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INVESTIGATIONS ON THE TOXICITY OF *KOCHIA SCOPARIA* (L.) SCHRAD (FIREWEED)

by

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Approved by

T. R. Oelrm

Major Professor

Dedicated to my parents,

Charles and Edythe Galitzer

who have waited a long time for this.

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EFFECTS OF CHLOROFORM ON SERUM SORBITOL DEHYDROGENASE AND ALANINE AMINOTRANSFERASE OF GUINEA PIGS

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Effects of Chloroform on Serum Sorbitol Dehydrogenase and Alanine Aminotransferase of Guinea Pigs. GALITZER, S. J. and OEHME, F. W. (1978). Toxicol. Appl. Pharmacol. 00,00-00.

Male guinea pigs were given 10-750 mg chloroform/kg body weight ip in 1, 2 or 3 successive doses at 48 or 72 hr intervals, and serum sorbitol dehydrogenase (EC 1.1.1.14; SD) and alanine aminotransferase (EC 2.6.1.2; ALT) levels were studied as indicators of liver necrosis. Increases in SD (3-129 times normal) and ALT (3 times normal) occurred at doses of 250 mg chloroform/kg and greater. Enzyme levels were greater and mortality was higher with dosing every 48 hr. SD levels peaked 24 hr after dosing and returned to normal by 96 hr. Two successive doses of chloroform in 48 hr resulted in greater SD increases than 1 dose or 3 successive doses every 48 hr. ALT increases were not as dramatic as SD increases. Dosing guinea pigs with 250-400 mg chloroform/kg every 72 hr produced hepatotoxicity, as evidenced by increased SD levels, without overt toxicity or mortality.

The primary toxic response of the liver to chloroform is necrosis. An early study in dogs (Whipple and Sperry, 1909) reported liver necrosis after 1-2 hr of chloroform anesthesia and showed young dogs more susceptible than adults. Von Oettington (1955) exposed mice, rabbits and dogs to chloroform vapors which resulted in respiratory arrest and hepatotoxicity. McLean and McLean (1969) found that enzyme induction in protein-depleted rats increased chloroform hepatotoxicity and its lethality. Male mice were more susceptible than females to chloroform hepatotoxicity, and only male mice were susceptible to its nephrotoxic effect (Eschenbrenner and Miller, 1945). The nephrotoxic effect varied with the strain of mouse (Krus and Zaleska-Rutczynska, 1970).

Chloroform administration to horses via inhalation caused an increase in glutamate dehydrogenase, ornithine carbamyl transferase, sorbitol dehydrogenase, alanine aminotransferase and aspertein transferase with concurrent liver necrosis (Thorpe et al, 1969). Divincenzo and Krasavage (1974) found after an ip injection of chloroform an increase in ornithine carbamyl transferase (10-30 times normal) in guinea pig serum before liver damage was observed.

Serum sorbitol dehydrogenase (EC 1.1.1.14; SD) is a liver-specific enzyme (Secchi et al, 1971); Gopinath and Thorpe (1968) found SD in high concentrations in rat, mouse, dog, cat and cattle liver lobules. A sharp increase in SD occurs with chloroform-induced liver necrosis in horses (Thorpe et al, 1969).

Alanine aminotransferase (EC 2.6.1.2; ALT) is found in liver cells and erythrocytes; this enzyme is liver-specific in non-human primates (Kruckenberg et al, 1972) and dogs, but not in horses, cattle and pigs (Cornelius et al, 1959).

This study was undertaken to determine the effect of single and repeated doses of chloroform on SD and ALT in guinea pigs.

METHODS

Animals

Male Dunkin-Hartley albino guinea pigs[1] weighing 250-300 g were utilized. Animals were group housed in stainless steel cages (63 cm x 69 cm x 28 cm). Sanicel[2] was used as litter. All guinea pigs were examined upon arrival, tattooed, and stabilized for five days before initiating treatment. Food[3] and water were provided *ad lib*. Ascorbic acid (250 ppm) was added to the water supply. Room temperature was maintained at 20°C, with relative humidity at 50%.

