AN ABSTRACT OF THE THESIS OF

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Title: A Study of Chronic Senecio jacobaea Toxicosis in Calves

and Ponies

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//A./ Morrie Craig /

An experiment was designed to simulate field conditions of tansy

ragwort poisoning in cattle and horses.

Four calves and four ponies were fed pelleted feed containing 5%

Senecio jacobaea and two ponies and two calves were used as controls.

The feeding continued for two months. Following this, the chronic

delayed response of the animals was determined. Liver biopsy was per-

formed every two weeks and serum activity of alkaline phosphatase,

gamma-glutamyl transferase and glutamate dehydrogenase was determined

at the same time.

Serum glutamate dehydrogenase activity rose within two weeks, in

many cases followed by the other two enzymes. The glutamate dehydro-

genase peaked and dropped to a lower level before death. Both the

alkaline phosphatase and the gamma-glutamyl transferase remained

elevated many weeks after the consumption of Senecio had ceased.

Some previously unreported microscopic lesions in the pony and

calf are described. One of the lesions was an intranuclear vacuole.

These vacuoles were seen in biopsy specimens only and not in post-

mortem tissues. These lesions had been previously described in rats

poisoned with pyrrolizidine alkaloids. An electron microscope was used to detect the lesions in the previously reported rat studies. They are reported to be invaginations of the cytoplasm into the nucleus. Some cytoplasmic invaginations were seen in a limited electron microscope study in this experiment. These nuclear changes were easily seen with the light microscope in liver biopsy tissue of both calves and ponies.

The serum alkaline phosphatase activity rose corresponding to biliary hyperplasia. The gamma-glutamyl transferase activity paralleled the alkaline phosphatase activity in most cases, but the magnitude of change was much greater. The gamma-glutamyl transferase activity was more indicative of active necrosis and returned to normal even with some remaining biliary hyperplasia. Glutamate dehydrogenase activity increased with active necrosis of cells in Rappaport's "zone one" of the liver lobule, the periportal areas.

A Study of Chronic Senecio jacobaea
Toxicosis in Calves and Ponies

by

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I. INTRODUCTION

The plant Senecio jacobaea has a world-wide distribution (1). It
is commonly called tansy ragwort in the Pacific Northwest, and stinking
willie in parts of Eastern Canada (2). Senecio jacobaea is a modified
biennial and will grow any place that gets 20 inches or more of annual
rainfall (3). It grows two to four feet tall in the flowering stage
and has many golden yellow daisy-like flowers. The leaves are divided
into lobed and toothed leaflets. The seed cases are equipped with many
white hairs resembling those of thistle down (4). The rosette stage
develops in the first year and grows near the ground.

Senecio jacobaea was first suspected of being poisonous in England
in 1787, and animal feeding experiments were conducted in New Zealand
as early as 1903 (5). There are numerous reports of its poisonous
effect on livestock (1, 4, 5). Cattle and horses are most commonly
affected and swine are also very sensitive. Sheep seem to be resistant
(1) and in fact have been advocated to control the weed and prevent
poisoning in cattle (6). Deer also seem to be somewhat resistant (7).
Senecio has produced poisoning in humans (8). It has been shown that
rats are able to transfer the toxic principales to their young in the
milk (9). There have been some suggestions that cattle could also
transmit the poison in the milk (10, 11, 12). Recent studies, however,
were unable to show lesions in calves fed milk from their dams who were
fed tansy ragwort until they were dead or moribund (13).

Tansy ragwort was first introduced into Tillamook County, Oregon in 1936 (1). Since that time it has spread throughout the western parts of Oregon, Washington, and Northern California. By 1971 over 500,000 acres in Oregon had become infested with the weed (14). It has been estimated that tansy ragwort costs Oregon agriculture over $20,000,000 a year, and produces more deaths to livestock in Oregon than all other poisonous plants combined. There is over 1.2 million dollars lost annually due to tansy poisoning by the cattle industry alone (15).

Most of the animals poisoned by tansy die within one to two weeks from when signs develop (1). In some cases this may be months after the consumption of the plant has stopped. There is as yet no satisfactory antidote for the poisoning.

A number of experiments have been carried out on Senecio jacobaea poisoning in horses and cattle in recent years, but all of these have been acute experiments in which the animals have been fed until they died (16, 17, 18). As of 1970 sequestial hepatic changes in domestic species receiving small or intermittent doses of the alkaloids in Senecio had not been studied (19). The disease produced by consumption of Senecio plants by grazing animals is characteristically chronic in nature. The disease is progressive and death may take place weeks to months after consumption of the plant has ceased (5). Although most of the signs of Senecio jacobaea poisoning may be due to failure of one or more liver functions, none of the signs is specific for liver disease let alone for Senecio jacobaea poisoning (12). The disease is difficult to diagnose due to the delay in signs (20). Also symptoms are secondary to primary damage of the liver. It is impossible to accurately diagnose

without liver function tests or liver biopsy (1).

This paper reports long-awaited studies on the chronic poisoning of calves and ponies. Serum activities of some liver enzymes not frequently used were studied along with sequential hepatic changes which were determined by liver biopsy.

II. REVIEW OF THE LITERATURE

Alkaloids in Senecio jacobaea

The poisonous substances in Senecio jacobaea are pyrrolizidine
alkaloids. Bull et al. (5), in a monograph of the pyrrolizidine
alkaloids, states that they are present in a number of plants. This
includes plants of the genera Senecio, Crotalaria, Amsinckia, Echium
and Heliotropium. James et al. (20) list Senecio, Crotalaria and
Heliotropium as three common genera of plants in Western United States
that contain pyrrolizidine alkaloids.

Most of the pyrrolizidine alkaloids contain hydroxy-methyl groups
and therefore are amino alcohols. Over 100 different pyrrolizidine
alkaloids are known. New alkaloids are continuing to be found in
Senecio. Segall (21) has isolated six different alkaloids from Senecio
jacobaea. He gives evidence that there are other pyrrolizidine
alkaloids in the plant, since a larger number of peaks are obtained
using high pressure liquid chromatography. Some of the peaks have not
yet been identified.

The Clinical Disease

It is reported by Muth (1) that cattle and horses usually avoid
eating the plant because of its bitter taste. They may, however, eat
it in the rosette stage when the pasture is short, and occasionally
they will develop a lethal addiction to it. The younger plants are
more apt to be eaten when mixed in with short grasses and cannot be
separated by cattle. Cockburn (22) published a report of Senecio

jacobaea killing a large number of cattle when it was in pelleted feed.
Hay containing Senecio is especially dangerous. Both Bull (23) and
James (20) claim that the poisoning is usually chronic in nature. Muth
(1) claims death may occur some five to six months after the ingestion
of the plant.

Although the clinical signs of Senecio poisoning can be related to
failure of one or more of the liver's functions, they are quite variable.
In cattle, diarrhea is a fairly common finding. A transient diarrhea
in "Pictou" cattle disease in Canada is reported by Tilt (2). Duby (24)
has found diarrhea sometimes with blood. According to Muth (1) the
disease may resemble Johne's disease with chronic diarrhea. Diarrhea
containing a sticky bile material is discussed by Harris (25). In
England, Cockburn (22) reported diarrhea in a group of 65 Friesian cows
poisoned by common ragwort. Four out of five cases reported by Pearson
(12) had diarrhea.

Tenesmus which is present in many cases is believed by Finn and
Tennant (26) to be due to hepatic encephalopathy. Hepatic encephalo-
pathy is the name given the central nervous system dysfunction which is
secondary to liver damage. Tenesmus in Senecio poisoning cases is also
reported by Bull (5), Duby (24), Cockburn (22), Fowler (10), Muth (1),
Pearson (12) and Tilt (2). Rectal prolapse is a common sequella of the
tenesmus. Duby (24) claims 30% of his cases had rectal prolapse, and
three out of five cases recorded by Pearson (12) had rectal prolapse,
as did one of Fowler's (10) cases.

Although there is extensive liver damage in Senecio poisoning,
icterus is not commonly reported (1, 10). Only three of five calves

experimentally poisoned by Ford and Ritchie (17) had increased bilirubin levels, and these occurred terminally. Most cases lose weight and may become emaciated (1, 2). Many non-specific signs of disease such as depression and a dull hair coat have been seen (22, 24).

Many signs suggesting a central nervous system dysfunction have been noted. Ataxia and dysmetria were seen in the cattle with hepatic encephalopathy reported on by Finn and Tennant (26). The two claves examined by Fowler (10) also had ataxia and dysmetria. Some of the animals acted blind (10, 22). Muscle tremors and jerking have been seen in several cases (10, 12, 26). There was bellowing in some calves described by Finn and Tennant (26). There may be a manic phase shortly before death of the animal (24). Terminally the animals are paralyzed, unable to rise, and progress into a coma (2, 10, 12). Spinal reflexes in the heifer described by Tilt (2) remained normal, as did the pupillary reflexes.

Duby (24) reports some degree of ascites in all of his cases. Two of the five cases described by Pearson (12) had marked ascites. Ascites without edema in other locations of the body is usually due to portal hypertension (27). Portal hypertension may be post hepatic and due to right heart failure. It also could be prehepatic if there were an obstruction of the portal vein. The blood flow is impaired in the liver itself in cases of hepatic injury. This may be either presinusoidal or postsinusoidal. In either case there is an increased intravascular hydrostatic pressure in the tissues drained by the portal vein which leads to the ascites (28). Heart failure could also cause ascites, but there would be other signs such as edema and distended peripheral veins

(28). A low serum albumin could reduce the osmolarity of the blood and contribute to ascites and edema. Serum protein levels must be quite low, usually below 4 gm/dl in order to cause ascites and edema.

In the horse, non-specific signs of debility and disease are often seen. McLintock and Fell (29) found inappetence and constipation in a horse with acute ragwort poisoning. They also claim most cases are without jaundice. Ford's (16) cases had a depraved appetite and loss of flesh.

Signs referable to central nervous system dysfunction are quite frequently seen in the horse. Ford (16) reports seeing ataxia, dullness, yawning, head pushing and intermittent coma in his experimental cases in horses. He assumed these were all part of hepatic coma. The case reported by McLintock and Fell (29) had a staggering gait, bumped into things, stood with its head in the corner and sometimes had its front legs crossed. It also was dull and yawned quite frequently. They found no pupillary reflex in this horse. Hill and Martin (30) also have seen nervous signs and suggest walking disease in Nebraska is a pyrrolizidine alkaloid poisoning.

Hepatic encephalopathy is often the most pronounced manifestation of pyrrolizidine alkaloid poisoning in horses. The signs described in the above paragraph can all be related to hepatic encephalopathy. It has been associated with an increased blood ammonia level (28). According to Reynolds (31) only the liver has enzymes capable of converting nitrogen degradation products into urea. There is evidence recently that increased blood ammonia may not be the sole cause of the nervous disturbance. Zieve et al. (32) found mercaptans and short chain fatty

acids worked synergestically with ammonia to produce coma in rats. Ammonia levels equal to those seen in hepatic coma will not consistently produce nervous signs in otherwise normal animals. Schenker et al. (33) suggest certain biologic amines and metabolic products may contribute to hepatic encephalopathy along with other neurotransmitters. They also say there may be an increased sensitivity of the nervous tissue. Strombeck et al. (34) found abnormal amino acid concentration in dogs with hepatic disease that could have altered the function of the central nervous system.

In dogs and humans with hepatic disease or portal-caval shunts, there is an increased concentration of certain aromatic amino acids in the plasma and a decreased concentration of some of the short branched-chain amino acids such as leucine, isoleucine and valine. The branched-chain amino acid concentrations may have been low because of an increased rate of utilization. Aromatic amino acids are precursors for some of the neurotransmitters. An increase in some of the inhibitory neurotransmitters such as serotonin could lead to some of the depressant signs seen in hepatic encephalopathy.

Pathology of Senecio Poisoning

Senecio has been considered by some to be synonymous with pyrrolizidine alkaloid poisoning. As has already been stated, there are many different pyrrolizidine alkaloids. It will be pointed out that their toxicity is not the same and their manifestation may vary in different species.

Some of the gross lesions described by Muth (1) in cases of

Senecio jacobaea poisoning in livestock are: Ascites and mesenteric edema, a hard yellow liver, and petechial hemorrhage. Duby (24) describes finding a distended gall bladder with thick walls on post-mortem examination of cattle dying of tansy ragwort intoxication. According to Smith and Jones (35), distention of the gall bladder occurs in simple stomached animals if there has been no recent meal eaten. Water is reabsorbed from the bile by the mucosa of the gall bladder and mucus secreted; as the bile remains in the gall bladder longer, it becomes more concentrated and contains more mucus.

Bull (5) describes the gross lesions in cattle. The livers are turgid, tough, atrophic and not fatty. There is often increased fluid in the peritoneal cavity (ascites), edema of the omentum and mesentery, and edema of abomasal folds. In horses he has described distended stomachs and hydrothorax, but no macroscopic central nervous system lesions.

The liver may respond to insult in a number of ways. In addition to the actual damage and death of hepatocytes, Jubb and Kennedy (19) cite three possible responses of the liver to insult. One is the actual regeneration of hepatic parenchymal tissue. This as we shall see later may actually be impaired in pyrrolizidine alkaloid poisoning. The second is fibrosis. Connective tissue may be laid down around areas of irritation or to replace dead cells. The last response reported is hyperplasia of bile ducts or their epithelium. This biliary hyperplasia may occur independent of parenchymal changes. It is often a response to certain poisons, and the excess ducts may disappear when the insult is removed. The biliary hyperplasia may accompany fibrosis.

Smith and Jones (35) say that necrosis of the hepatocyte may be preceded by cloudy swelling or in some cases fatty degeneration. Pyknosis of nuclei and an acidophilic cytoplasm are early signs of cell necrosis. Jubb and Kennedy (19) describe the cloudy swelling to be apparent by the swollen cells with finely granular cytoplasm. The cloudy swelling is not often seen in biopsies because it is agonal. The granular clumping of the cytoplasm may be preceded by pyknosis of the nuclei.

Smith and Jones (35) also describe proliferation of connective tissue, newly formed bile ducts and a distorted architecture as characteristic of the cirrhosis of chronic toxic liver disease. They claim one effect of portal cirrhosis is interference with blood flow through the liver which results in congestion of the spleen, digestive organs, and all structures drained by the portal system.

In order to understand the action of toxic substances on the liver, it is important to know the functional organization of the liver. The anatomical unit of the liver has for a long time been considered the hexagonal liver lobule with the central vein in the middle and several portal triads at the periphery. Rappaport (36, 37, 38), however, has proposed a functional unit related to the blood supply to the liver. He and his co-workers used various dyes injected into branches of the portal vein to identify the acinar structure supplied by the same blood source. He found the hexagonal lobule was formed by halves of six different acinar units around the periphery. The central vein is at the periphery of his acinar unit, and the acinus supplies central veins of two adjacent hexagonal lobules. His functional unit is therefore a

berry-like mass of parenchyma around the trio of terminal branches of the artery, vein and bile duct.

Rappaport (36) has divided the functional liver unit into three zones. His zone one is nearest the portal triads. Zone two is located between one and three, and zone three is a half circle of cells interconnecting two central veins. Vascular twigs from the portal vein and hepatic artery empty into the sinusoids of zone one. Zones two and three receive blood from the sinusoids of zones one and two respectively.

Rappaport (36) also claims the enzymes vary in the different zones. The respiratory enzymes are more concentrated in the zone one cells where the oxygen tension is greatest.

Grisham (39) has injected labeled deoxyribonucleic acid (DNA) prior to mitosis to show that most of the replication and growth takes place in the areas near the portal tracts. Later it extends toward the central veins.

The most characteristic microscopic structural change in pyrrolizidine alkaloid poisoning is hepatic megalocytosis. The term megalocytosis was used by Bull (40) to describe changes he considered at the time to be pathognomonic of pyrrolizidine alkaloid poisoning. There is an increase in size of both the cell and the nucleus. Jubb and Kennedy (19) claim the volume of the hepatocyte may be increased 20 times normal. They also describe thick nuclear membranes, scanty fragmented chromatin and an enlarged nucleolus. Bull (40) also says there may be four to six times as many nuclei in an area of normal liver than in an area with megalocytosis. Some cells may also be multinucleated.

Bull (40) also describes eosinophilic globules in the hepatocytes.

These are from 2 to 30 microns in diameter and vary in intensity of staining. He has seen them in pyrrolizidine alkaloid poisoning, but not in carbon tetrachloride poisoning. Some of these eosinophilic bodies are PAS positive according to Bull et al. (5).

Biliary hyperplasia is commonly seen in cases of pyrrolizidine alkaloid poisoning as it is in other causes of hepatic insult. Muth (1) reports a proliferation of biliary epithelium. Bull (23) describes a proliferation of bile duct epithelium along with an increased number of bile ducts. Thrope and Ford (41) found ductule proliferation in some of their experimental cases. Jubb and Kennedy (19) suggest that the biliary hyperplasia may be an attempt to regenerate parenchyma for which the liver has lost its ability. Hooper (42) did not find bile duct hyperplasia in mice acutely poisoned with Senecio jacobaea. Ford (16) did find new bile duct formation in horses experimentally poisoned with ragwort.

There is obviously eventual death of hepatocytes in pyrrolizidine alkaloid poisoning. Jubb and Kennedy (19) describe the cells as dying slowly not as a tissue. Muth (1) reports central lobular necrosis with hemorrhage. Thorpe and Ford (41) also report centrolobular necrosis. Hooper (42) has seen central lobular necrosis in mice. Bull (23), however, has found that in chronic cases the central lobular area is unaffected. In the chronic cases there is more mid-zonal and peripheral lobular involvement. Only in acute poisoning with high doses is the central lobular area involved first.

Fibrosis will usually eventually occur. Muth (1) describes a marked hepatic fibrosis with collagen fibers isolating masses of

parenchymal cells and disrupting the architecture. Thorpe and Ford (41) describe cirrhosis in their experimental calves. Bull (23) says fibrosis occurs in the portal areas first along with bile duct proliferation. He also says fibrosis is seen more in cattle than in horses and much less in rats.

