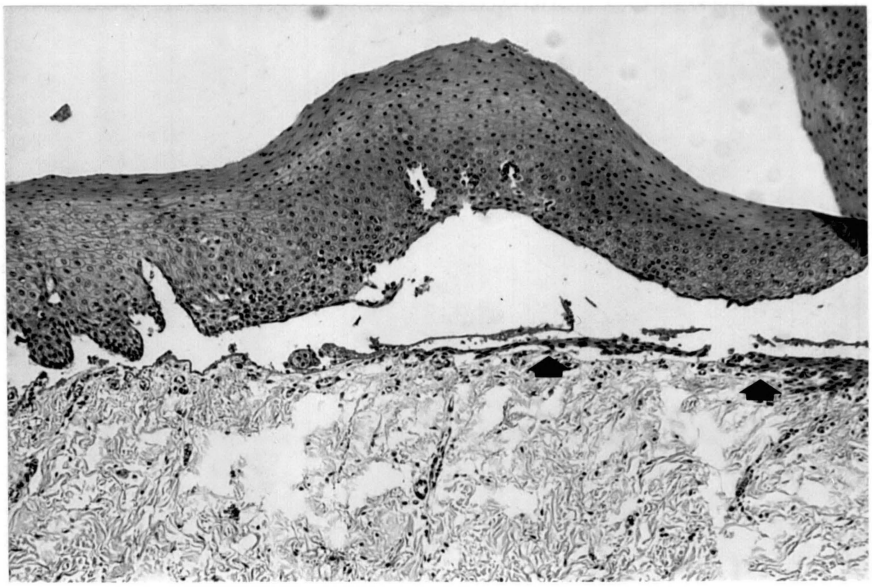

Figure 8. Esophagus. Separation of superficial from deep layers of epithelium. A layer of basal cells remains attached to the basement membrane (arrows).

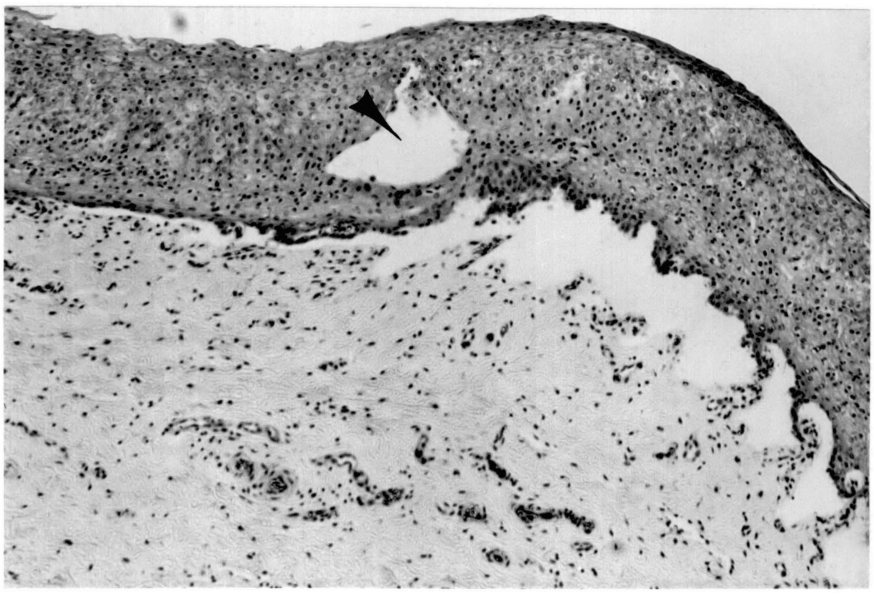

Figure 9. Stomach. Cytoplasmic swelling and nuclear pyknosis of epithelial cells. Intraepithelial space containing cell debris (arrow). Separation of epithelium from lamina propria is artifactual.