Chloroform Injection

Each guinea pig was dosed ip with chloroform in 0.5 ml corn oil, except as noted. Dosing regimen was accomplished in two phases. Ten guinea pigs were each given a single dose of 300 mg chloroform/kg body weight (phase I). Later (phase II), 83 guinea pigs were dosed with 10-750 mg chloroform/kg every

48 or 72 hr (Table 1). Ten to 50 mg chloroform/kg was added to 0.5 ml olive oil. Six control guinea pigs received 0.5 ml olive oil ip every 48 hr.

Serum Collection

Blood samples were taken by cardiac puncture or decapitation. In the first study (phase I), blood was collected from two animals each at 0, 16, 24, 48, 72 and 96 hr after dosing. In the later study, (phase II) blood samples were taken 48 hr after the last chloroform dose.

Enzyme Assays

Serum during phase I and II was assayed for SD by a spectrophotometric method[4]. Serum from phase II guinea pigs was assayed for ALT by a colorimetric method[5]. SD was reported in Sigma Units per ml (SU/ml)[6]. ALT was reported as Sigma-Frankel Units per ml (SFU/ml)[7].

Statistical Analysis

Differences in chloroform lethality were evaluated by Chi square analysis.

RESULTS

Phase I

The mean SD level in the pre-dose serum samples was 2929 SU/ml (±201 SE). The SD reached peak levels in excess of 100,000 SU/ml 24 hr after chloroform administration; by 96 hr the SD level approached normal (Fig. 1).

Phase II

The mean SD level of the control samples was 3179 SU/ml (±202 SE). Treatment with olive oil alone produced no significant effect (Fig. 2).

The lowest dose of chloroform to elicit an increase in SD was 250 mg/kg measured after 48 hr (Fig. 2). The increase in all groups (≥ 250 mg/kg) due to chloroform ranged from 3-139 times the baseline serum level. Guinea pigs receiving two successive doses at 48 hr intervals had a greater increase in SD than guinea pigs receiving 1 or 3 successive doses of chloroform at 48 hr intervals (Fig. 2). Guinea pigs dosed at 48 hr intervals had a greater increase in serum SD than those dosed at 72 hr intervals (Fig. 3).

The mean serum ALT of the control samples was 11.92 SFU/ml (±3.19 SE). Olive oil produced no significant effect. Guinea pigs receiving 250, 500 and 750 mg chloroform/kg every 48 hr had ALT levels that increased two to three fold (Fig. 4).

The difference in lethality between groups dosed every 48 hr or 72 hr was highly significant ($P < 0.01$). Thirty-three percent of the guinea pigs died when dosed every 48 hr while only 4% died when dosed every 72 hr. Death did not occur at doses of 10, 30 or 50 mg chloroform/kg.

DISCUSSION

Chloroform injected ip at 72 hr intervals was less toxic than that administered at 48 hr intervals. Doses between 250 and 400 mg/kg produced a detectable increase in serum SD. Since SD is a liver specific enzyme, any increase in SD serum levels indicates liver damage. The SD reached a maximum level 24 hr after single injections of chloroform and approached normal by 72 hr, indicating peak liver cell damage within 24 hr of chloroform administration.

The lowered SD level in animals receiving three chloroform doses suggests a tolerance of liver cells to chloroform insult. The mechanism may be increased metabolism, increased excretion, or incorporation of the insulting compound into the cell's metabolic scheme (Casarett, 1975).

Alanine aminotransferase is also an indicator of liver damage in the guinea pig as evidenced by the ALT level increases. Since the ALT increases were not as dramatic as those of SD, the latter appears a more sensitive indicator of chloroform-induced liver damage in the guinea pig.

Chloroform doses of 250-400 mg/kg given ip at 72 hr intervals did not cause general biological toxicity or overt mortality. However, the increased serum SD levels suggests that guinea pigs receiving this regimen could be a good biological model for studying liver dysfunction and its influence on the biological effects of other foreign compounds.