Pyrrolizidine alkaloid poisoning has long been considered a veno-occlusive disease. Bras and Hill (43) describe cases of veno-occlusive disease in children in Jamaica who had consumed plants containing pyrrolizidine alkaloids. There was occlusion of the central lobular vein and the hepatic vein. This resulted in congestion and a non-portal cirrhosis. They said it was similar histologically to _Senecio_ poisoning in cattle. Berry and Bras (44) report on veno-occlusive disease due to consuming _Crotalaria_ and _Senecio_. There are ascites, edema and a nutmeg liver with central lobular congestion. Obliterating lesions of the hepatic venous tree are due to subendothelial swellings. They claim the condition is similar in animals and humans. Veno-occlusive lesions were described by Thorpe and Ford (41) in their experimental calves. They found thickening of the walls of central and hepatic veins. Ford (16) also found veno-occlusion in experimental horses, with cirrhosis occluding the central veins. Jubb and Kennedy (19) claim perivenous fibrosis causes veno-occlusive disease and that the lesion is not constant in pyrrolizidine alkaloid poisoning.

Jubb and Kennedy (19) bring out the point that the effect of pyrrolizidine alkaloid poisoning may be mimicked by aflatoxicosis. There may be nucleolar changes and megalocytosis in both. Chronic aflatoxicosis has been reviewed by Newberne (45).

In other species with different pyrrolizidine alkaloids and at unusual doses, other organ systems may be affected. Pulmonary vascular lesions are reported in monkeys fed monocrotaline by Chesney and Allen (46). Pulmonary vessels were obliterated by enlargement of the endothelium and hypertrophy of the medial musculature. Carstens and Allen (47) describe hyalinization of glomeruli in rats fed crotalaria. The basal lamina obliterated the glomerular capillaries. Muth (1) reports hyperepithelialization of the alveoli of the lungs of pigs with tansy poisoning. Harding and Lewis (48) also describe lung lesions in pigs experimentally poisoned by Senecio jacobaea. In the mice poisoned by Hooper (42), there were increases in the size of bronchial epithelial cells. Nuclear inclusions were also seen in the bronchial epithelium. In the kidneys of these mice, tubular epithelial cells were enlarged in both the proximal tubule and in the loop of Henle. Allen and Carstens (49) found vascular lesions in the lungs of rats given the alkaloid monocrotaline, or fed Crotalaria spectabilis. There were hyalin thrombi and bronchial hyperplasia in addition to the large hepatocytes.

Some of the first changes in pyrrolizidine alkaloid poisoning occur in the nucleus. Jubb and Kennedy (19) report a separation of the granular portion of the nucleolus. Bull (5) also describes a fragmentation or spatial rearrangement of the nucleolar constituents. This may occur within one half hour and revert to normal within 72 hours. Svoboda and Soga (50) found primary nucleolar changes in rats one hour after injecting pyrrolizidine alkaloids, using the electron microscope. They also found cytoplasmic invaginations of the rat hepatocyte nuclei using the electron microscope. Allen et al. (51), using the electron microscope,

found nuclear inclusions which turned out to be cytoplasmic invagina-
tions in hepatocytes of rats fed Crotalaria spectabilis. There were
numerous nuclear inclusions in each nucleus. Most of them were flask
shaped and remained connected to the cytoplasm, although a few broke
loose and formed sequestra in the nucleus. Novikoff and Essner (52)
claim nuclear inclusions are usually invaginations of the cytoplasm.

The brain lesions described in some cases of pyrrolizidine alkaloid
poisoning are probably not a direct effect of the alkaloid, but a part
of hepatic encephalopathy. Finn and Tennant (26) report hepatic
encephalopathy in cattle with megalocytes in the liver. The brain
lesions in these cases consisted of polymicrocavitation of the cortex
and juxta cortical white matter. There was also astroglial hypertrophy
and myelin degeneration in the medulla. Hooper et al. (53, 54) also
reported brain lesions with pyrrolizidine alkaloid poisoning. They
found a correlation between the brain changes and the blood ammonia
level. The lesions consisted of spongy degeneration of white matter
and sub-cortical grey matter. The glutamine content of the cerebral
spinal fluid was also increased.

Foley (55), describing hepatic encephalopathy in general, lists
hypertrophy of the protoplasmic astrocytes as the most common micro-
scopic change in the brain. He says the nerve cells are not morphologi-
cally changed and does not believe the astrocyte changes are specific.
Schenker et al. (33) list hypertrophy of astrocytes, neuronal degenera-
tion and demyelination as the most commonly reported lesions in hepatic
encephalopathy. They consider the condition mainly a metabolic dis-
arrangement.

Metabolism of the Alkaloids

According to McLean (56) the pyrrolizidine alkaloids disappear from the blood quite rapidly. Over one-half of the alkaloid is eliminated in the urine with its basic structure intact. Another large fraction is broken down and appears in the bile. Labeled alkaloid adheres to liver, intestines and kidney but not other tissues. Some of the metabolites such as the N-oxides and glucuronide conjugates are less toxic than the alkaloid.

Mattocks (57) claims the pyrrolizidine alkaloids themselves and some of their metabolites are not toxic. He found there is a good degree of correlation between the degree of hepatotoxicity and the amount of pyrrole found in the liver. Pyrroles are found in the urine and in the liver after administering pyrrolizidine alkaloids. Some are bound strongly to tissues of the liver. If pyrroles are given intravenously, lung damage will occur. Mattocks and White (58) later showed how pyrrolizidine alkaloids or their derivatives could be dehydrogenated to dihydropyrrolizidine or pyrrole derivatives. These derivatives were toxic and reactive. Mattocks and White (59) then used rat liver microsomes in vitro to convert pyrrolizidine alkaloids to pyrrole derivatives and N-oxides. The enzymes had characteristics of mixed function oxidases. Induction of the enzymes with phenobarbital or DDT induces a greater production of pyrrole than N-oxides. Inhibitors of the liver microsomal enzymes decreased the formation of pyrroles. They assumed there was a separate pathway for the two conversions.

Mattocks (60) reports the capacity of liver microsomal preparations to convert retrorsine to pyrrole metabolites is low in very young

rats. After one to 17 days of age the capacity was about the same as adults. The susceptibility to retrorsine poisoning had comparable age differences. Jago (61) was also able to convert heliotridine based pyrrolizidine alkaloid to a pyrrolic derivative, dehydroheliotridine, in vitro, by using rat liver microsomes. Only trace amounts were formed from N-oxides. Jago (62) also was able to enhance the toxicity of lasiocarpine and heliotrine to rats by stimulating their microsomal enzymes with phenobarbital. Allen et al. (63) demonstrated more severe lesions in pyrrolizidine-treated rats after stimulating the microsomal enzymes with phenobarbital. If they were pretreated with the enzyme inhibitor chloramphenical there was an absence of mortality and less severe lesions.

Shull et al. (64) demonstrated a decline in pyrrole production one hour after dosing with alkaloids from Senecio jacobaea. They suggested the pyrroles may inhibit the enzymes involved in their own production and have a direct action on microsomal enzymes. Buckmaster et al. (65) showed that cysteine treatment considerably increased the survival time.

There is considerable evidence that these pyrrole derivatives are toxic metabolites in pyrrolizidine alkaloid poisoning. Mattocks and White (66) observed that alkaloids of greater toxicity are more rapidly converted to pyrrole derivatives in vitro by microsomal preparations. Shull et al. (67) claim that with the exception of the rabbit and chicken there is a direct relationship between the rate of pyrrole production in vitro and the susceptibility of the species to pyrrolizidine alkaloid poisoning. Armstrong and Zuckerman (68) were able to induce changes in both liver and lung cells in culture by using retronecine
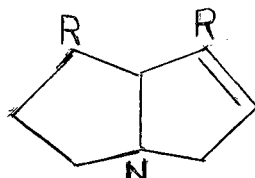
pyrrole. The alkaloids lasiocarpine and retrorsine were toxic only to liver cells. They concluded that the lungs were unable to metabolize the pyrrolizidine alkaloids to the toxic pyrroles, but the liver could. Lasiocarpine applied to human embryo liver cells resulted in inhibition of DNA, RNA and protein synthesis and the formation of megalocytes.

Cheeke (69) in a recent review of the subject says the pyrrolizidine alkaloids themselves are relatively non-toxic, but are metabolized by the liver to produce very toxic metabolites called pyrroles. McLean (56) claims the pyrroles are chemically reactive themselves and capable of alkylating, unlike the parent alkaloids or N-oxides. McLean (70) in a more recent article says the pyrrolizidine alkaloids are metabolically activated by microsomal fractions of liver cells to pyrrole derivatives that can cause either cell necrosis and veno-occlusive disease or abnormal division and megalocytosis in cattle. Jago (61) mentions the pyrrolic derivative is a highly reactive alkylating agent.

In the past, it has been suggested that sheep might be resistant because rumen microorganisms changed the pyrrolizidine alkaloids to a less toxic product. Lanigan (71) reported this was done by certain bacteria in the sheep's rumen. Russet and Smith (72) claimed a gram negative coccus in the rumen of sheep caused a reductive fission of hepatotoxic pyrrolizidine alkaloids. More recently, Shull et al (67) incubated pyrrolizidine alkaloid with sheep rumen fluid and could show no effect on the toxicity of the alkaloid.

Theories of Action

Schoental (73) has tried to relate variability in response to different pyrrolizidine alkaloids to variation in molecular structure. A double bond in the moiety seems to be essential for hepato toxicity. There is no effect from products of hydrolysis. The basic structure for toxicity is:

McLean (56) claims three essentials are necessary for toxicity. The first is a double bond at position one-two of the ring nuclei. Second, it must carry esterfied hydroxyl groups, and third, at least one of the ester side chains must contain a branched carbon chain.

There is considerable biochemical and histopathological evidence that nuclear damage occurs in pyrrolizidine alkaloid poisoning. Direct damage to DNA has been suspected. Culvenor et al. (74) claim the fundamental biochemical lesion in the cell nucleus results from alkylation. Culvenor et al. (75) also have shown the alkylating ability of pyrrolizidine alkaloids is weak but definite. They suggested a possible metabolite caused the antimitotic effect. Alkaloid labeled with ^{14}C was slowly incorporated into liver protein and nucleic acid. Black and Jago (76) were able to bind dehydroheliotridine with calf thymus DNA in vitro to form a soluble complex. Shull et al. (64) report that the pyrrole derivatives are alkylating agents and capable of binding to protein and nucleic acid.

McLean (56) reports a profound drop in DNA mediated RNA synthesis. She relates this to the ultrastructural change in the nucleolus. Reddy

et al. (77) demonstrated a decreased DNA mediated RNA synthesis after lasiocarpine administration. There was an inhibition of labeled uridine incorporation into rat liver nuclear RNA after the rats had been treated with lasiocarpine. There was also a reduction in labeled ribosomal RNA and a reduction in nuclear RNA:DNA ratio. A similar effect could be produced with actinomycin D and aflatoxin. However, Hsu et al. (78) reported a suppression of DNA synthesis resulting in inhibition of cell division and megalocytosis. They used monocrotaline and a metabolite, monocrotaline pyrrole. The pyrrolic derivative had a greater effect in supressing DNA synthesis than did the parent compound.

There is also evidence of decreased protein synthesis by the hepatocyte following pyrrolizidine alkaloid poisoning. Harris et al. (79) found that lasiocarpine caused an inhibition of protein synthesis by a disaggregation of the Polyribosomes. They believed it was unlikely that it was due to decreased RNA synthesis since it occurred so rapidly. They did consider the polyribosomal disaggregation a direct effect. Using lasiocarpine poisoned rats, Svoboda et al. (80) found the RNA polymerase activity the same as in normal liver. They considered the translational mechanism was intact since they were able to induce tryptophan pyrrolase with hydrocortisone.

Schoental (81) proposed that the toxic action of pyrrolizidine alkaloids was due to a depletion of the nicotine adenine dinucleotide (NAD) cofactor. Either a leakage or decreased synthesis could occur. Nicotinamide was protective. Earlier, Christie and Lepage (82) had reported a reduction in the liver's ability to synthetize NAD after poisoning with heliotrine, a pyrrolizidine alkaloid. The mitochondria

would not oxidize certain substances. The failing enzyme systems were all pyridine nucleotide dependent.

Pathogenesis of the Lesions

There seems to be considerable agreement that the pyrrolizidine alkaloids have an antimitotic effect. Downing and Peterson (83) performed partial hepatectomies on rats to demonstrate the inhibition of mitosis by hepatotoxic pyrrolizidine alkaloids. The usual burst of mitosis following partial hepatectomy is inhibited by certain pyrrolizidine alkaloids given prior to the operation. Jago (84) concluded that megalocytosis was due to long-lasting mitotic inhibition. A stimulus for growth such as loss of cells at the same time as the mitotic inhibition accelerated the megalocytosis. DNA replication was not inhibited and the megalocytosis did not disappear. Culvenor et al. (75) also mention an antimitotic effect of pyrrolizidine alkaloids. A stimulus to growth caused by an acceleration of cell death leads to megalocytosis if there is also an antimitotic activity. Jago (85) suggests the severity and extent of megalocytosis is a measure of the persistent antimitotic action of the alkaloid. Experiments by Samuel and Jago (86) demonstrated that both the pyrrolizidine alkaloid and its metabolite stopped mitosis but not the wave of DNA synthesis. Allen and Hsu (87) injected the pyrrole derivative dehydroretronecine subcutaneously into rats. It produced an inhibition of cell mitosis in the gastrointestinal tract. The bone marrow became devoid of precursor cells and there was inhibition in the seminiferous tubules. The antimitosis was most devastating in the rapidly dividing cells. McLean (56) suggests the alkaloid

may inhibit mitosis similar to nitrogen mustard and mitomycin C by linking themselves across two strands of DNA.

Bull (5) has put together a number of ideas on the pathogenesis of the lesions. There is an inhibition of cell division but not cell growth. DNA is still built up causing an enlarged nucleus. The anti-mitotic activity is zonal in nature being located in the mid zone and periphery of the liver lobule. There is an increased amount of DNA in the nucleus of the megalocytes. The alkaloid may pick out the most susceptible cells, those preparing for division in the outer part of the lobule.

Necrosis of hepatocytes may be due to several other possible mechanisms. McLean (56) has listed several possibilities proposed by different investigators. Protein synthesis may be decreased. This may be due to the failure of DNA mediated RNA synthesis. There may be a failure of pyruvate oxidation. A loss of glycogen may occur. Structural damage of the mitochondria has been reported. Failure of mito-chondrial NAD dependent enzymes and failure of nuclear NAD synthesis has been mentioned by Schoental (81) and Christie (82).

The pathogenesis of the lesion and the resulting type of lesion may depend mainly on the rate of dosing. Bull (5) states that with high acute doses there is central lobular necrosis and hemorrhage. In these cases there is less fibrosis and new bile duct formation. In chronic continuous dosage that is more characteristic of the grazing animal, megalocytosis, small atrophic lobules, new bile duct formation, and new fiberous tissue are seen in the liver.

McLean (56) reports three changes present in the acutely damaged

livers. First, cytoplasmic protein synthesis falls rapidly. Second, nuclear changes occur; and third, there is a disaggregation of the polyribosomes. In the chronically damaged liver there is megalocytosis apparently related to the antimitotic activity. Bile duct proliferation and fibrosis occur as a result of the insult and there is a proliferation of the rough endoplasmic reticulum as seen by the electron microscope. Little glycogen is present in the megalocytes. Svoboda et al. (80), however, claim the megalocytes are functionally normal cells except there is cellular hypertrophy and incapability of division due to the potent antimitotic action.

The pathogenesis of the veno-occlusive lesion is somewhat debatable when it does occur. Butler et al. (88) report that the pyrroles are extremely reactive and can cause damage to the endothelium of the central vein. When the alkaloids are injected into the mesenteric vein liver infarcts result. When they are injected into the tail vein lung vascular lesions result. McLean (56) lists four possible causes of veno-occlusive disease. Central lobular necrosis may press on sinusoids blocking the flow of blood. Sinusoidal outlet blocks may occur, most probably caused by necrosis of the wall of the central vein. There may be necrosis of the endothelium. Platlet agglutination may block the flow of blood through the liver. Some alkaloids seem to be more prone to producing veno-occlusive disease than others.

Both Bull (5) and McLean (56) list reports of the carcinogenic effect of pyrrolizidine alkaloids. Bull (5) does not believe that chromosomal damage and mutation play any significant part in the chronic lesions of pyrrolizidine alkaloid poisoning.

Previous Studies of Toxicity

There have been several recent studies on the toxicity of Senecio jacobaea in horses and cattle. For the most part these have been acute studies which involved feeding the animals until they died. There have also been studies made of a few groups of clinical cases. Betty and Markson (89) used liver biopsy to diagnose ragwort poisoning in a herd of cattle in England. They found four clinical cases and then performed a liver biopsy on the remaining 26 head. The affected animals were not treated but converted to meat before they became sick. Vardiman (90) used both liver biopsy and the bromsulfulein liver function test in diagnosing Senecio poisoning in cattle. He considered the biopsies to be good indicators of cirrhosis and to be simple and not dangerous. The sulfabromophthalein retention test was sensitive but time consuming. The tests were performed on calves he had fed Senecio longilobus and Senecio riddellii.

Mortimer and White (91) fed Senecio weeds to calves until they died. It took 55 gm per kilogram of body weight to kill the calves when it was fed at the rate of 68 gm per day. When it was fed at 34 gm per day it took 26 gm per kilogram of body weight to kill the calves. They also claimed it was toxic to sheep when fed at 3 gm per kilogram per day. Dickinson et al. (13) administered Senecio jacobaea intra-rumenally through rumen fistulas in four cows to determine if the alkaloid would be transferred in the milk. The cows were given from 0.25 to 1.00% of their body weight in tansy ragwort each day until they died or became moribund. The cows all lost weight and developed a persistent diarrhea after 7 to 14 days. Plasma protein, especially the

albumen fraction, dropped continually until they died. According to the published graphs, all of the cows were dead in about 40 days. The calves did not become ill.