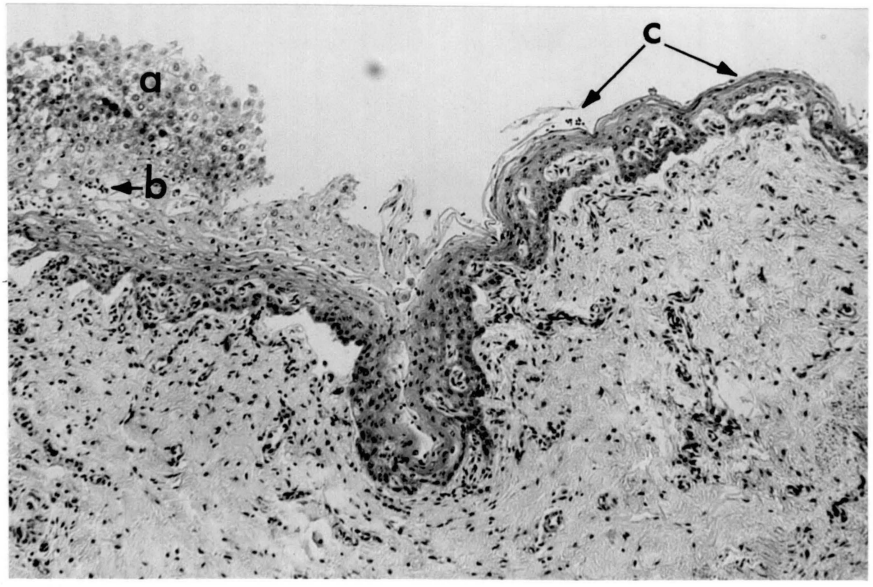

Figure 10. Stomach. Degenerated epithelial cells (a), neutrophils in epithelium (b), area of loss of superficial and middle layers (c).

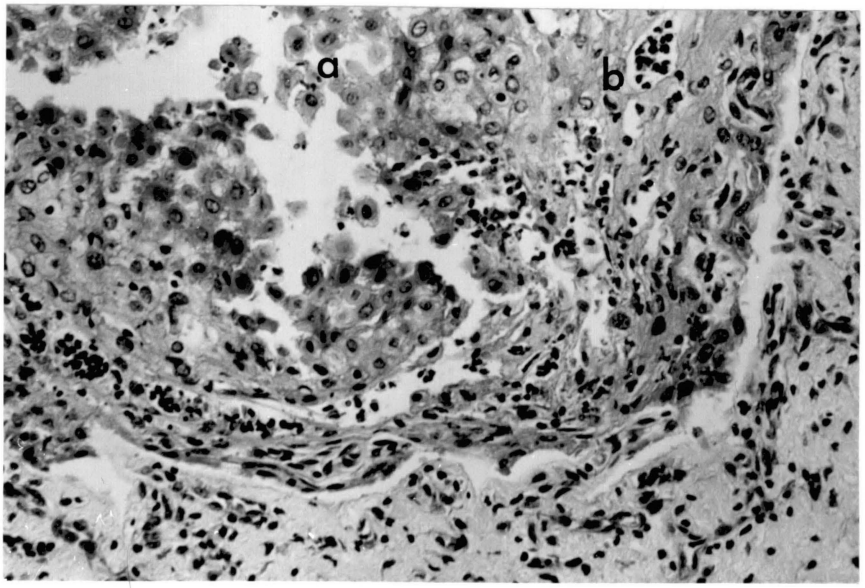

Figure 11. Stomach. Degenerated, loosely attached epithelial cells (a), intraepithelial spaces containing neutrophils and debris (b). Lack of normal basal cells.

Figure 12. Stomach. Submucosal edema.