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Table 1. Chloroform Dose, Dosing Interval, Enzyme Levels and Mortality in Guinea Pigs Receiving Chloroform by ip Injection

Chloroform Dose (mg/kg)	Number of Guinea Pigs	Number of Doses	Interval hr	Sorbitol Dehydrogenase (SU/ml)	Alanine Aminotransferase (SFU/ml)	Mortality
10[a]	2	1	48	3,654	-	0/2
10[a]	2	2	48	3,828	23	0/2
10[a]	2	3	48	4,350	15	0/2
30[a]	2	1	48	2,668	0	0/2
30[a]	2	2	48	3,306	9	0/2
30[a]	2	3	48	2,407	1	0/2
50[a]	2	1	48	3,045	-	0/2
50[a]	2	2	48	3,074	4	0/2
50[a]	2	3	48	3,045	12	0/2
250	4	1	48	3,915	34	3/4
250	2	2	48	11,426	46	1/2
250	2	3	48	4,176	28	1/2
300	2	1	48	4,486	29	0/2
300	2	2	48	21,750	13	0/2
300	2	3	48	4,060	9	0/2
400	2	1	48	-	-	2/2
400	2	2	48	30,580	21	0/2
400	2	3	48	9,860	12	0/2

Chloroform Dose (mg/kg)	Number of Guinea Pigs	Number of Doses	Interval hr	Sorbitol Dehydrogenase (SU/ml)	Alanine Aminotransferase (SFU/ml)	Mortality
500	4	1	48	27,260	34	2/4
500	2	2	48	440,800	18	2/2
500	3	3	48	28,420	17	1/3
750	6	1	48	66,990	36	2/6
750	3	2	48	247,080	>125	3/3
750	2	3	48	-	-	2/2
75[b]	2	1	72	2,755	-	0/2
75[b]	2	2	72	3,625	-	0/2
150[b]	3	1	72	2,903	-	0/3
150[b]	5	2	72	3,198	-	0/5
150[b]	2	3	72	3,190	-	0/2
300	4	2	72	3,883	10	1/4
300	3	3	72	4,407	5	0/3
400	2	2	72	8,990	20	0/2
400	2	3	72	11,600	21	0/2

[a] Chloroform in 1/2 ml olive oil, all others in 1/2 ml corn oil.

[b] Guinea pigs received 300 mg/kg initially followed by noted dose after 72 hr.

Figure 1. Serum sorbitol dehydrogenase (SD) levels (±SE) in guinea pigs following single ip administration of 300 mg chloroform/kg.