Ford and Ritchie (17) and Thorpe and Ford (41) fed five calves varying amounts of feed containing 20% ragwort. One calf was fed six pounds per day, two were fed four pounds per day, and two were fed two pounds per day. They were all fed until they died. Ford and Ritchie (17) reported on the serum changes in these calves. Enzyme activity in the serum for glutamate dehydrogenase (GD), sorbitol dehydrogenase (SDH), ornithine carbomyl transferase (OCT), and glutamic oxylacetic transaminase (GOT) were measured. There was increased activity of all of these enzymes by the 25th day for most calves. Serum enzyme activity peaked in from 30 to 50 days depending on the rate that the Senecio was fed and the enzyme involved. Some of the enzyme activity then fluctuated or reached a lower plateau before death. Also before death both SDH and GD fell to normal in the calves receiving 0.4 pounds of ragwort a day. In the calves receiving 0.8 pounds of ragwort a day, all four fell to normal by 45 to 50 days. In the calf receiving 1.2 pounds of ragwort per day, the level of activity of all four enzymes was reduced but still elevated above normal at the time of death.

Thorpe and Ford (41) reported on the histopathological changes in these calves. They reported minimal structural changes when the enzymes first rose. One of the earliest changes reported was karyomegaly. No megalocytosis occurred until day 39 in the calves fed 0.8 pounds of ragwort per day. In the later stages focal megalocytosis and enlarged portal tracts were reported. Veno-occlusive lesions did not occur

until day 136 in the calves fed only 0.4 pounds of ragwort per day. A reduced amount of cytoplasmic RNA was detected. A margination of the chromatin in the nuclei was seen in some of the hepatocytes of the calf fed 1.2 pounds per day. These workers concluded that there was little if any relationship between the progress of the liver structural changes and changes in serum enzyme concentration.

Johnson (18) recently reported on experimentally feeding Senecio jacobaea to cattle. Forty-five calves and cows were given Senecio jacobaea daily by stomach tube.. Feeding at the rate of 1.5 g of plant per kg of body weight a cummulated dose of plant equal to 3 to 4% of the animal's body weight was lethal. Cattle were able to tolerate up to 1.5% of their body weight of dried plant when it was consumed within a 15 day period. Cattle died if 2% of their body weight was consumed within a 20 day period.

A rise in activity of some of the serum enzymes were noted in the above experiment. Lactic dehydrogenase, aspirate aminotransferase and gamma-glutamyl transpeptidase activity was increased. Upon post mortem examination of these animals, several histopathological changes were noted. Nuclear changes consisted of karyomegaly, vesiculation and hyperchromicity. Hepatocyte swelling was seen along with fibrosis which replaced hepatic necrosis. Bile duct proliferation with extensive fibrosis was one of the most prominent features.

In horses, Hill and Martin (30) reported on cases of veno-occlusive disease with blockage of the central veins either by connective tissue or fibrous thrombi. Ascitic fluid was present along with a nutmeg liver. They also found a thickening of the intima of the central

lobular veins. A central lobular fibrosis was described as due to the veno-occlusive disease. Ford (16) reported on poisoning horses with ragwort. He found megalocytosis and veno-occlusive disease. The central veins were occluded with fibrosis. Blood ammonia levels up to four times normal were the significant finding in the terminal disease. Ford (17) also reported an altered activity of serum enzymes in these horses. He found no structural changes at the beginning of the release of these enzymes.

Liver Function Tests

Cornelius (92) states that there are over 100 standard liver function tests for domestic animals. No single test gives status of all of the magnitude of liver functions. Liver function may be interlocked but not evenly impaired with liver damage. Extensive damage is required to impair function due to the great reserve capacity of the liver. Routh (93) divided liver function tests into three categories: metabolic tests, excretion tests and serum enzymes. Zamcheck et al. (94) would add another category, the flocutation tests.

Since the liver has many important metabolic functions, a failure of certain functions may lead to an altered blood level of certain compounds. Most of the plasma protein including all of the serum albumin is synthesized by the liver. Sherlock (95) claims there is a decrease in serum albumin synthesis in liver disease, but since the pool of albumin is so large the rate of decrease in the serum is slow. Ford (17) found no marked change in serum protein in his ragwort poisoned horses. The albumen:globulin ratio decreased before death in

some but not all of the horses.

Gluconeogenesis and other processes of carbohydrate metabolism might be altered. Routh (93) mentions the possibility of using a galactose tolerance test but claims it is insensitive. Since cholesterol is both synthesized and excreted by the liver, the use of serum cholesterol levels is considered by Sherlock (95) but it is not specific. He also claims the determination of bile salts in the serum is a sensitive indicator of liver damage but also a complicated procedure. Cornelius (92) lists several compounds in the blood that could be measured as an index to liver function. These include glycoproteins, blood uric acid and amino acids. Strombeck and Rogers (34) found a reduced amount of branched chain amino acids such as valine, leucine and isoleucine, along with an increased amount of aromatic amino acids such as phenylalamine and tyrosine in dogs with liver damage. Arginine is greatly reduced in massive liver necrosis due to the release of arginase from hepatocytes.

Cornelius (92) also reports deamination of amino acids is decreased after 85% of the effective liver parenchyma is lost. The transmaination to convert ammonia to urea by the liver is also reduced. Schenker et al. (33) report raised ammonia levels as a common finding in hepatic encephalopathy. Some blood ammonia levels were over 400 micrograms per dl in the sheep described by Hooper et al. (53) with spongy degeneration of the brain and hepatic necrosis. Bull (23) also reports a high ammonia level in pyrrolizidine alkaloid poisoned animals. He claims the loss of capacity to form urea caused a rise in ammonia and a rapid death. In the experiment by Ford (16) on ragwort poisoned horses, there were

blood ammonia levels over four times normal.

Zimmerman (96) reports there may be a decrease in some of the clotting factors that are produced by the liver if the liver is damaged. These factors include fibrinogen, prothrombin and factors V, VII, IX and X. This, however, is usually a late change in chronic hepatic disease.

Measuring the liver's ability to excrete certain substances is a test of its excretory function. Bilirubin, a product of heme breakdown is normally excreted by the liver. With impairment of its uptake, conjugation, and secretion by the liver there may be increased serum bilirubin levels. Cornelius (92) reports there may be an increased unconjugated bilirubin in the serum in both hemolytic and hepatotoxic disease in the horse. In cattle serum bilirubin is only slightly increased in diffuse and severe hepatic disease. In experimental work by Ford and Ritchie (17) only three of five calves poisoned with Senecio jacobaea had a significant rise in serum bilirubin and this was only a terminal change.

Several foreign dyes may be injected into an animal so their rate of removal by the liver can be measured as an index of the liver's excretory function. Cornelius (92) lists four dyes that have been used for this purpose. They are sulfobromophthalein (BSP), ^{125}I rose bengal, phenaltetrachlorophthalein and indocyanin green. Sulfobromophthalein has been used most extensively. In the horse BSP is rapidly removed. Determining the clearance time by finding the half-life of the dye in the serum is less time consuming than the BSP retention test.

Vardiman (90) used BSP retention in diagnosing Senecio poisoned

cattle along with liver biopsy. He considered it too sensitive and too time consuming. Finn and Tennant (26) found a decreased rate of BSP excretion in their calves with hepatic encephalopathy. Cherry and McLellan (97) did not believe BSP retention gave any greater evidence of hepatic disease than did serum gamma-glutamyl transpeptidase activity. They considered it time consuming and somewhat hazardous due to possible anaphylactoid reactions.

According to Cornelius (92) floculation tests are based on the assumption that certain colloidal solutions are stable with serum albumin but tend to precipitate in serum globulin. Routh (93) lists three floculation tests commonly performed in human medicine: the cephalin cholesterol floculation, the zinc sulfate turbidity and the thymol turbidity tests. Since they respond to an increased serum globulin and a decreased serum albumin, they give no more information than serum electrophoresis which tells more about the state of protein in the serum. Lloyd (98) used an iodine floculation test in the diagnosis of ragwort poisoning in cattle. He claimed it to be very sensitive since all of the cattle that reacted showed some cirrhosis of the liver when they were slaughtered. Hindson (99) also used the test and claims it was reliable in cases of cirrhosis and fascioliasis, although there were some false negatives.

Serum Enzymes

A change in the activity of certain enzymes in the serum may be the earliest indication of liver damage. Mullen (100) describes several possible reasons for an alteration of the normally expected amount of

enzyme in the serum. Necrosis of the parent cell may result in the release of the enzyme into the circulation. Altered permeability of the cell membranes may allow the enzymes to escape from their normal compartmentalization within the cell. The ability of the body to break down or eliminate these enzymes may be changed. Some damaged cells may actually increase their production of certain enzymes. A decreased production may also occur when the cell is sufficiently damaged or even replaced by other tissue. According to Cornelius (101) the increased permeability of cells may be due to alteration of energy dependent transport systems as a result of hypoxia or toxins. In these cases cytoplasmic enzymes are more easily released.

There would be an ideal situation if one enzyme were present in only one tissue. In such a case, if that particular tissue were damaged, the level of its specific enzyme in the serum would change. Ford (102) lists arginase, sorbitol dehydrogenase and ornithine carbomyl transferase as liver specific enzymes. He also claims that there is a high quantity of glutamate dehydrogenase in the liver of ruminants. Kachmar (103) enumerates some criteria for useful enzymes to study. The assay should be simple. The data should be of diagnostic significance. The enzyme should be present in blood, urine or other available tissue. Finally, the procedure for determining the enzyme activity should not be complex.

The transaminases have been used to detect damage to various tissues. Aspartate amino transferase, formerly called glutamic oxaloacetic transaminase, is one of these enzymes. Kachmar (103) reports the enzyme's activity is highest in heart, liver and skeletal muscle.

The action of the enzyme is to catalyze the conversion of amino acids to alpha ketoacids by transfer of the amino group. Routh (93) claims with acute destruction of tissue there is release of the enzyme into the serum. Dixon and Webb (104) claim the transaminases are present mainly in the supernatant or cytosol portion of the cell. Shaw (105) found that bruised animals showed a marked rise in serum glutamic oxaloacetic transaminase (GOT), so it is not specific for liver damage. The calves poisoned with ragwort by Ford et al. (17) had increased serum GOT levels at some stage of the poisoning but many of those that were given lower doses had activities falling to normal before death. Anderson et al. (106) found some increase in aspartate amino transferase in calves with experimental fascioliases but not as large a ratio between the infected animal and controls as was found with other enzymes.

Alanine aminotransferase, formerly called glutamic pyruvic transaminase (GPT), is used as an indicator of liver damage in the human and dog, but it has been shown by Cornelius et al. (107) that there is a low level of the enzyme in livers of horses, cattle and pigs. They found an absence of a rise in the serum GPT after carbon tetrachloride administration to these animals. It is, therefore, not considered a good indicator of liver damage in the large herbivors.

Lactate dehydrogenase (LDH), according to Kachmar (103), is present in liver, heart, kidney, skeletal muscle and whole blood. It catalyzes the oxidation of L-lactate to pyruvate using the co-factor nicotine adenine dinucleotide (NAD) as the hydrogen ion acceptor. The enzyme can be stored at room temperature for two to three days without significant loss in activity. There are five different isoenzymes of

LDH according to Lehninger (108). Each tissue has its own pattern of
isoenzymes. Routh (93) claims these isoenzymes can be separated by
starch-gel electrophoresis, also the hepatic LDH isoenzyme can be
destroyed by heating buffered serum to 65°C for 30 minutes. Keller
(109) reports that serum LDH is higher in cattle with liver disease
than in horses or sheep. The LDH isoenzymes are more significant than
the total enzyme level. Some fractions increase faster than others.
There is a moderate increase in serum LDH in human viral hepatitis but
a greater increase in myocardial infarction or pulmonary embolism.
Dixon and Webb (104) indicate LDH is present mainly in the cytoplasm or
supernatant part of the hepatic cell. Sherlock (95) indicates it is a
less sensitive indicator of liver disease than some other enzymes. In
the calves infected with liver flukes by Anderson and his co-workers
(106) there was not a large ratio between the infected calves serum LDH
concentration and the concentration in the control calves.

Isocitric dehydrogenase is also present in all cells but predomin-
ately in the liver according to Kachmar (103). It catalyzes the oxida-
tive decarboxylation of isocitric acid to alpha-glutamic acid. Outside
the liver it is highest in skeletal muscle, kidney and adrenal. The
activity of the enzyme is maintained for 24 hours at room temperature
and for three weeks under refrigeration. Kackmar also claims it is
elevated in the serum only in diseases of the liver parenchymal tissue
and not elevated in myocardial infarction unless there is a secondary
congestion of the liver. Red blood cells have 100 times the level of
serum. Routh (93) claims it is elevated in human viral hepatitis but
only moderately elevated in obstructive jaundice or cirrhosis.

Freedland et al. (110) found it elevated in carbontetrachloride and
halothane poisoning in horses.

Sorbitol dehydrogenase (SDH) is present mainly in liver tissue
according to Routh (93). It catalyzes the conversion of sorbitol to
fructose. Little, if any, of the enzyme is present in normal serum.
He reports there is little SDH in the serum with obstructive jaundice
or cirrhosis but it is markedly elevated in people with acute hepatitis.
Cornelius (92) claims it is easily measured and located in sub-cellular
elements. Freedland et al. (110) claim it is the enzyme of choice in
hepatic necrosis in the horse but in their carbontetrachloride poisoned
horses it returned to normal in 144 hours. Mullen (100) notes that SDH
is located in the ribosomes of the hepatocyte and is liver specific.

Shaw (105) says SDH is of great value in diagnosing liver disease
in ruminants. It did not rise in animals bruised before slaughter
although GOT did, so it is useful in differentiating muscle from liver
damage. He found an average of 3.2 IU of SDH in the serum of normal
cattle. Dickinson et al. (13) concluded that SDH was a valuable aid in
the diagnosis of liver damage in tansy poisoning. In Ford and Ritchies'
(17) experiment with ragwort poisoned calves the serum SDH rose to a
peak then dropped off. Finn and Tennant (26) did not find a consistent
rise of this enzyme in the serum of calves with hepatic encephalopathy.
Anderson et al. (106) found a 4.7 fold increase in the enzyme in the
serum of calves experimentally infected with liver flukes. Ikeda et al.
(111) took blood from 50 healthy race horses and found the mean serum
level of SDH to be 1.4 IU/1. When carbontetrachloride was given to
horses at the rate of 0.4 ml/kg the serum SDH reached a peak in 24 to
48 hours.

Ornithine carbomyl transferase, according to Cornelius (92) is in high concentration in liver tissue. deDuve et al. (112) claim the enzyme is mainly in the mitochondria. Mullen (100) claims it is liver specific. The level of the enzyme in the serum of calves experimentally infected with liver flukes increased 5.8-fold according to Anderson et al. (106). In the calves reported on by Ford and Ritchie (17), there was a peak in the serum level of the enzyme in from 25 to 50 days from the beginning of the feeding of Senecio jacobaea; the time of the peak varied with the rate the ragwort was fed. There were two peaks in one calf fed 0.4 pounds of ragwort per day but the level fell to normal again by 115 days. Wolf et al. (113) claim arginase is a liver specific enzyme in the horse. The serum arginase along with isocitric dehydrogenase is greatly elevated in horses two days after chloroform anesthesia when there is evidence of central lobular necrosis of the liver. The enzyme concentration returned to normal by the 13th day in these horses. Cornelius (92) says arginase is highly concentrated in livers of ureotelic mammals. Serum levels rose rapidly in the horse, calf, sheep and dog following administration of carbontetrachloride but returned to normal within three to four days. Serum GOT remained elevated for a longer time.

Sherlock (95) reports that glucose 6 phosphatase and 5' nucleotidase are two other enzymes present only in the liver at any significant amount. Seymour et al. (114) found 5' nuceotidase activity was surpassed only by gamma-glutamyl transferase of the membrane bound liver enzymes.

Alkaline phosphatase is an enzyme with broad, non-specific activity;

it hydrolyzes many esters of phosphoric acid. According to Routh (93) it is present in all tissues but in higher concentration in liver, bone, intestinal epithelium, kidney tubules, leukocytes and placenta. Dixon and Webb (104) state that alkaline phosphatase is in the mitochondria of the hepatocyte but Mullen (100) claims it is present in the cytoplasm of the hepatocyte and excreted into the bile. The amount of the enzyme in the liver declines with age in horses, swine and, to a lesser extent, cattle. There is a great variation in serum levels in horses, cattle, sheep and swine. deDuve et al. (112), however, report there are two types of alkaline phosphatase in the liver. One type is insensitive to magnesium and present in the nuclear and microsomal fractions. The other is sensitive to magnesium and present in the supernatant (cyto-plasm). Seymour and Peters (114) claim it is membrane bound. Kachmar (103) believes most of the alkaline phosphatase in the serum originates in the liver. The enzyme maintains most of its activity for 24 hours at room temperature but it is denatured at 57°C. Under refrigeration its activity falls very slowly.

Forman et al. (115) used low voltage immuno-electrophoresis to determine that placental alkaline phosphatase was immunologically dis-tinct from the others. Saini and Saini (116) found intestinal mucosa a rich source of alkaline phosphatase, which occurred in the blood as an isoenzyme. In the dog the hepatic enzyme is the most anodal compon-ent. Anticanine-intestinal alkaline phosphatase serum did not react with the other components. The main source of serum alkaline phospha-tase in the dog was determined to be the liver in these studies.

According to Routh (93) the level of alkaline phosphatase in the

serum depends on the rate of release from the tissues as well as the rate of inactivation and excretion. With liver damage there is an increased liberation of the enzyme from the sinusoidal surface of the hepatocytes. There is also some regurgitation of biliary isoenzyme back into the serum. He concludes it is a more sensitive index of bile stasis than serum bilirubin.