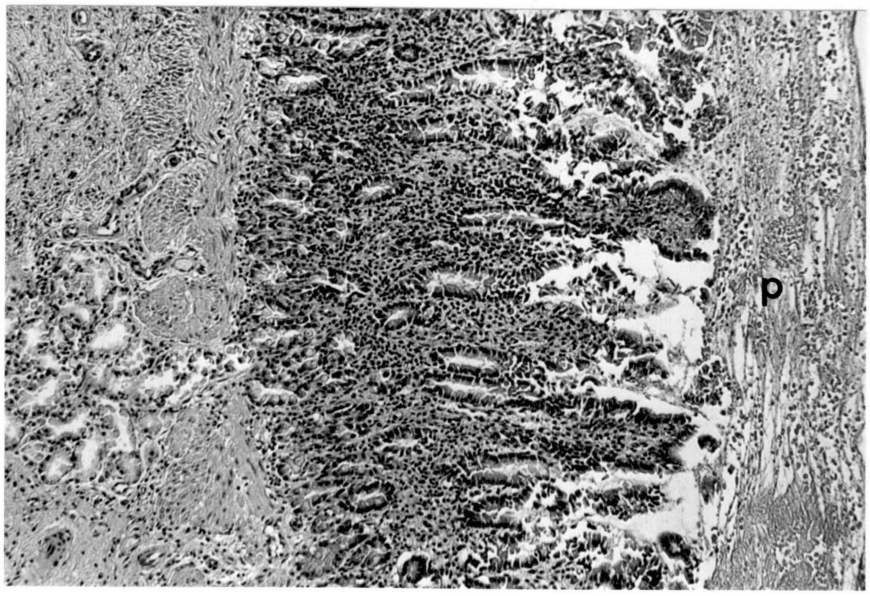

Figure 13. Duodenum. Pseudomembrane of fibrin and cell debris (p).

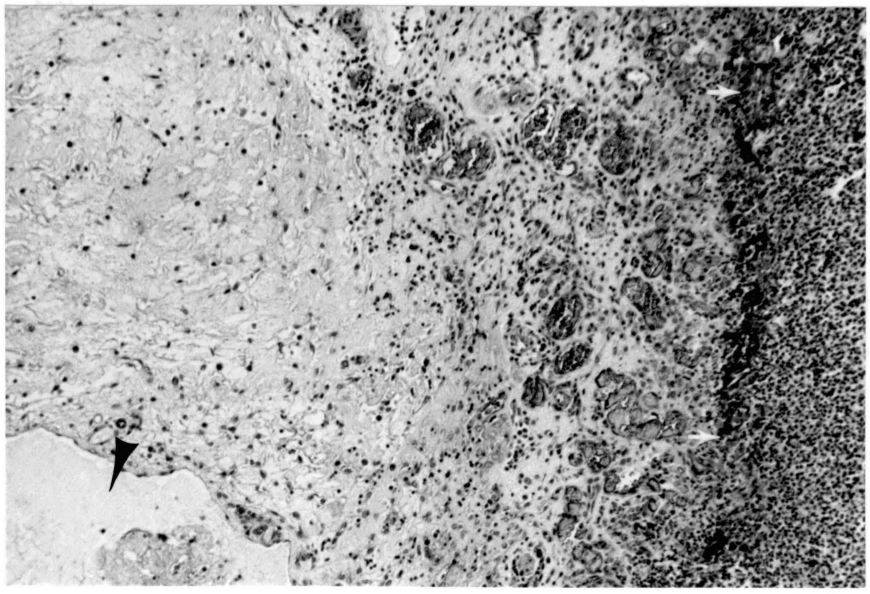

Figure 14. Colon. Deep ulcer covered with exudate and debris (white arrows). Submucosal edema and inflammation. Dilated lymphatic containing protein and debris (black arrow).