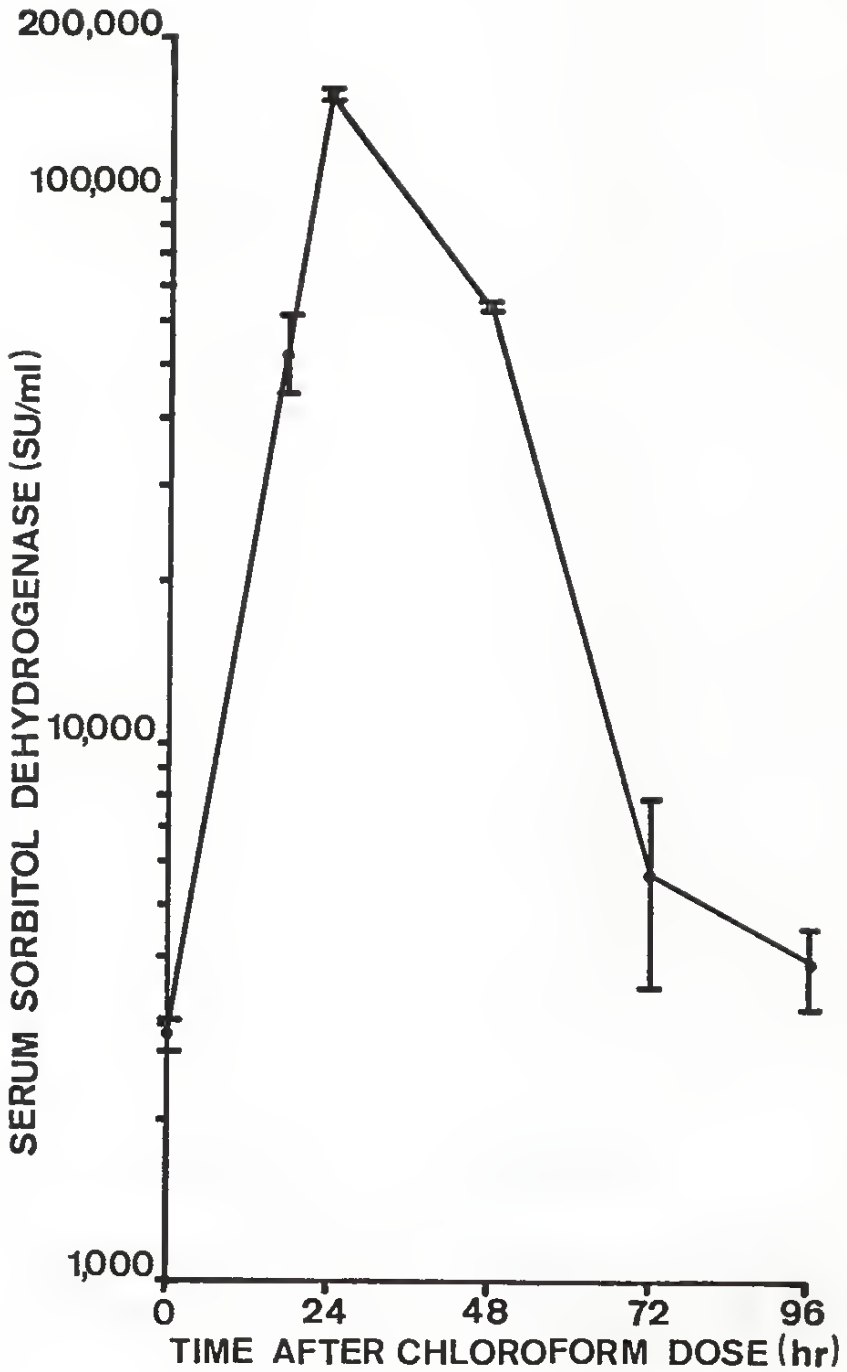

200,000
100,000
10,000
1,000
SERUM SORBITOL DEHYDROGENASE (SU/ml)
0
24
48
72
96
TIME AFTER CHLOROFORM DOSE (hr)

Figure 2. Serum sorbitol dehydrogenase (SD) levels (±SE) following single or multiple (every 48 hr) ip administrations of chloroform at various dose levels; (^{a}n = 1 due to mortality from multiple injections). Values represent samples collected 48 hr after the last dose of chloroform.