Cornelius (92) claims the highest levels of serum alkaline phosphatase are seen in extra-hepatic obstructive icterus. It is also elevated in human hepatitis, fibrosis of the liver, amyloidosis of the liver and bone diseases. He found a wide range of serum activity in normal cattle. Pekarthy et al. (117) found that removal of part of the liver in rats resulted in an increased serum alkaline phosphatase activity due to increased activity in cell membranes. It was especially high in membranes lining bile canaliculi and in the cytoplasm. The control of the level of activity was tied to the metabolism of phosphorylcholine.

The serum alkaline phosphatase activity did not rise significantly in calves experimentally infected with liver flukes by Anderson et al. (106). Experiments on dogs by Van Vleet and Alberts (118) revealed an increased serum alkaline phosphatase level shortly following carbon-tetrachloride administration and later following bile duct ligation.

Gamma-glutamyl transferase, according to Lehninger (108) plays an important role in amino acid transport across cell membranes. Hagenfeldt et al. (119) report the gamma-glutamyl cycle involves adding an amino acid to glutathione to yield gamma-glutamyl amino acid. Rico et al. (120) analyzed the tissue of normal horses and found the highest concentration of the enzyme to be in the kidney followed by the pancreas

and then the liver. All other tissues tested had a very low activity of the enzyme. Rico et al. (121) reported on a similar analysis in cattle. Gamma-glutamyl transferase was highest in kidney, especially the cortex. Pancreatic concentrations were higher than liver. All other tissues had very low concentrations. The liver had only 8.2% of the enzyme activity of the renal cortex. Hachler (122) also reports the highest enzyme activity is in the kidney but there is a greater increase in serum enzyme activity with certain liver diseases.

Lum and Gambine (123) found the enzyme in the microsomes near the canaliculi brush border of hepotocytes and in renal tubular epithelium. deDuve et al. (112) found the enzyme in rabbit liver nuclear fraction and in the microsomal fraction of rat and pig kidney. Seymour and Peters (114) assayed five membrane bound enzymes in the liver and found gamma-glutamyl transferase to have the highest activity. They also found a relatively low activity in normal serum.

Zein and Discombe (124) claim liver cells actually increase the production of gamma-glutamyl transpeptidase with noxic stimuli. They also claim that although the enzyme is in high concentration in normal kidney the serum activity does not increase with renal disease. The serum levels are high in most liver diseases. Serum levels are increased in both hepatic carcinoma and alcoholics even when alkaline phosphatase levels are not. They concluded that the serum level of gamma-glutamyl transpeptidase is a sensitive and specific index to liver damage. Toro and Ackerman (125) report that serum gamma-glutamyl transpeptidase activity in the serum is a strong indicator of liver disease. They say it is not a feature of kidney disease and only

moderate elevations are seen in acute pancreatitis.

Ikeda et al. (111) reported the gamma-glutamyl transferase response persisted for three weeks following carbontetrachloride administration to horses and was of more value in chronic liver disease. Hackler (122) believes it is a more sensitive indicator of liver disease than aspartate amino transferase or alanine amino transferase. It was elevated in the serum of 75% of the heavy drinkers tested and often it was the only enzyme elevated. He concluded it could detect liver damage in the early and reversible stages.
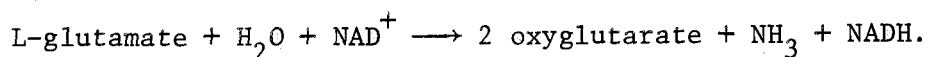
Lum and Gambino (123) found serum gamma-glutamyl transpeptidase activity to be above normal in all forms of liver disease studied. They also found it more sensitive than alkaline phosphatase. There was a disproportionate increase in serum activity of the enzyme with intra- or extrahepatic biliary obstruction. The serum activity was normal in bone disease and renal failure. Dickson and Beck (126) report that gamma-glutamyl transferase activity often decreases in human sera as bilirubin increases. Rico et al. (120) claim renal lesions are followed by urinary escape of the enzyme but an increased serum level is not expected in renal disease.

Rico et al. (120) reported the mean serum level of gamma-glutamyl transferase in horses was 12.7 IU/l with a standard deviation of 6.1. Rico et al. (121) tested the blood of 54 normal cows and found the mean gamma-glutamyl transferase activity to be 18.6 IU/l with a standard deviation of 6.2. Sorbiech and Sorbiech (127) found the serum gamma-glutamyl transferase activity in calves to be three times that of adult cattle. Zein and Discombe (124) claim the enzyme is stable for two

days at room temperature.

In calves experimentally infected with Fasciola hepatica by
Anderson (106) there was a 13.8-fold increase in gamma-glutamyl trans-
ferase.

Glutamate dehydrogenase is an enzyme only recently used for
diagnostic purposes. Dixon and Webb (104) describe the reaction cata-
lized by glutamate dehydrogenase as:

$$\text{L-glutamate} + H_2O + NAD^+ \longrightarrow 2 \text{ oxyglutarate} + NH_3 + NADH.$$

They claim it is mainly in the mitochondria of liver cells. deDuve et
al. (112) claim it is solely in the mitochondria. Toro and Ackerman
(125) found glutamate dehydrogenase in liver, heart and brain. The
serum levels were usually not elevated in non-hepatic disease.
Freedland et al. (110) report a significant amount of the enzyme only
in liver. Lesser amounts were found in kidney and brain. There were
low levels in normal horse serum. After giving the horses 250 or 500
ml of carbontetrachloride the serum levels rose markedly but fell to
normal again after 144 hours. Rico et al. (120) claim there is a 14
times higher concentration of the enzyme in horse liver than in kidney.
Ford (101) found a high quantity of glutamate dehydrogenase in ruminant
livers. Ikeda et al. (111) reports the mean serum activity of glutamate
dehydrogenase in 50 healthy race horses to be 1.5 IU/l with a standard
deviation of 1.1. After giving carbontetrachloride a peak in the
enzyme level was reached in three days.

Boyd (128) found glutamate dehydrogenase is the enzyme most affec-
ted by liver damage in calves. There was a marked increase in the
enzyme following carbontetrachloride administration. A peak in the

enzyme level occurred in 24 to 48 hours at the time most of the necrosis was taking place. He concluded this was due to a leakage of the enzyme during necrosis. In the calves with experimental fascioliasis reported on by Anderson et al. (105) there was a 17.6-fold increase in glutamate dehydrogenase in the serum. Nelson et al (129) could find no evidence of isoenzymes of glutamate dehydrogenase in humans by using electrophoretic techniques.

Ford et al. (17) determined the serum glutamate dehydrogenase activity in calves poisoned with Senecio jacobaea. In one calf fed 1.2 pounds of ragwort per day the serum level rose by the 25th day, peaked by the 40th day and then fell. In the two calves fed 0.8 pounds of ragwort per day, the serum glutamate dehydrogenase activity increased by the 15th day, peaked at 30 and 40 days, but returned to normal before death. The enzyme level rose in both calves fed 0.4 pounds a day but fluctuated in one and dropped to normal 60 days before death.

III. MATERIALS AND METHODS

Six apparently healthy calves and six ponies were used for the experiment. The animals were from the Eastern Oregon area and had not been exposed to Senecio jacobaea. The feces of the calves were examined for liver fluke eggs and found to be negative. All of the animals were fed a balanced ration of pelleted feed except that the feed of four ponies and four calves contained 5% Senecio jacobaea by dry weight. Two calves and two ponies were used as controls and underwent the same procedures as the animals receiving the Senecio. The feeding of the Senecio continued for two months. At the end of the two months time the four calves had consumed Senecio jacobaea equal to 5.8% of their body weight. Two of the ponies had consumed Senecio equal to 5.8% of their body weight and the other two had eaten 6.7% of their weight in Senecio. After the Senecio feeding stopped all the animals were kept on pasture supplemented by a balanced pelleted feed.

The animals were observed throughout the feeding and during the months which followed. Every two weeks the animals were weighed, examined physically and bled. A blind percutaneous needle liver biopsy was performed on each animal at the same time. The method of liver biopsy in cattle has been described in the literature (130). The calves were confined in a squeeze chute and a nose lead applied. The skin over the site of biopsy was clipped and prepared for aseptic insertion of the needle. A local infiltration of 2% lidocaine helped reduce the animals' reactions but some still flinched when the pleura and peritoneum were penetrated. A small stab wound was made through the skin at the site of insertion with a number 11 Bard-Parker blade.

In the bovine the puncture site can be located by extending a hori-
zontal line anteriorly from the middle of the paralumbar fosa. The
needle is inserted where this line crosses the 11th intercostal space
on the right side. Figure 1 illustrates the relationship between the
lungs, liver and ribs in the bovine. The lungs do not extend as far
posteriorly in the bovine so there is more liver exposed. The needle
is directed slightly anteriorly and ventrally to keep it in the liver
parenchyma. Either a 3-inch or 4 1/2-inch needle was used for the calf
liver biopsy.

The method of liver biopsy used on the ponies is a modification of
techniques described in the literature (131). The site of insertion is
clipped, scrubbed and disinfected for aseptic insertion of the needle
the same as with the calves. The ponies were usually cross tied and a
nose twitch applied. A local infiltration of 3 to 4 ml of 2% lidocaine
was used to anesthetize the skin over the site of insertion. A small
stab wound was made through the skin with a number 11 Bard-Parker blade.
The right 12th intercostal space is recommended as a site for insertion
of the biopsy needle in some texts (132). When this location was used,
the lung was penetrated and pulmonary hemorrhage and hematoptysis
resulted. The site of needle insertion used in this experiment was the
right 14th intercostal space at the intersection of a line drawn from
the tubercoxae to the point of the shoulder. Figure 2 illustrates the
site of insertion in the horse. The needle is directed slightly anteri-
orly and ventrally. By angling the needle in this direction it usually
remains in the liver parenchyma. If the needle is directed straight in
perpendicular to the body surface it is more apt to pass on through the

liver and into the right kidney, the right dorsal colon or the pancreas. Angling the needle also avoids some of the larger veins on the visceral surface. Figure 3 illustrates these relationships in a frontal view. Either a 4 1/2-inch or 6-inch needle was used for the pony liver biopsy.

A number of instruments were used for biopsy in this experiment. A large aspiration trochar designed by Bone (130) was used in some cases. A modified Vim-Silverman needle was used part of the time. It had the drawback of a more complicated procedure since once the instrument was in place the stilet had to be removed and the split needle used for cutting the tissue inserted. Most of the biopsies were made using the disposable "Tru-Cut" biopsy needle produced by Travenol Laboratories. This disposable needle is used primarily for human breast biopsies. The same needle was chemically resterilized and used from 10 to 30 times in this experiment. The needle is all in one piece and the sample is removed by sliding the cutting needle over the specimen notch. Figure 4 shows the various instruments used in this experiment.

The biopsy tissue was placed immediately in a solution of 2 1/2% buffered formalin. The 10% buffered formalin commonly used resulted in more tissue dehydration than the 2 1/2% solution. Tissue taken with Bone's large aspiration trochar was placed in 10% buffered formalin. Some of the specimens were placed in glutaraldehyde for fixing for a limited electron microscope study. For this study one pony and one calf were sampled prior to the experiment and at weeks 8 and 20.

The tissues were imbedded in paraffin and sectioned at five microns thickness. Tissue sections from each sample were stained with hematoxalin and eosin. Some of the sections were stained with Mason's trichrome

method and by the periodic acid-Schiff reaction (PAS). Some were stained by the Feuglen method for DNA and some with Toluidine blue. An iron stain was used to identify hemosiderin in the tissues and some of the tissue was frozen, sectioned and stained for fat. The sectioning and staining was performed by the Histology Section of the Veterinary Diagnostic Laboratory using standard procedures for these stains (133).

The approximately 15 ml of blood drawn from the jugular wein of each animal was allowed to clot. It was then centrifuged at 1500 rpm and the serum poured off to be used for assays of enzyme activity. Serum activity of alkaline phosphatase, gamma-glutamyl transferase and glutamate dehydrogenase was determined. In each case the assays were run in duplicate to avoid error. Human sera of known enzyme concentration were analyzed each time the experimental samples were tested to insure uniformity in the results. A spectrophotometric method was used for each enzyme. Our own standard curve was developed comparing the change in absorbance with the enzyme concentration. These were developed using sera of known concentration and for each kit lot and each spectrophotometer. A Coleman 55, a Boeckman and a Cary spectrophotometer were used.

Alkaline phosphatase activity was determined by the method of Hausamen et al. (134). A commercial kit was used in which P-nitrophenylphosphate was hydrolized to P-nitrophenol and phosphate. The increased yellow color due to the release of P-nitrophenol was proportional to the amount of enzyme present. The analysis was conducted at 30°C and light absorbance was measured at a wave length of 405 nanometers.

Gamma-glutamyl transferase activity was measured by a modification

of the method of Szazz (135). A commercial kit was used here also in which glycylglycine was added to an L-gamma-glutamyl compound liberating 5-amino-2-nitrobenzoate which increased the yellow color of the solution. The analysis was carried out at 30°C and light absorbance was measured at 405 nanometers.

Glutamate dehydrogenase activity was determined using a commercial kit imported from Germany. It used a method similar to that used by Freedland et al. (110). The test principal involved: a-oxoglutarate + NADH + ammonia ion being converted by glutamate dehydrogenase to glutamate + NAD + water. The decrease in color was due to a decrease in NADH. The test was conducted at 25°C and the absorbance of light was measured at 340 nanometers.

When an animal died or became moribund a necropsy was performed. The organs were examined grossly and representative tissues were fixed in 10% buffered formalin and stained similar to the biopsy tissue for histopathological examination.
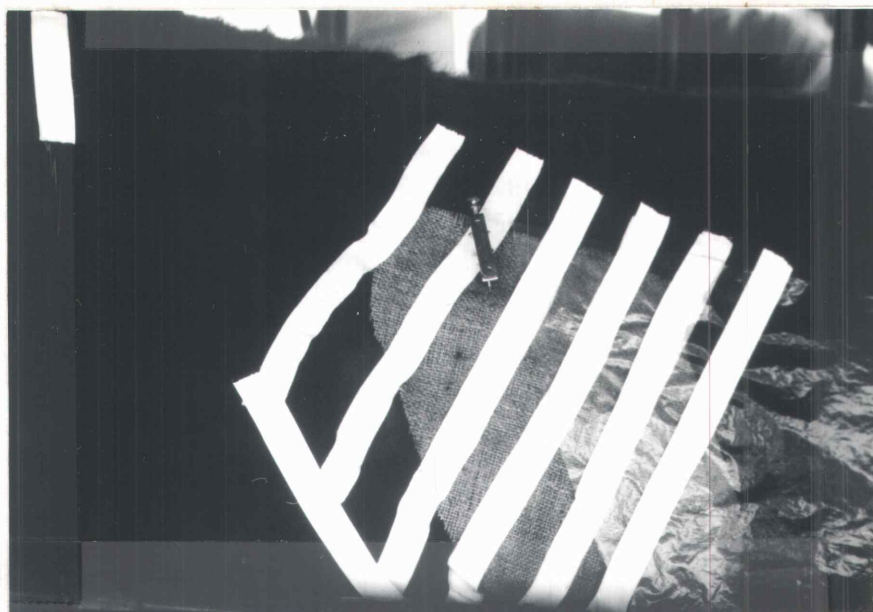
Figure 1. The location of liver biopsy in the calf. The white tape
shows the location of the ribs and tubercoxae. The lungs
are represented by plastic and the liver by cloth. The
11th intercostal space at the level of the middle of the
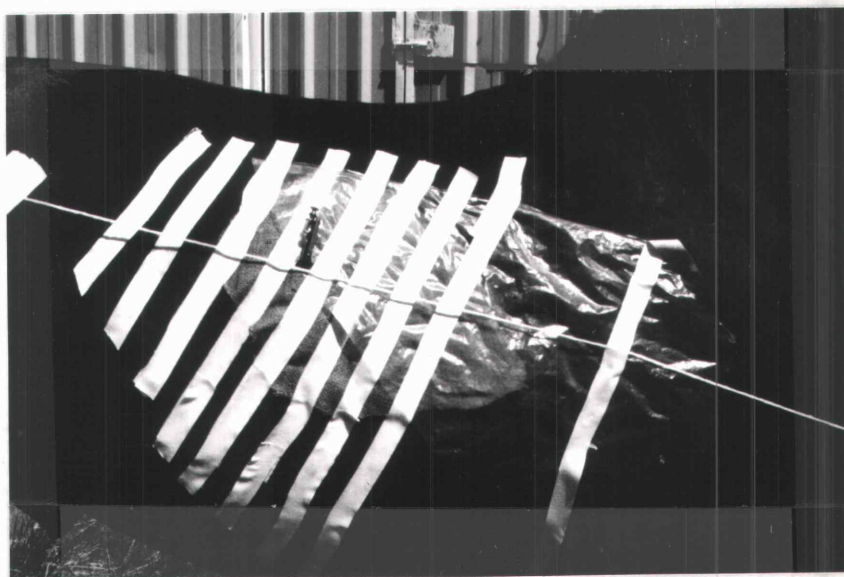paralumbar fossa was the site used for liver biopsy in the
calves.