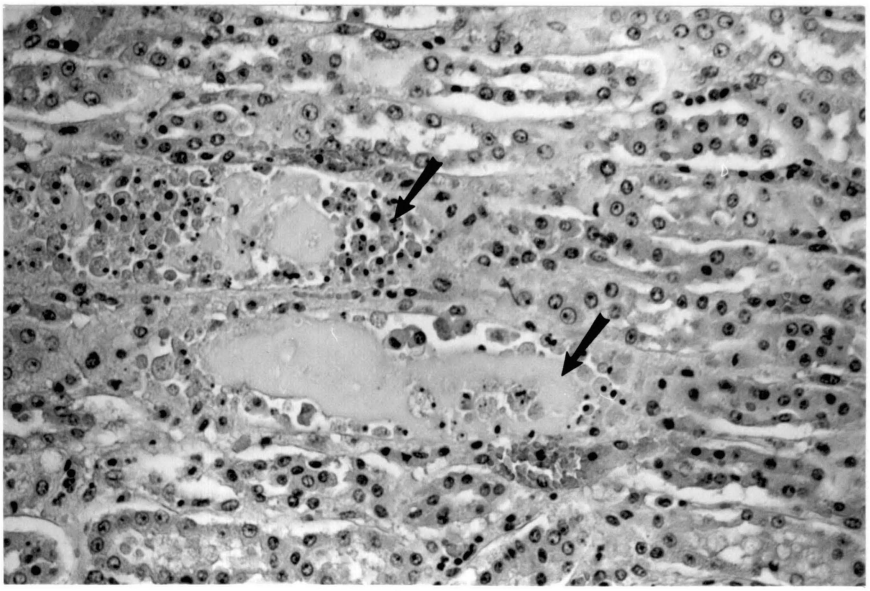

Figure 15. Kidney. Necrotic cells and protein in two tubules of a medullary ray (arrows).

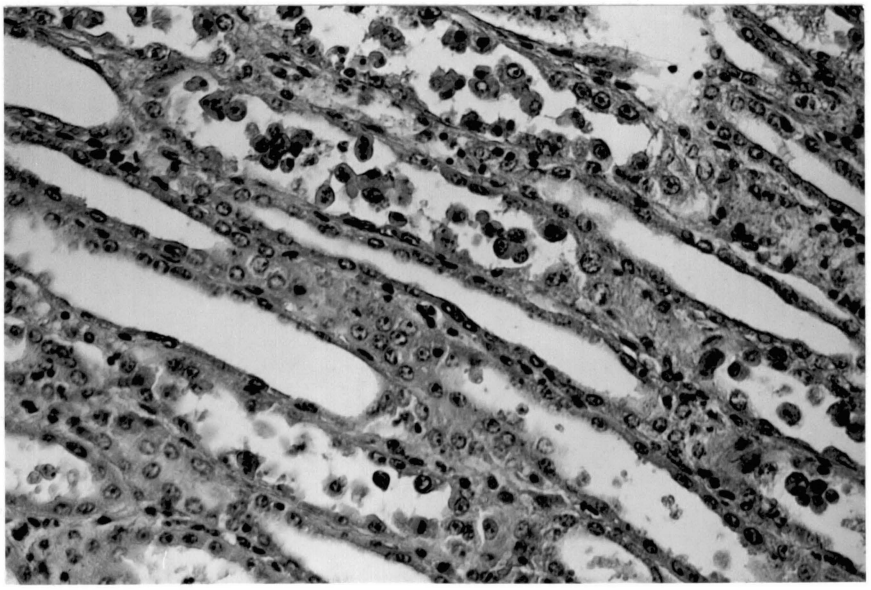

Figure 16. Kidney. Degeneration and necrosis of tubular epithelium in medulla.