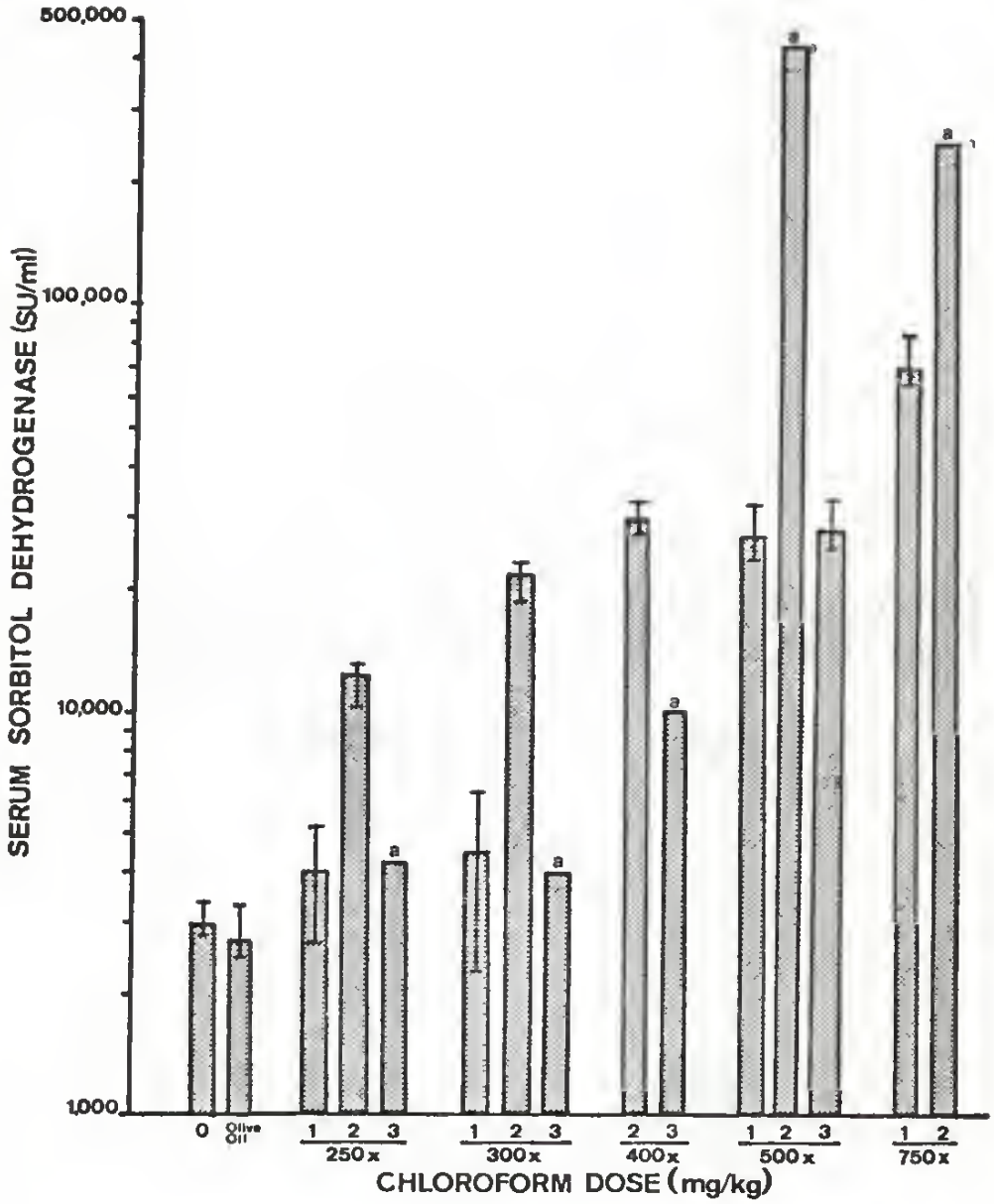

SERUM SORBITOL DEHYDROGENASE (SU/ml)
500,000
100,000
10,000
1,000
0
Olive Oil
1 2 3
250x
1 2 3
300x
2 3
400x
1 2 3
500x
1 2
750x
a
CHLOROFORM DOSE (mg/kg)

Figure 3. Serum sorbitol dehydrogenase (SD) levels (mean ±SE) in guinea pigs before (0) and 48 hr after receiving chloroform doses every 48 or 72 hr ($n_0 = 146$; $n_{48} = 26$; $n_{72} = 24$).