Figure 2. The location of liver biopsy in the pony. The white tape
shows the location of the ribs and the tubercoxae. The
string represents a line drawn from the tubercoxae to the
point of the shoulder. The lungs are represented by plastic
and the liver by cloth. The 14th intercostal space at the
intersection of a line from the tubercoxae to the point of
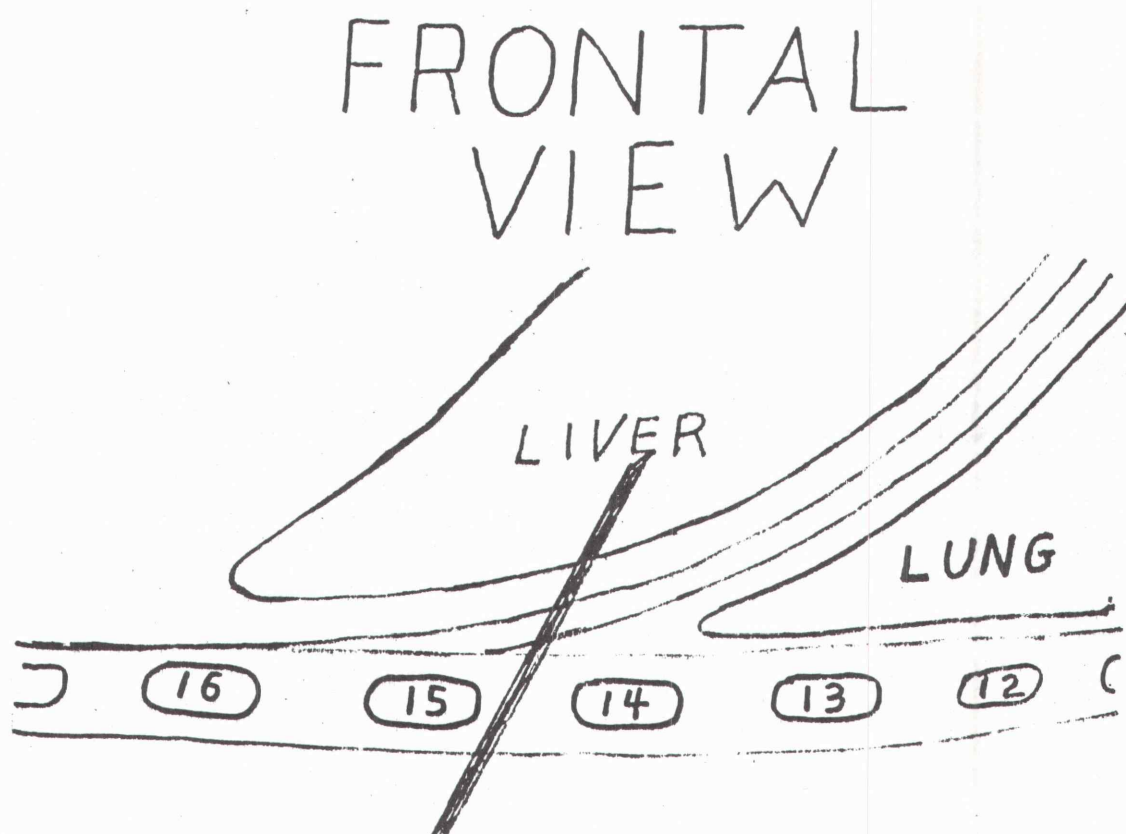the shoulder was the site used for liver biopsy in the pony.

Figure 3. Frontal view of relative positions of ribs, lungs, liver and other viscera in the pony.
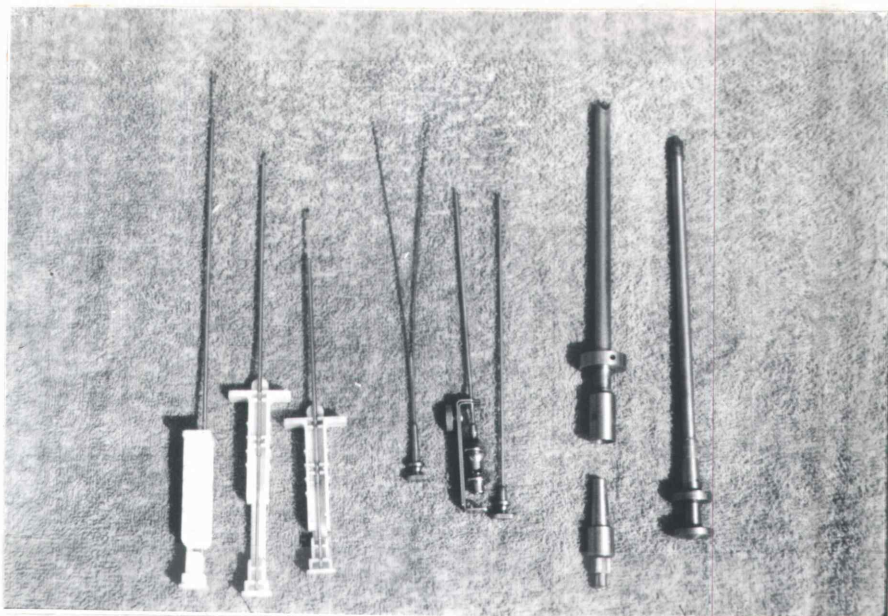
Figure 4. Instruments used in liver biopsy. The three instruments
at the left are "True-Cut" disposable biopsy needles. In
the center is the modified Vim-Silverman needle, and at
the right an aspiration biopsy punch designed by Bone.

IV. RESULTS

The response of the animals to the feeding of Senecio jacobaea was quite varied. Three types of reaction were seen in the calves. Two of the calves behaved similarly to those in a previous acute experiment and died at about the time the Senecio feeding was stopped. The enzyme response and the histological changes were similar to those noted in previous experiments in which the animals were fed the poison until they died. One of the calves had a delayed response which occurred 17 weeks after the consumption of the poison had stopped. One of the calves did not receive a critical amount of tansy. It would apparently need to consume additional tansy next year if it were to die from the poison since it did not die during the experiment and showed improvement in serum enzyme levels as well as some structural changes in the liver.

Various responses were also seen in the ponies. Two of the ponies died acutely while the tansy was still being fed in a manner similar to those in a previous acute experiment. In these two ponies the serum enzyme response and the histopathological changes were similar to those in other experiments where the ponies were fed tansy until they died. Two of the ponies survived the experiment and would seemingly need to consume more tansy or have other liver insults before they died from the effects of the poison. Both of these surviving ponies had marked structural changes in the liver. One of these ponies had resolution of some of the structural damage. Some of the measured serum enzymes returned to pre-exposure levels in this pony and were reduced in the other surviving pony.

The clinical signs of Senecio poisoning in these calves and ponies were not different from those already described in the literature. In most cases no signs were apparent until within a few days of the death of the animal. One of the calves, #3, developed ascites at week 12 which was three weeks after the consumption of Senecio jacobaea had ceased. This ascites was clinically detectable until week 24, but at necropsy there was no excess peritoneal fluid. Calf #2 did not die and 23 weeks after the consumption of Senecio stopped there were no clinical signs of the disease.

Pony #2 did not die and also had no clinical signs of disease 23 weeks after the exposure to Senecio. Pony #4 was also alive at week 32, 23 weeks after the consumption of the weed had ceased. Pony #4 did have some obscure changes in its temperament and behavior. It became less friendly and cooperative and was seen yawning more frequently than the others.

The gross necropsy of the calves and ponies revealed no unexpected lesions since all changes found had been previously described in the literature.

Serum Enzymes

Serum enzyme activities for alkaline phosphatase, gamma-glutamyl-transferase and glutamate dehydrogenase increased during the feeding of Senecio jacobaea (Figures 5-16). The first enzyme to rise was glutamate dehydrogenase. In some of the calves its level of activity in the serum had increased more than 20-fold at the end of only two weeks of feeding Senecio jacobaea. At this time they had consumed dried Senecio jacobaea

equal to 1.7% of their body weight. In the other two calves a 20-fold increase did not occur until week six at which time they had consumed Senecio equal to 5.4% of their body weight. The mean serum glutamate dehydrogenase activity for 75 calf samples, both control and pre-exposure, was 6.8 IU/l with a standard deviation of 3.3.

Two of the ponies had an eight-fold increase in serum glutamate dehydrogenase activity at week two. They had consumed Senecio equal to 2% of their body weight at this time. Pony #3 did not show an increase until week six, after it had consumed Senecio equal to 3.7% of its body weight. The serum activity of glutamate dehydrogenase in this pony then dropped and the pony died at week nine. Pony #4 did not have a two-fold rise in serum glutamate dehydrogenase until week eight after it had consumed 5% of its body weight in Senecio. The mean activity of this enzyme in the serum of 71 samples taken from pre-exposure and control ponies was 3.8 IU/l with a standard deviation of 3.4.

In both the calves and ponies there was a peak rise in the serum enzyme activity which was followed by a drop in activity. In all of the calves and in two of the ponies this peak occurred while the Senecio was still being consumed. In pony #2 the peak occurred at week 12, three weeks after the consumption had ceased. This pony did not die during the experiment. In pony #4, the peak occurred at week ten and this pony also did not die during the experiment.

Serum alkaline phosphatase showed at least a two-fold rise in activity by week four in all of the calves, at which time they had consumed Senecio equal to 3.8% of their body weights. The peak activity occurred at or near the time of death in the two calves that died

acutely. In calf #3, the peak occurred at week 20, 11 weeks after it had stopped consuming Senecio. In calf #2, which survived the experiment, there were two peaks, one at week four and a lower peak at week 26. The mean serum alkaline phosphatase activity in 80 samples from calves unexposed to Senecio was 214 IU/1 with a standard deviation of 53.

The ponies exhibited a more varied response. The two animals that died acutely did not have a great rise in serum alkaline phosphatase until just prior to their death. Pony #2 did not have a two-fold rise in the serum enzyme activity until week 12 which was three weeks after the exposure had ceased. The peak level of activity, 760 IU/1, was measured at this time. Following week 12, the level of activity decreased but remained above the pre-exposure level at week 30 which was 21 weeks after exposure was stopped.

Pony #4 had an early rise and then another rise after week 12. Two peaks were present, one at week two and the other at week 20 which was 11 weeks after the animal was no longer receiving Senecio. The mean of 62 samples from ponies that had not been exposed to Senecio was 217 IU/1 with a standard deviation of 84.

There was a continuing greater magnitude of increased activity of serum gamma-glutamyl transferase than the other enzymes. In the calves a two-fold rise occurred by week four to six. The peak level in one calf that died acutely occurred just prior to death whereas the other peaked at week four, eight weeks before it died. In the calf with the delayed response, the peak occurred at week 20 which was 11 weeks after exposure had stopped. The level of serum activity of the enzyme then gradually decreased but was more than four times the pre-exposure

activity at the time of its death, 17 weeks after it had stopped con-

suming Senecio. Calf #2 survived the experiment and its peak serum

gamma-glutamyl transferase activity occurred at week ten, only one week

following the time the Senecio feeding had stopped. Eighty samples

from calves not exposed to Senecio had a mean serum gamma-glutamyl

transferase activity of 12.5 IU/1 with a standard deviation of 2.7.

At least a two-fold rise in serum gamma-glutamyl transferase

activity occurred by week two in three out of four ponies. In the

other pony it did not occur until week eight. Both of the animals that

died acutely while still being fed Senecio had a peak level near the

time of their death. Pony #2, which survived the experiment, had a

peak level of activity at weeks four and 12. This was followed by a

gradual return to the pre-exposure levels. Pony #4, which also survived

but had extensive liver damage that was evident on histological sections,

had a peak activity of the enzyme at week 20. Following this peak there

was a gradual decrease in activity but at week 30 which was 21 weeks

following the last exposure to Senecio the activity was almost four

times that of unexposed animals. The mean serum activity of 62 samples

from ponies not exposed to Senecio was 13.6 IU/1 with a standard devia-

tion of 4.8.

Histopathology

A number of structural changes were observed using the light micro-

scope in liver tissue taken by liver biopsy. The earliest changes

noted were in the nuclei of hepatocytes. In many cases a large number

of nuclei contained intranuclear vacuoles. These areas within the

nucleus did not take on the usual basophilic chromatin stain. A denser stained area was present at the periphery of the vacuole as well as at the nuclear membrane (Figure 17). These vacuoles did not stain using the Feuglen method for DNA. Some of the tissue was frozen and stained for fat and the vacuole did not take on the fat stain. The vacuoles were visible using the Masson's trichrome stain and with the periodic acid-Schiff reaction but they were not PAS positive. The vacuoles did not stain with toluidine blue but the remainder of the nucleus did. Some of the tissue was embedded in plastic and sectioned at two to three microns. The vaculoes were visible in these sections also when the tissue was stained with hematoxylin and eosin, or toluidine blue.

Using the electron microscope numerous large invaginations of the cytoplasm into the nucleus could be seen. There was also a margination of the chromatin at the periphery of the nucleus (Figure 18).

The vacuoles or invaginations were present in both calves and ponies. These nuclear changes were seen in every case receiving Senecio but not every week. Only rarely was a somewhat similar structure seen in the control animals. These changes were never seen on post-mortem specimens. In some cases as many as 80% of the nuclei contained these invaginations or vacuoles. In other cases only 1 or 2% of the hepato-cytes were involved. When a large percentage of hepatocytes contained these nuclear changes the animals did not survive long. Calf #3 had many intranuclear vacuoles at week 24 which was 15 weeks after the Senecio feeding had stopped (Figure 20).

The next earliest histological change seen in these animals was biliary hyperplasia. There was an increase in biliary epithelial cells

as well as an increased number of bile ducts in the portal tracts (Figure 21).

Biliary hyperplasia was evident by the eighth week in two of the calves. These were the two calves that died acutely shortly after they stopped consuming Senecio. In calf #2, which survived the experiment, it was not readily apparent until the 12th week and in calf #3, which had the delayed response, it could be detected by week 14. In this calf with the delayed response the biliary hyperplasia increased until about week 20 and following this became gradually and partially resolved.

In the two poinies that died acutely while they were still being fed Senecio the biliary hyperplasia was not apparent until near the time of death and was seen on tissue taken at post-mortem examination. Pony #2, which survived the experiment, had biliary hyperplasia that was distinct by the eighth week. It gradually increased until about week 16 and then became partially resolved. Pony #4, which also survived the experiment but developed severe liver damage, had distinct biliary hyperplasia by week 12; this increased until about week 20 and then partially resolved.

Hepatic megalocytosis, which has been a hallmark of the pyrrolizidine alkaloid poisonings, was not consistently seen in these biopsy specimens. It was seen sporadically in some of the calves and ponies. It could usually be detected in post-mortem tissue.

Some binucleated hepatocytes could be seen, especially in both the calf and ponies with a delayed response.

Portal fibrosis in most cases followed the biliary hyperplasia. New collagen and fibroblasts were first seen around the bile ducts.

The ponies that died acutely did not have a lot of new connective tissue laid down until near the time of death. In the two ponies with the delayed response fibrosis was not marked until several weeks after the feeding of Senecio was stopped. Although the area of the portal tracts appeared smaller later in the course of the experiment it was probably due to a resolution of bile ducts and not connective tissue. In cases that survived a long time the portal tracts became joined by connective tissue. It seemed as though many of the cells in Rappaport's zone 1 were replaced by connective tissue. Pony #3, which had a delayed response, had a gradual progression of biliary hyperplasia and portal fibrosis followed by some resolution of biliary hyperplasia (Figures 22-24).

The calves that died acutely shortly after the Senecio feeding stopped did not have marked hepatic fibrosis until near the time of their death. Calf #3, which had a delayed response, did not have a marked fibrosis until several seeks after the feeding of Senecio was stopped. Both biliary hyperplasia and fibrosis progressed gradually in this calf (Figures 25 and 26). Calf #2, which survived the experiment, did not develop any marked fibrosis but some biliary hyperplasia developed following the feeding of Senecio (Figures 27 and 28). There was a definite lack of fibrosis in this case as compared to the other cases which were exposed to the same amount of Senecio.

The ponies that died acutely while still being fed Senecio developed a diffuse necrosis of hepatocytes. Nuclear changes could be seen along with biliary hyperplasia and connective tissue that replaced the lost hepatocytes (Figure 29). At post-mortem examination there were few

normal hepatocytes left (Figure 30).

In addition to the above changes the animals with a delayed response had a gradual loss of hepatocytes which were not replaced. Small necrotic foci were seen throughout the liver lobules. No certain zone was solely involved since as many foci were found in zones one and two as in zone three. There was an infiltration of polymorphonuclear leukocytes into the area. Some of the hepatocytes had karyorrhexis of the nuclei. These ponies, especially pony #4, had these necrotic foci present throughout the later part of the experiment (Figures 31 and 32).

Calf #3, which had a delayed response, also had many microscopic necrotic foci (Figure 33). Later in the experiment this calf had small focal areas of fibrosis within each lobule. First a cell infiltration was seen which was followed by the accumulation of collagen and fibroblasts. This new connective tissue was stained very distinctly with Masson's trichrome stain (Figures 34 and 35).

Figure 5. Serum glutamate dehydrogenase activity in calves #1 and #4.