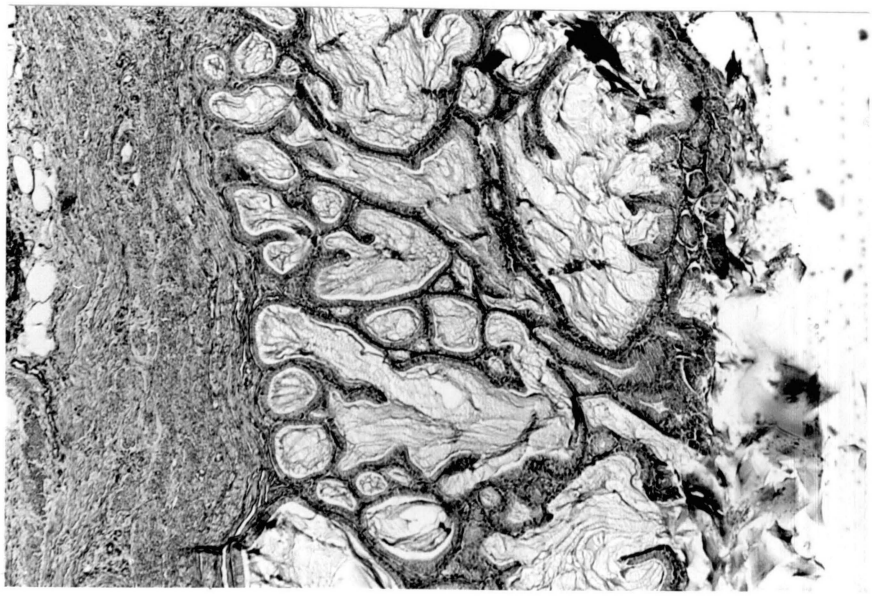

Figure 17. Renal pelvis. Dilation of mucous glands with excessive mucus.

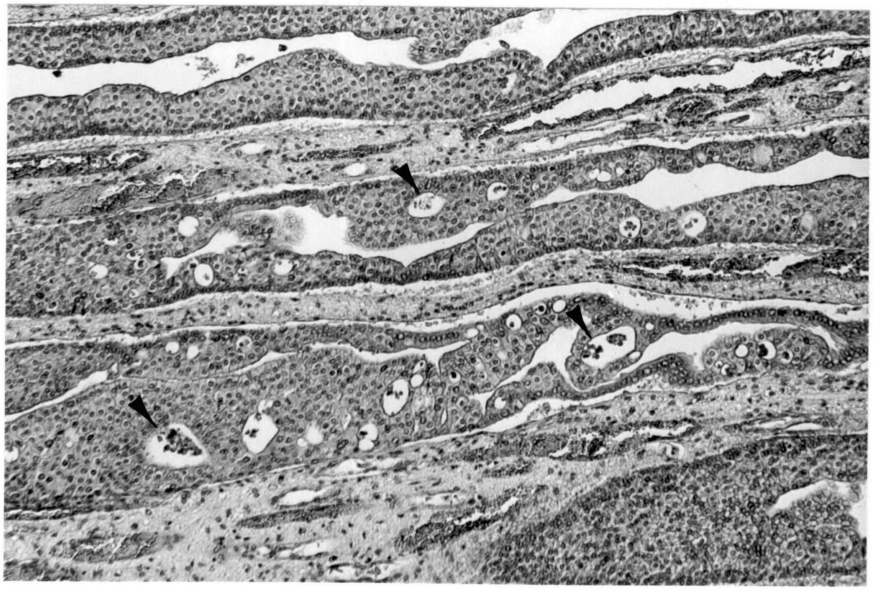

Figure 18. Renal crest. Spaces, some containing cell debris, in transitional epithelium.