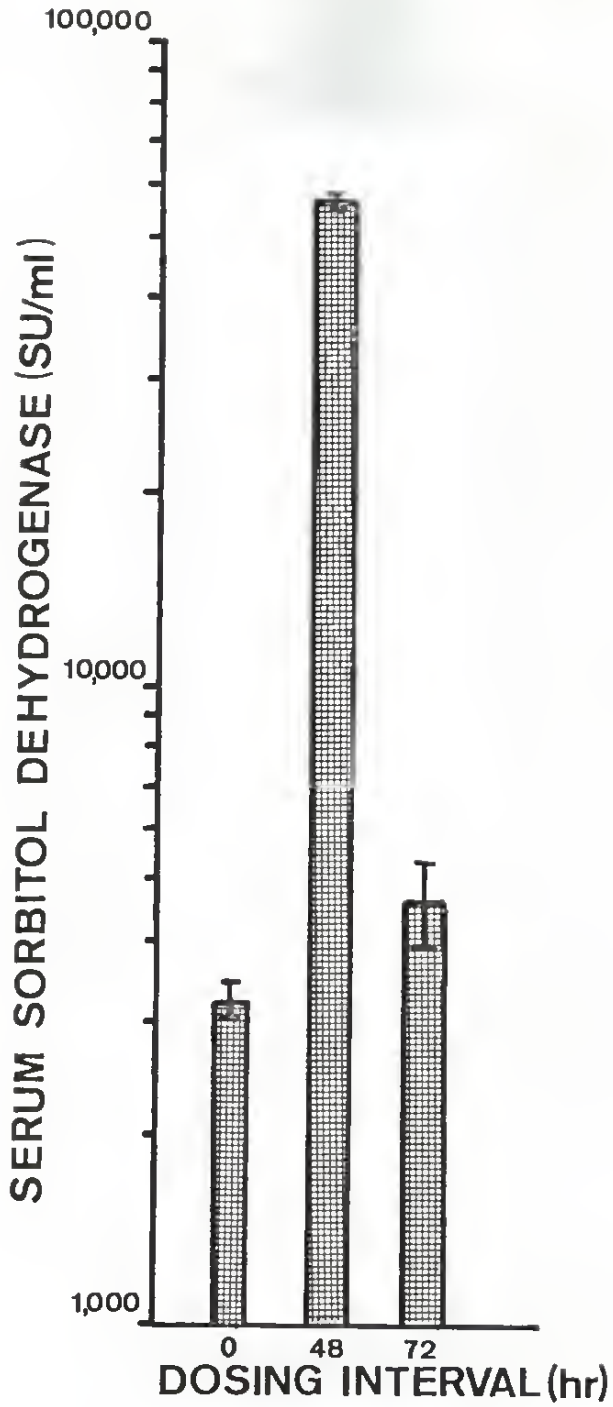
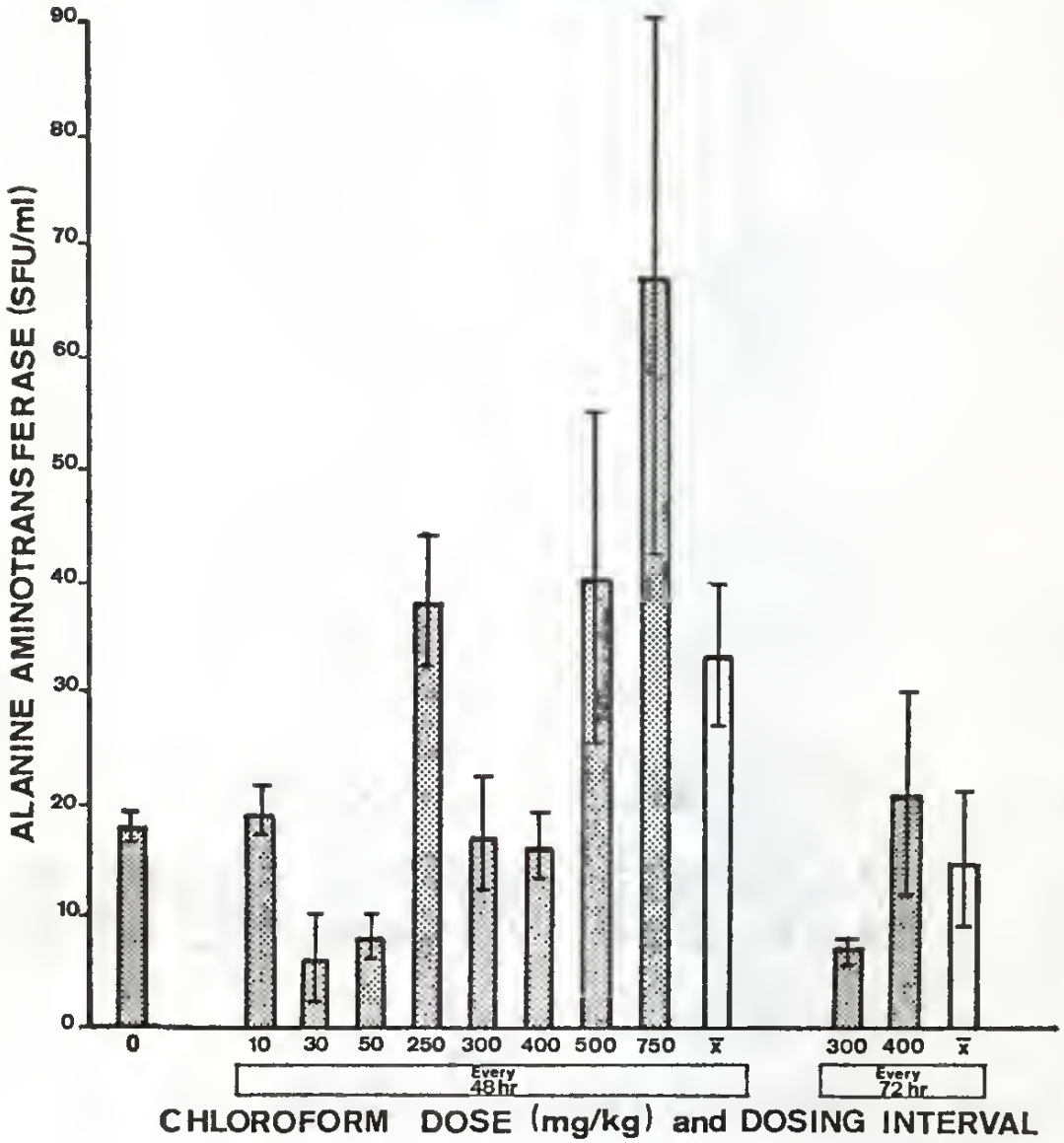