CALF NO. 2
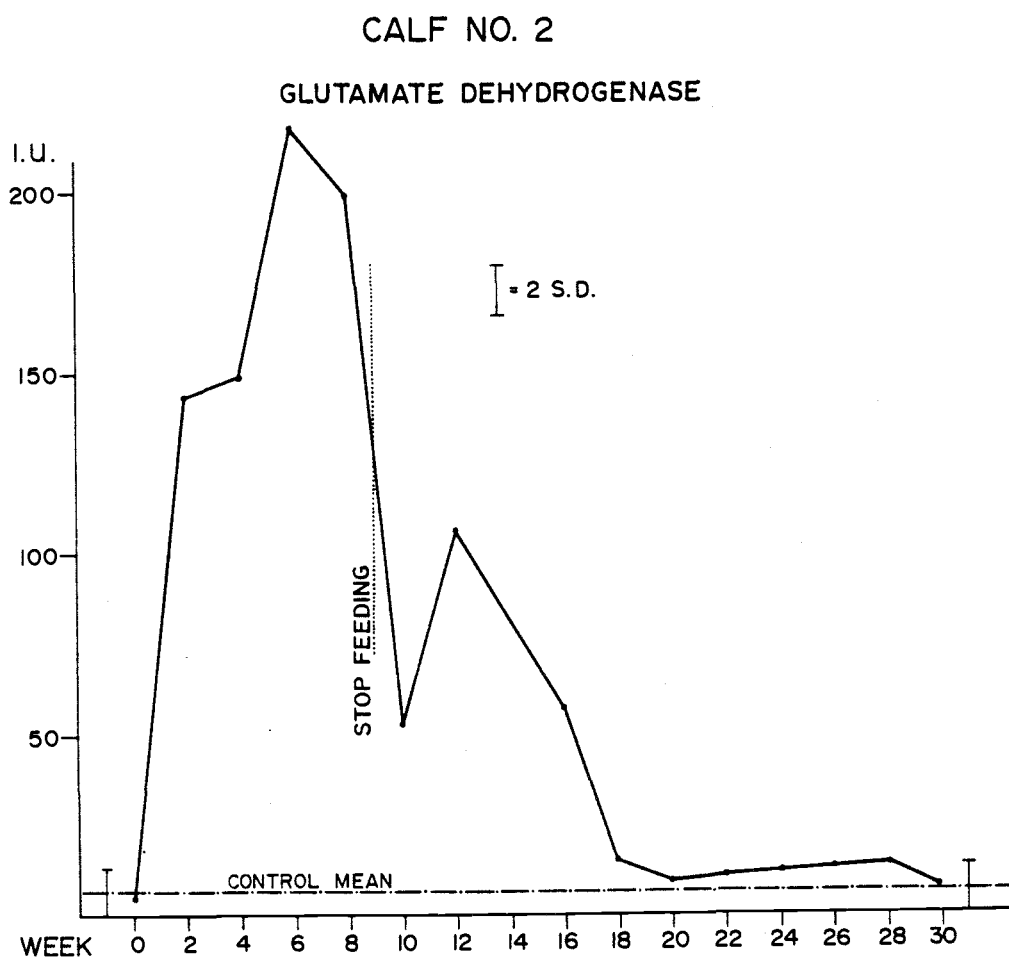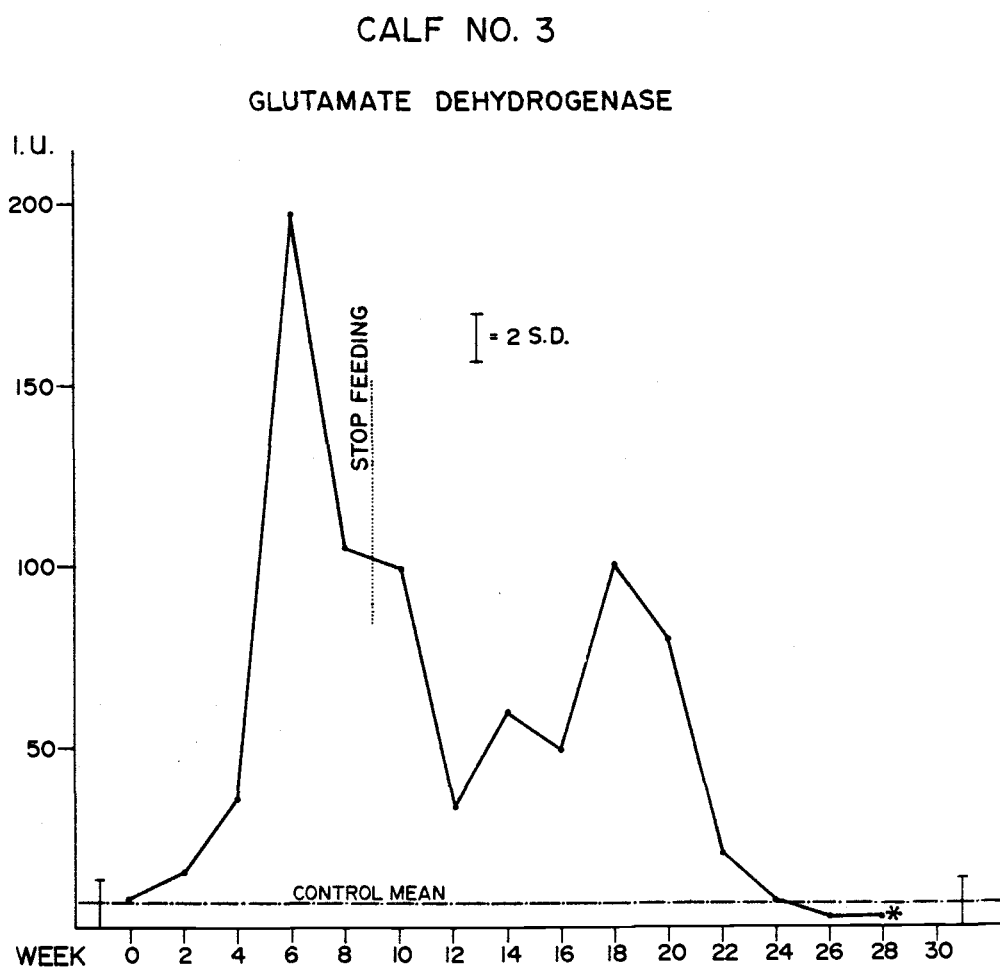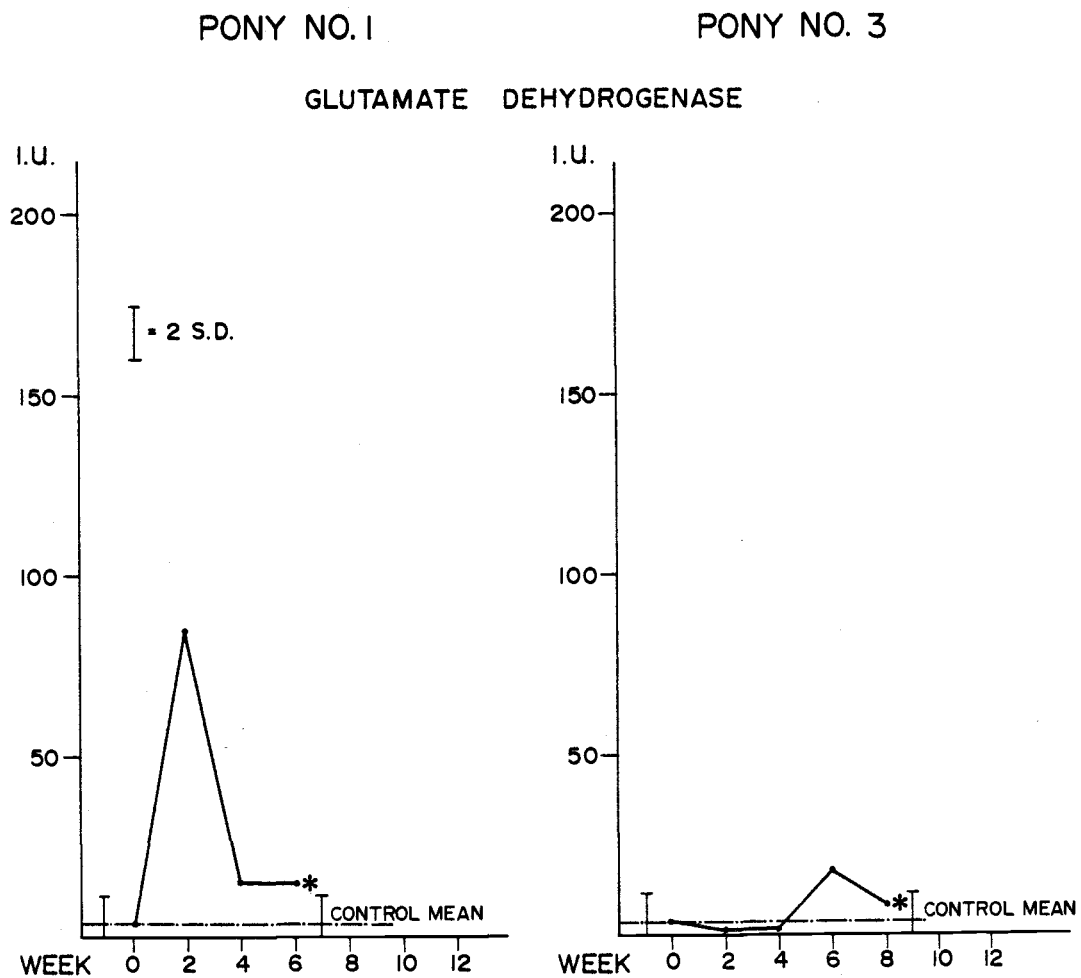
GLUTAMATE DEHYDROGENASE

Figure 6.   Serum glutamate dehydrogenase activity in calf #2.

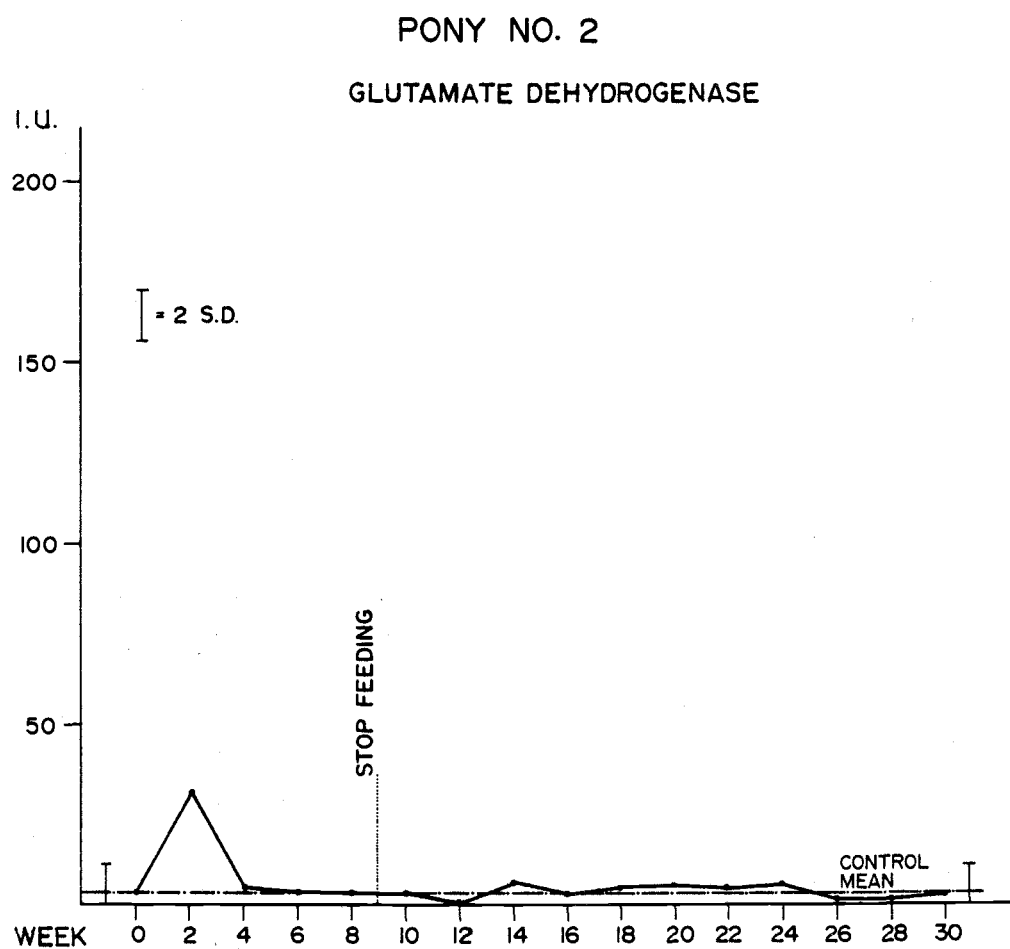Figure 7. Serum glutamate dehydrogenase activity in calf #3.

PONY NO. 1   PONY NO. 3

GLUTAMATE   DEHYDROGENASE

* ANIMAL DIED

Figure 8.   Serum glutamate dehydrogenase activity in ponies #1 and #3.

PONY NO. 2

GLUTAMATE DEHYDROGENASE

Figure 9.   Serum glutamate dehydrogenase activity in pony #2.

PONY NO. 4

GLUTAMATE DEHYDROGENASE

Figure 10.   Serum glutamate dehydrogenase activity in pony #4.

CALF NO.1   CALF NO. 4

ALKALINE PHOSPHATASE

GAMMA GLUTAMYL TRANSFERASE

NUCLEAR CHANGES ●
BILIARY HYPERPLASIA
PORTAL CIRRHOSIS
* ANIMAL DIED

Figure 11.   Serum alkaline phosphatase and gamma-glutamyl transferase
             activity in calves #1 and #4.   The lower line is mean of
             unexposed calves; the vertical bar indicates 2 standard
             deviations.   Shaded area at bottom indicates weeks histo-
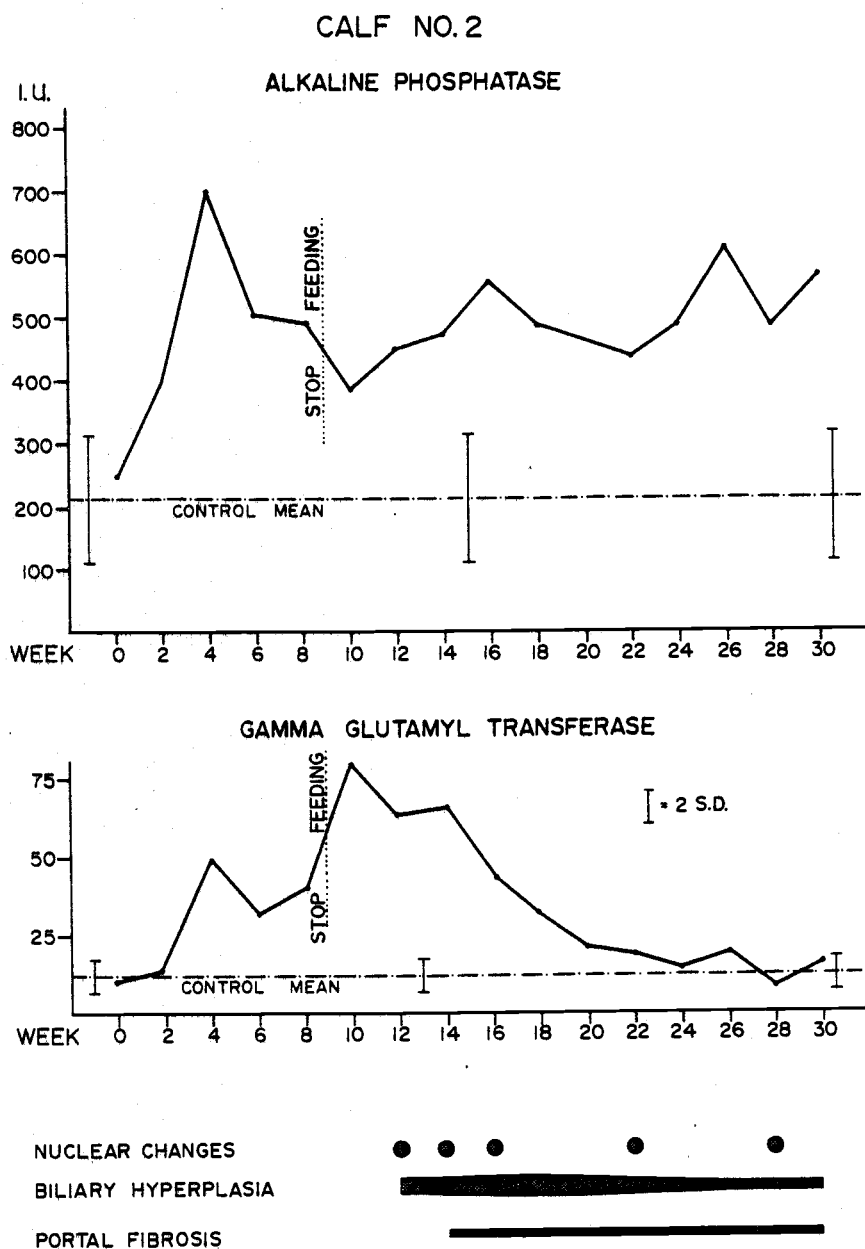   pathological lesions were seen.

Figure 12. Serum alkaline phosphatase and gamma-glutamyl transferase activity in calf #2. Shaded areas indicate weeks microscopic changes were seen.
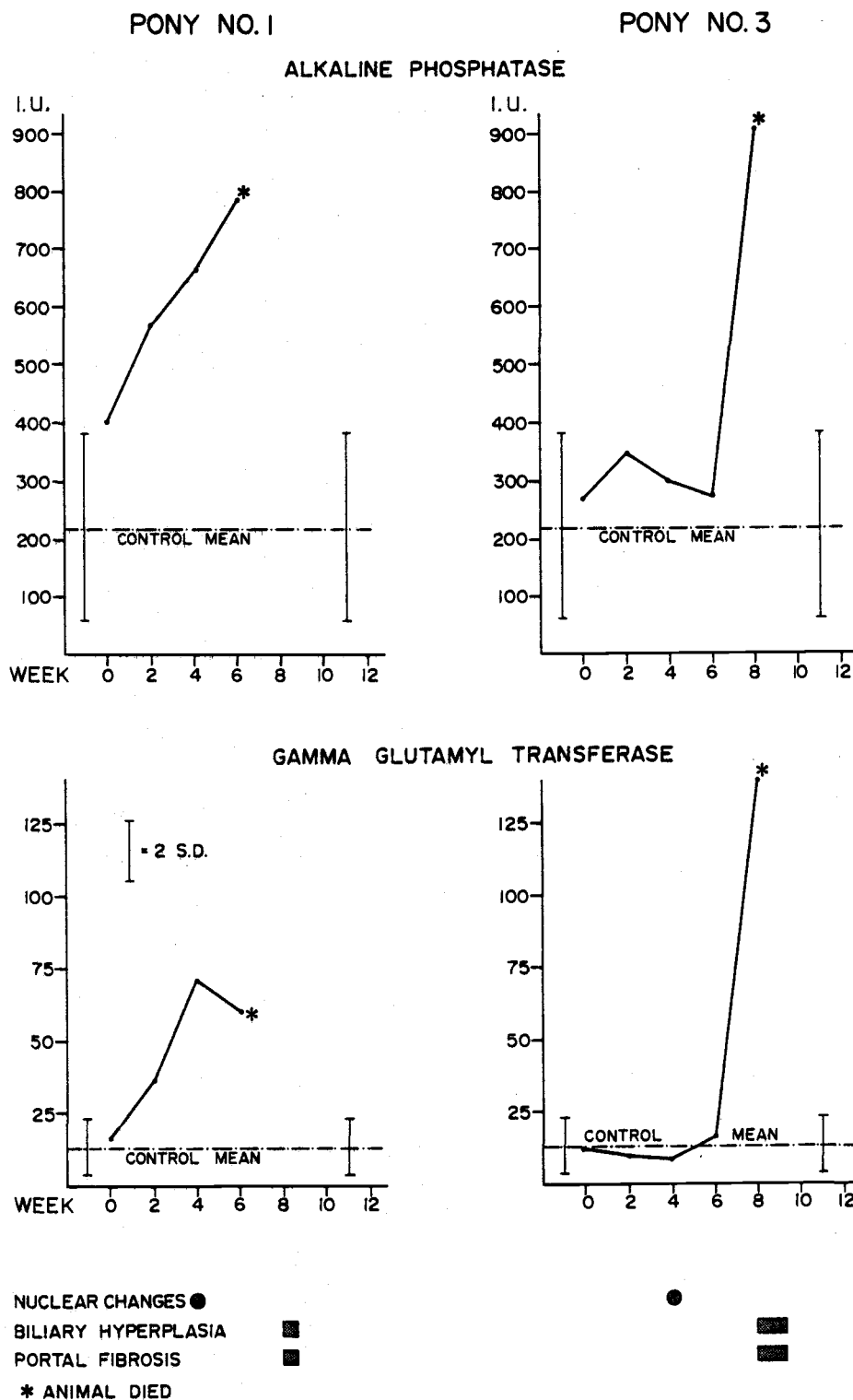
CALF NO. 3

ALKALINE PHOSPHATASE

I.U.