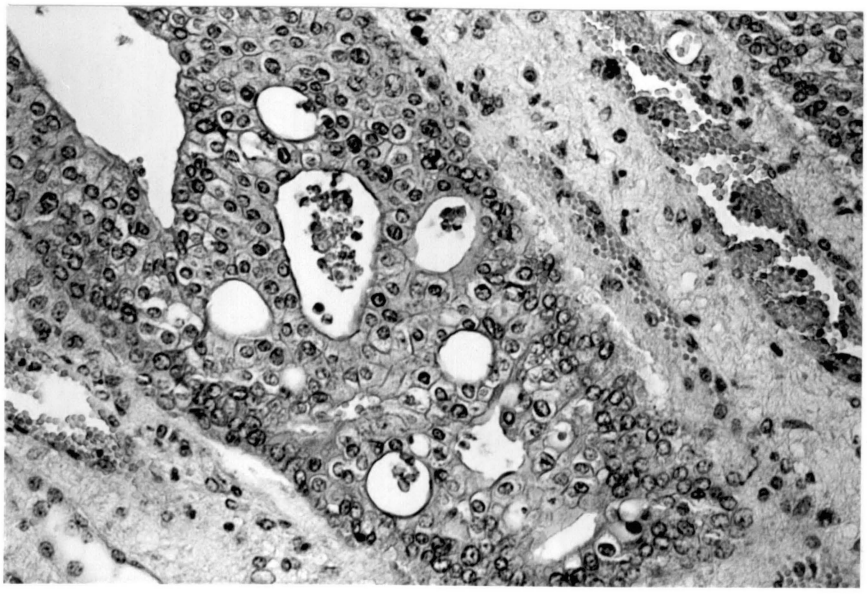

Figure 19. Renal crest. Spaces, some containing cell debris, in transitional epithelium.

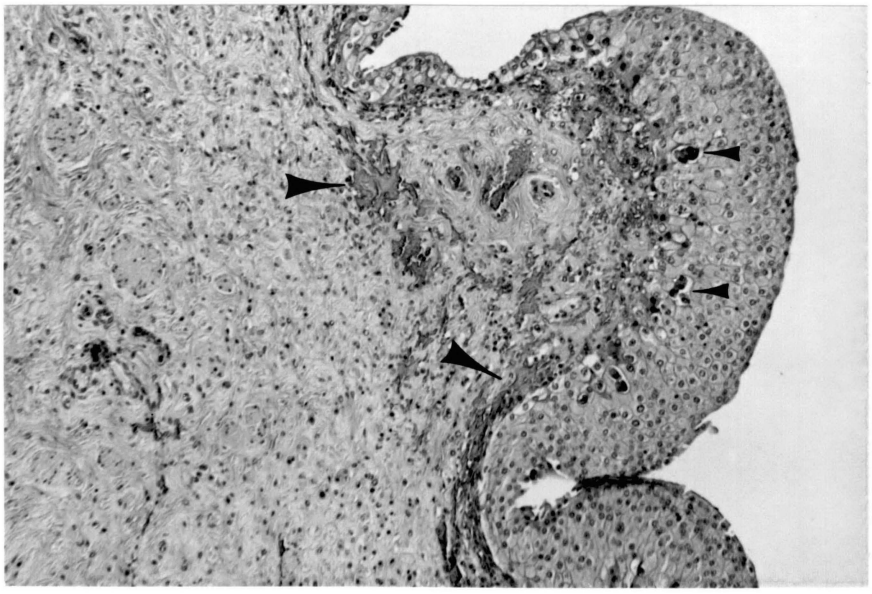

Figure 20. Urinary bladder. Necrosis of epithelial cells (small arrows) and hemorrhage in lamina propria (large arrows).

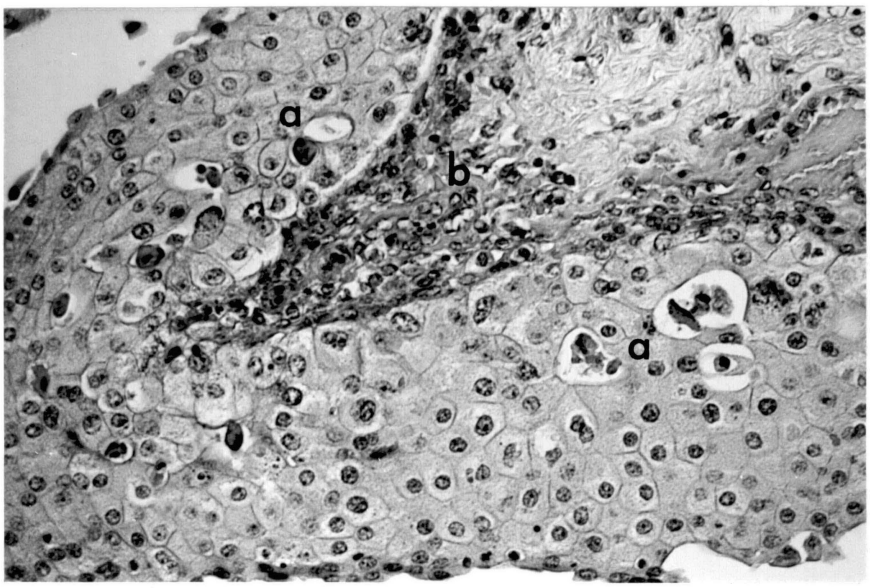

Figure 21. Urinary bladder. Epithelial cell necrosis (a) and hemorrhage in lamina propria (b).

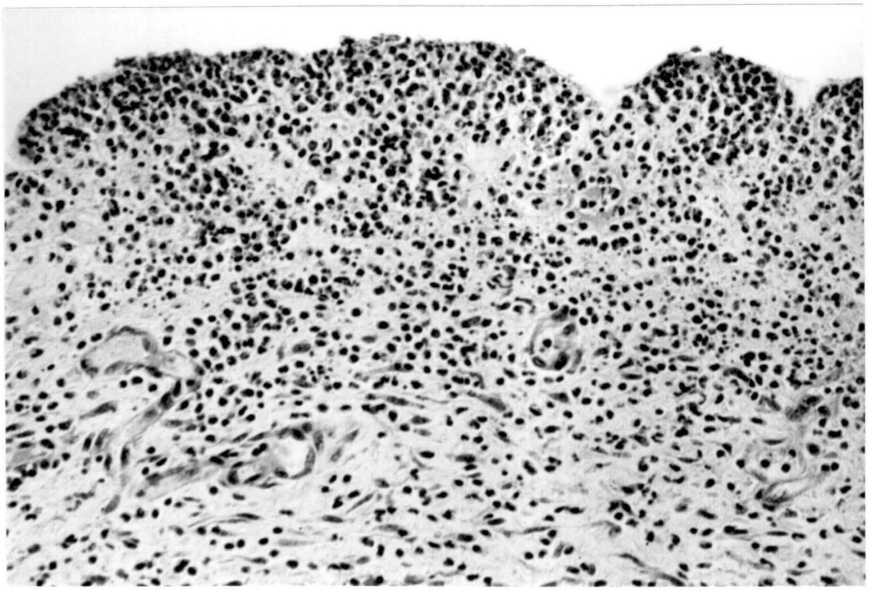

Figure 22. Urinary bladder. Epithelium denuded and lamina propria acutely inflamed.

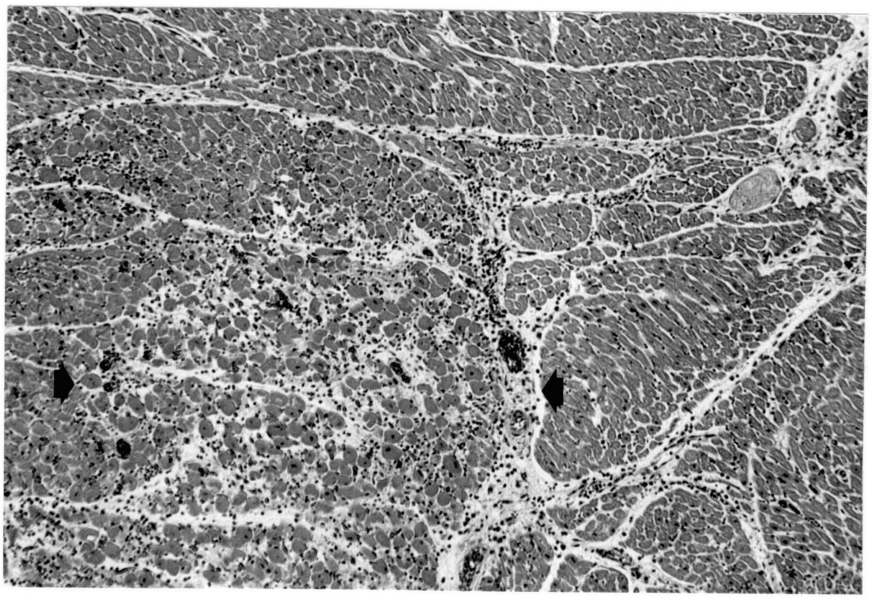

Figure 23. Myocardium. Area of acute necrosis (arrows), with swollen fibers separated by edema and a few neutrophils.