GAMMA GLUTAMYL
TRANSFERASE

NUCLEAR CHANGES ●   ●   A ● ● ●   ● ● ●

BILIARY HYPERPLASIA

PORTAL FIBROSIS

A = ASCITIES NO SAMPLE

* ANIMAL DIED

Figure 13.   Alkaline phosphatase and gamma-glutamyl transferase activity
in calf #4.   Shaded areas indicate weeks microscopic changes
were seen.

PONY NO. 1   PONY NO. 3

ALKALINE PHOSPHATASE

GAMMA GLUTAMYL TRANSFERASE

NUCLEAR CHANGES ●
BILIARY HYPERPLASIA ▨
PORTAL FIBROSIS ▨
* ANIMAL DIED

Figure 14. Alkaline phosphatase and gamma-glutamyl transferase activity
in ponies #1 and #3. Shaded areas indicate weeks microscopic
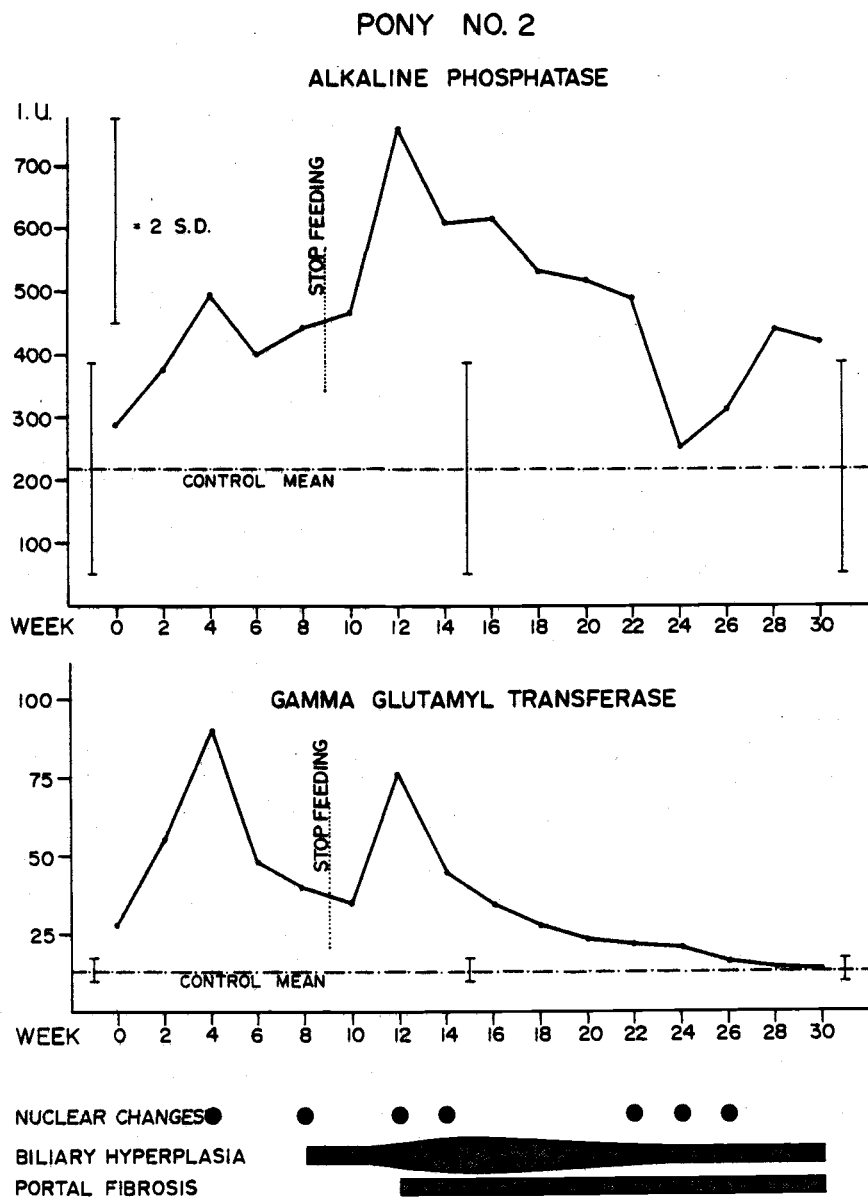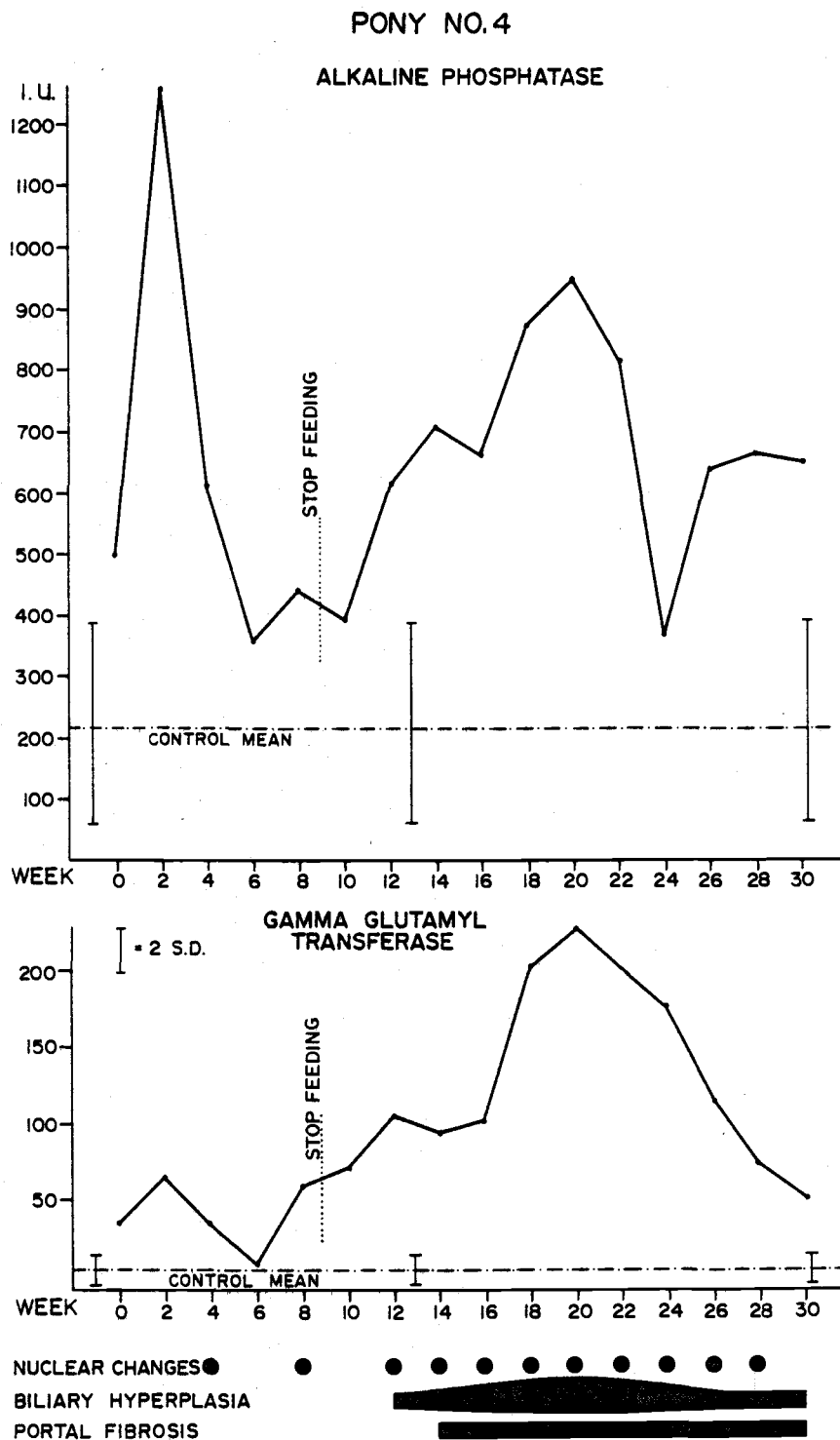changes were seen.

Figure 15. Alkaline phosphatase and gamma-glutamyl transferase activity in pony #2. Shaded areas indicate weeks microscopic changes were seen.
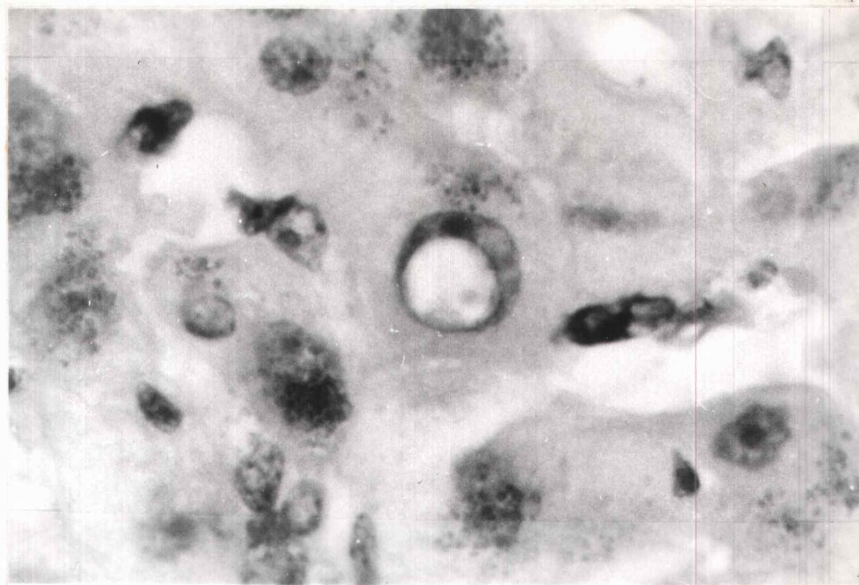
PONY NO. 4

ALKALINE PHOSPHATASE

GAMMA GLUTAMYL
TRANSFERASE

Figure 16. Alkaline phosphatase and gamma-glutamyl transferase activity
in pony #4. Shaded areas indicate weeks microscopic changes
were seen.

Figure 17. A large intranuclear vacuole in a hepatocyte of one of the ponies.

Figure 18. An electron microscope photograph showing the cytoplasmic invaginations into the nucleus of a hepatocyte from an animal fed Senecio jacobaea.
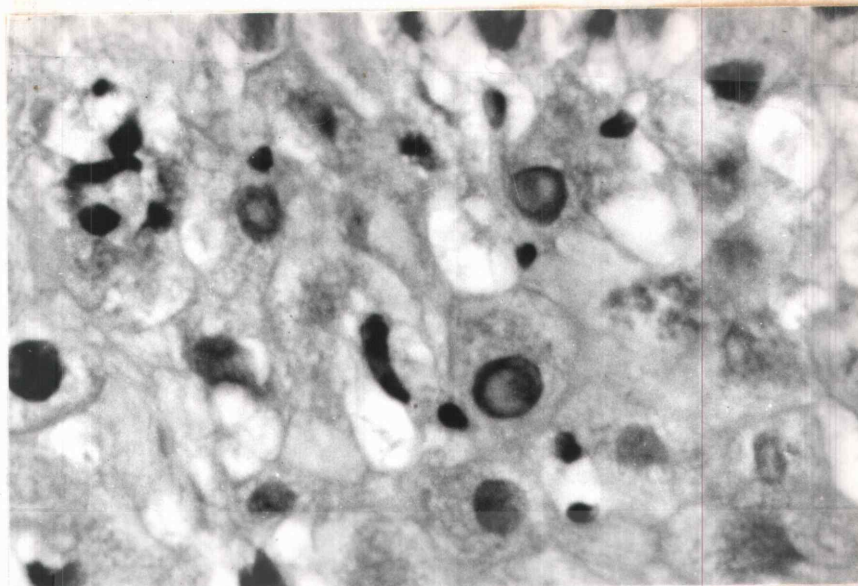
Figure 19. Many intranuclear vacuoles in pony #3 at week eight. This pony died one week after this biopsy was taken.
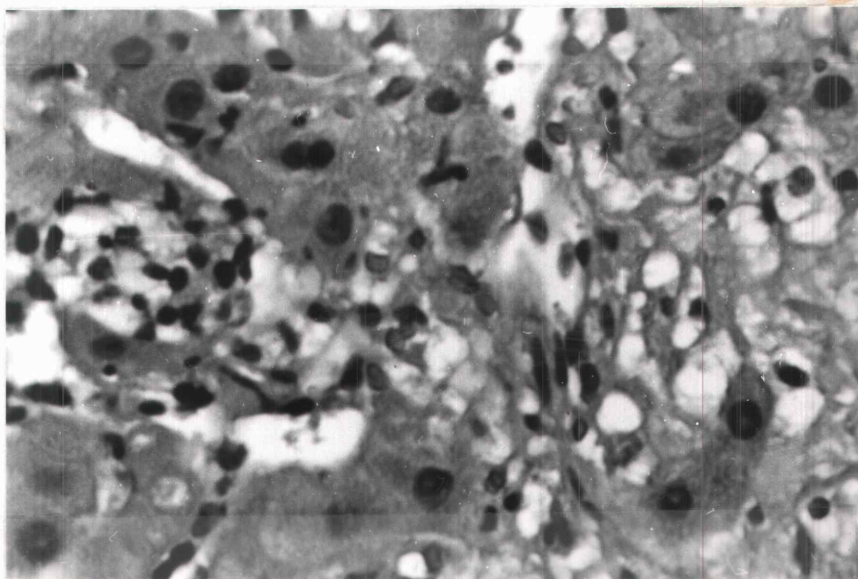

Figure 20. Intranuclear vacuoles in several hepatocytes in calf #3 at week 24, 15 weeks after exposure to Senecio. A loss of normal hepatocytes in the area is also seen.
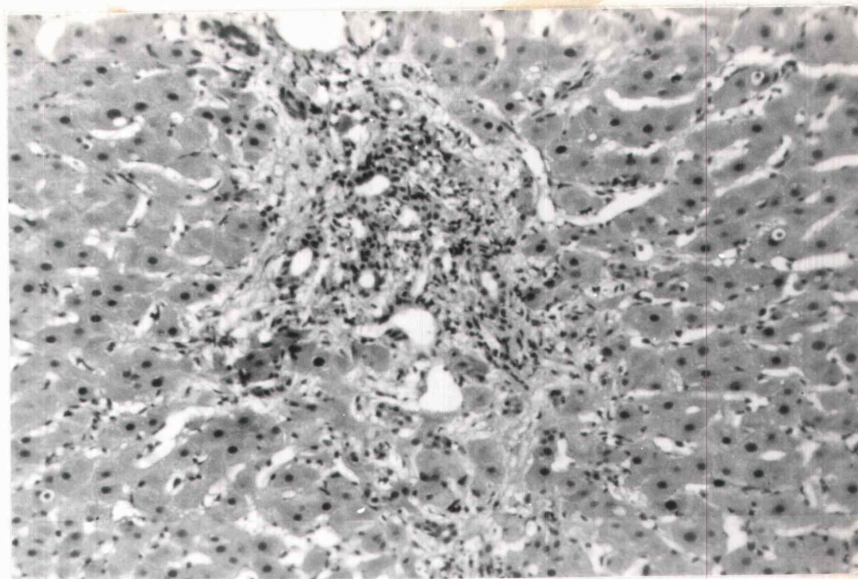
Figure 21.   Extensive biliary hyperplasia and some portal fibrosis in
calf #3 at week 20.