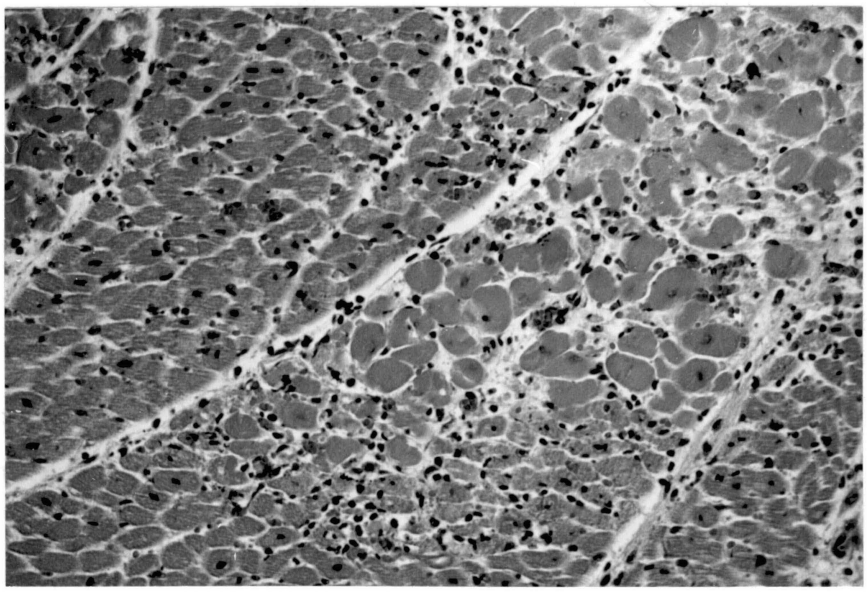

Figure 24. Myocardium. Focus of swollen fibers with karyolysis and homogeneous cytoplasm, and slight neutrophilic reaction.

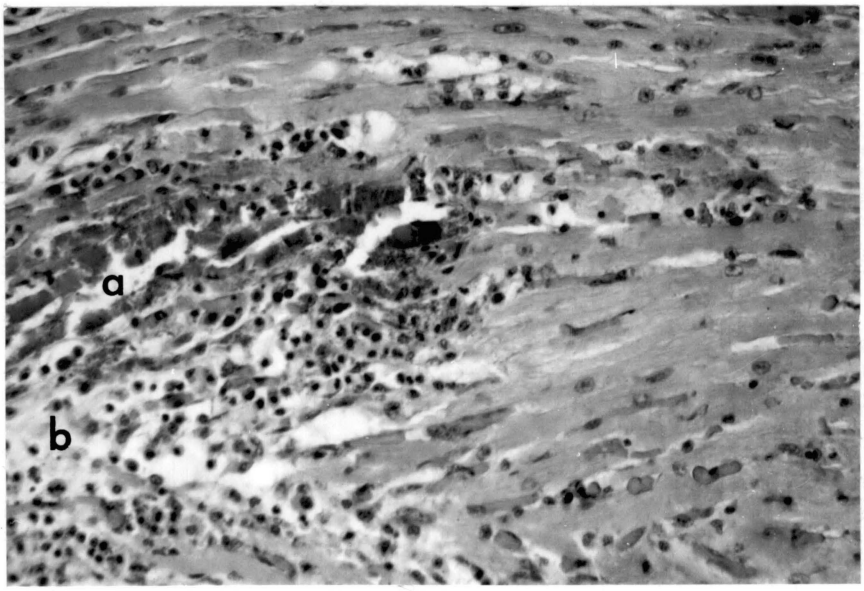

Figure 25. Myocardium. Fragmentation and mineralization of necrotic muscle fibers (a) with cellular reaction (b).

VITA

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