Figure 22.   A portal tract in pony #4 at week eight showing biliary
hyperplasia and portal fibrosis at the time _Senecio_ feeding
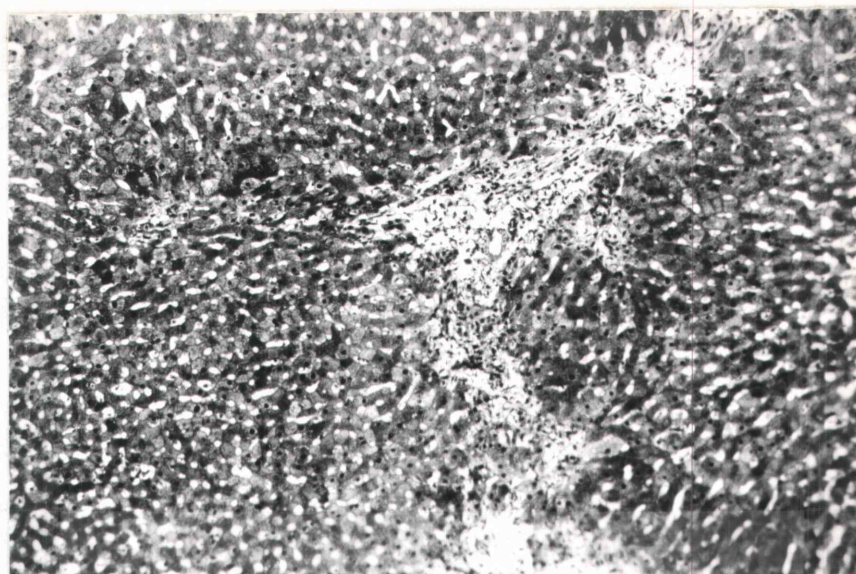had stopped.

Figure 23.   A portal tract in pony #4 at week 14, five weeks after feeding of Senecio had stopped
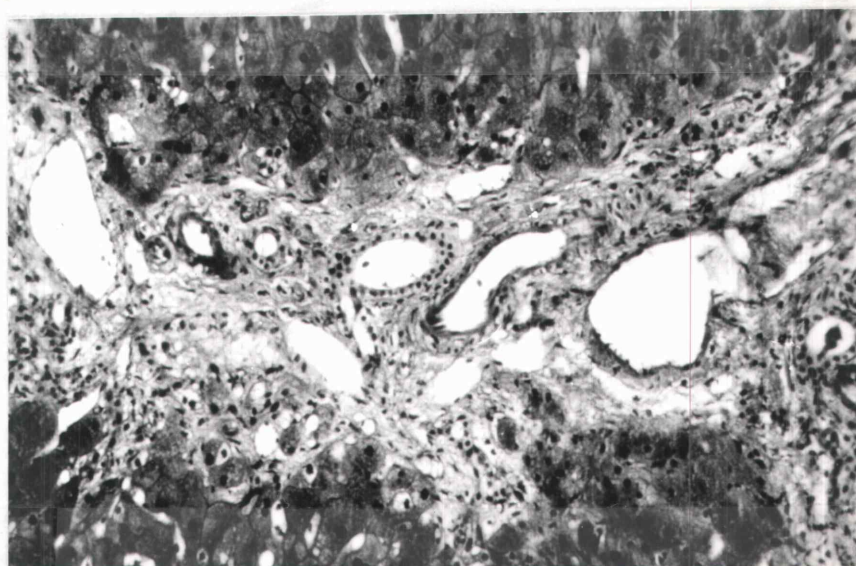

Figure 24.   A portal tract in pony #4 at week 20, 11 weeks after Senecio feeding had stopped.
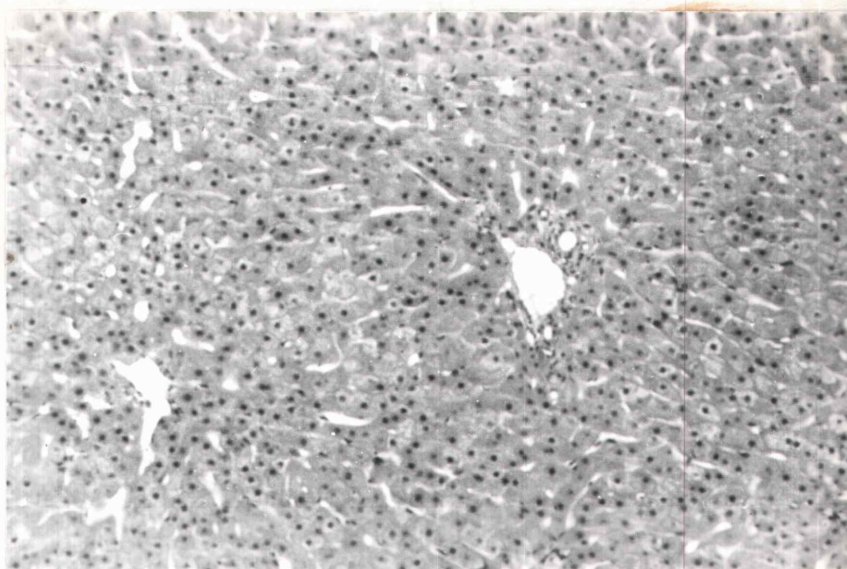
Figure 25.   A liver lobule in calf #3 at week eight near the time the
             Senecio feeding was stopped.
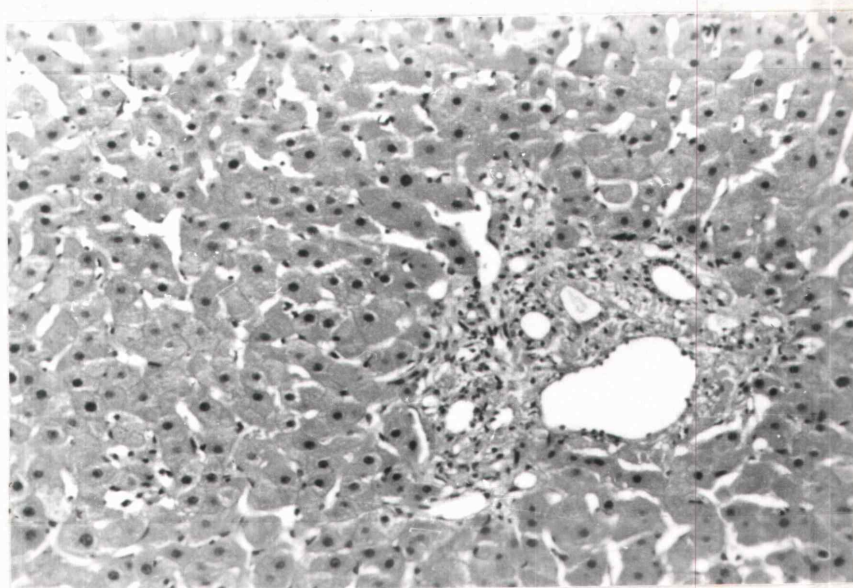

Figure 26.   A portal tract and the adjacent hepatocytes in calf #4 at
             week 16.

Figure 27. A liver section of calf #2 at the time the Senecio feeding was completed. This calf survived the experiment.

Figure 28. Liver of calf #2 at week 16, seven weeks after the Senecio feeding had stopped. Notice the limited amount of fibrosis as compared to the cases that died.

Figure 29. Liver of pony #3 at week eight, one week before its death. A large number of cells have intranuclear vacuoles and there are extensive biliary hyperplasia, fibrosis and loss of hepatocytes.

Figure 30. Section of liver taken at post-mortem from pony #3. There are fibrosis, fatty degeneration and only a few normal hepatocytes remaining.

Figure 31. Necrotic foci in pony #4 at week 16. There is an infiltra-
tion of polymorphonuclear leukocytes in the center of the
picture.

Figure 32. Another necrotic foci in pony #4 at week 22. There are also
binucleated hepatocytes visible.

Figure 33. Necrotic foci in calf #3 at week 20.

Figure 34. Liver tissue of calf #3 at week 22 showing fibrotic foci at
upper left with fibroblasts and collagen.

Figure 35. Fibrotic foci in calf #3 at week 24. There are fewer new bile ducts at this time than at week 20.

V. DISCUSSION

Some of the structural changes reported here have not been pre-viously described for the bovine and equine. The intranuclear vacuoles which are considered to be cytoplasmic invaginations have been reported in rats poisoned with pyrrolizidine alkaloid (50, 51). The electron microscope was necessary in these rat experiments to detect these nuclear changes. In our experimental cases the nuclear changes were readily visible with the light microscope on biopsy specimens. It has been claimed that most nuclear inclusions are actually invaginations of the cytoplasm (52). Karyomegal, vesiculation and hyperchromicity of nuclei have been reported in calves experimentally poisoned with _Senecio jacobaea_ but the lesions were not pictured or completely described (18). It is possible these nuclear changes are seen only on biopsy speci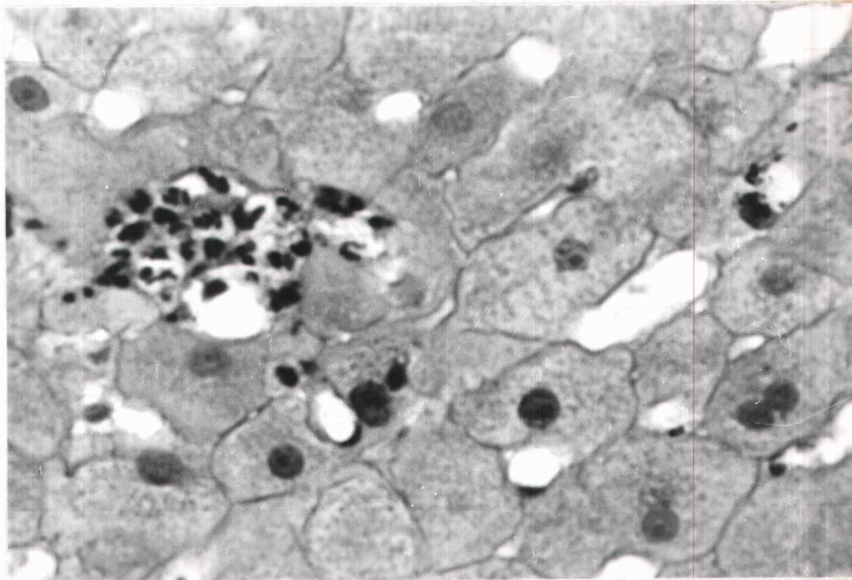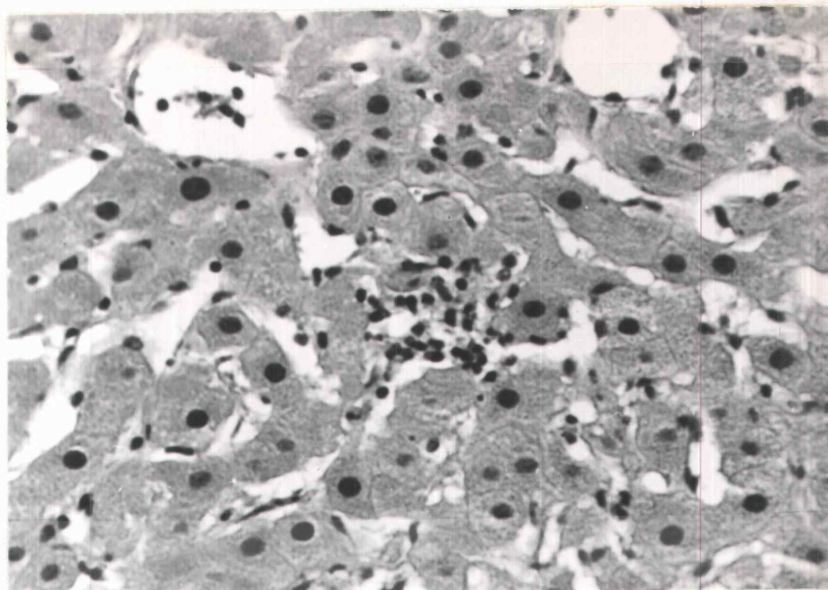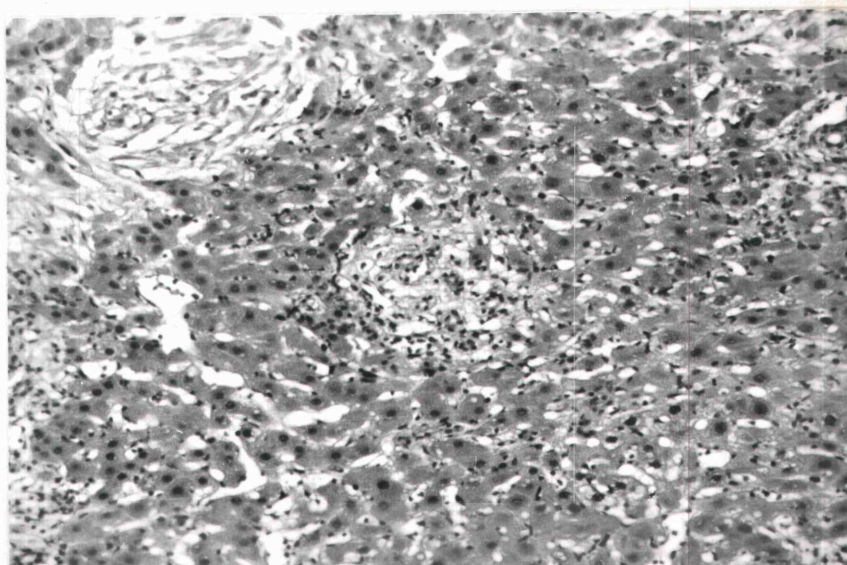mens and not on post-mortem tissue because of the delay in fixation. Most of the biopsy tissue here was in the fixative within a few seconds which is not possible on post-mortem specimens. Also, many of the cells containing the vacuoles were possibly dead before the time of post-mortem examination.

It is not expected that these nuclear changes are specific for pyrrolizidine alkaloid poisoning but since they do occur early and farily frequently in biopsy specimens, they should be a useful aid to the histopathologist when presented with liver biopsy tissue. Finding large numbers of these intranuclear invaginations seemed to indicate the imminent death of the hepatocyte involved and death of the animal in the near future. The large number of cytoplasmic invaginations into the nucleus of hepatocytes seen on electron microscope photographs of

tissue from poisoned animals were not seen in tissue taken from the animals prior to feeding the tansy. The micronecrotic foci in the liver are also not commonly seen in post-mortem specimens or in acutely poisoned animals. It is apparently evidence of the slow continual dying of hepatocytes. In the chronic delayed cases the accumulation of fibrous tissue is evidence that these cells are not replaced by new hepatocytes but are replaced by connective tissue. This could be due to the antimitoic activity of the pyrrolizidine alkaloid and failure of regeneration (56, 83). The histopathologist should not rule out pyrrolizidine alkaloid poisoning because he sees evidence of limited but acute cell death.

The other changes seen in this experiment have been previously reported. The lack of consistent visible hepatic megalocytosis on the biopsy specimens can be explained in two ways. First, the changes have been reported to be focal and could have been missed on liver biopsy (41). Second, in the chronic cases with a delayed response the number of megalocytes at a given time may have been limited.

The enzymes used in this experiment to evaluate the effect produced by pyrrolizidine alkaloids were all responsive to the damage. Glutamate dehydrogenase was the first enzyme to rise in most cases. As with other dehydrogenases and respiratory enzymes, the serum levels dropped off before the death of the animal. In other experiments, lactate dehydrogenase and sorbital dehydrogenase have peaked and dropped to normal before the animal died (17). Glutamate dehydrogenase is a good indicator of active liver damage but because of the drop in serum level after a peak and before death it may not be as good a diagnostic

aid in chronic delayed field cases as the other enzymes.

Alkaline phosphatase is not specific for liver damage but is responsive to the pyrrolizidine alkaloid induced damage. Some of the rise in pony #4 may not have been due to liver damage. When this pony was first put into the pen with the other three ponies at week zero he was badly mutilated by them. The first peak in alkaline phosphatase serum level occurred at this time but was not accompanied by a corresponding increase in the other two enzymes. The serum alkaline phosphatase level remained elevated longer than the other enzymes following pyrrolizidine alkaloid damage to the liver. In calf #3, with the delayed response, the level was 657 IU/1 just before the death of the animal 17 weeks after it was no longer being fed _Senecio_. In the surviving calf and the two ponies that have survived thus far the serum alkaline phosphatase is well above the normal range 21 weeks after the consumption of _Senecio_ was stopped.

Gamma-glutamyl transferase was rapid to respond although slightly behind glutamate dehydrogenase in most animals. Other authors have agreed it is a sensitive indicator of liver disease (97, 122). The serum level of the enzyme remained elevated for a longer time in all animals with persistent liver damage. In the calf with the delayed response the serum level was over four times pre-exposure level at the time of its death, 17 weeks after the _Senecio_ feeding had stopped. In the pony and calf that seemed to recover the level dropped to a normal range after many weeks. The pony that survived with a delayed response of considerable liver damage had a serum gamma-glutamyl transferase level well above normal 21 weeks after the _Senecio_ feeding had ceased.

Because the serum gamma-glutamyl transferase level was very respon-
sive to liver damage by _Senecio_ the magnitude of increase was great and
the level remained elevated for a long time; this enzyme may be of
greatest diagnostic value in chronic delayed field cases.

Most previous studies have not attempted to correlate serum enzyme
levels with liver structural changes (16, 18, 41). In this experiment
there seemed to be some relationship between serum alkaline phosphatase
levels and biliary hyperplasia for both calves and ponies. In both
cases the serum enzyme activity of alkaline phosphatase rose at about
the same time as biliary hyperplasia became evident. In both the calf
and the pony with the delayed response the maximum number of excessive
bile ducts and bile duct epithelial cells were seen at week 20. Week
20 was also the time at which the serum alkaline phosphatase levels
peaked in both of these animals. Following this the serum alkaline
phosphatase activity decreased and there was a gradual resolution of
some of the biliary hyperplasia.

Serum gamma-glutamyl transferase activity was high most of the time
the alkaline phosphatase activity was increased. It was increased at
all times in which either extensive biliary hyperplasia or active
necrosis of the hepatocytes was present. In cases of resolution of
biliary hyperplasia and no observable hepatocyte necrosis such as pony
#2 and calf #2 the serum gamma-glutamyl transferase activity returned
to the pre-feeding levels.

Serum glutamate dehydrogenase activity increased greatly at the
time of necrosis of zone one hepatocytes. Once these cells were des-
troyed the serum activity was reduced. It did not remain elevated in

those cases where only portal fibrosis or biliary hyperplasia remained.

Some micronecrotic foci could be seen within the liver lobule without

a rise in serum glutamate dehydrogenase activity.

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