100,000
10,000
1,000
SERUM SORBITOL DEHYDROGENASE (SU/ml)
0
48
72
DOSING INTERVAL (hr)

Figure 4. Serum alanine aminotransferase (ALT) level (mean ±SE) following ip administration of chloroform every 48 or 72 hr at various dose levels ($n_0 = 75$; $n_{48} = 24$; $n_{72} = 7$). Values represent samples collected at 48 hr after the last chloroform dose.


ALANINE AMINOTRANSFERASE (SFU/ml)
90
80
70
60
50
40
30
20
10
0
0
10
30
50
250
300
400
500
750
x̄
300
400
x̄
Every 48 hr
Every 72 hr
CHLOROFORM DOSE (mg/kg) and DOSING INTERVAL

[1]Ancare Corp., Manhasset, Long Island, NY.

[2]Sanicel[R], Paxton Processing Co., Inc., Paxton, IL.

[3]Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, MO.

[4]Bulletin 50-UV, Sigma Chemical Co., St. Louis, MO.

[5]Bulletin 505, Sigma Chemical Co., St. Louis, MO.

[6]This value can be converted to milli-International Units by dividing the data by 60.

[7]This value can be converted to International Units by multiplying the data by 48.

INDEX TERMS: Chloroform, Guinea Pigs, Enzymes, Sorbitol Dehydrogenase, Alanine Aminotransferase, Hepatotoxicity.

STUDIES OF THE COMPARATIVE TOXICITY OF *KOCHIA SCOPARIA* (L.) SCHRAD (FIREWEED)

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Studies of the Comparative Toxicity of Kochia Scoparia (L.) Schrad (Fireweed). GALITZER, S. J. and OEHME, F. W. (1978). Toxicol. Appl. Pharmacol. 00, 00-00. Dunkin-Hartley albino guinea pigs were fed Kochia scoparia (L.) Schrad (fireweed), a plant which caused photosensitization in domestic animals, and exposed to ultraviolet light daily for 15 days. Guinea pigs were also dosed with chloroform (300 or 400 mg/kg ip) to create liver dysfunction. Serum sorbitol dehydrogenase (EC 1.1.1.14; SD) and alanine aminotransferase (EC 2.6.1.2; ALT) were measured to evaluate liver damage. Kidneys were examined for oxalate crystals. Guinea pigs receiving chow and chloroform had elevated SD levels which returned to normal after 72 hr. Fireweed-fed animals also receiving chloroform had SD levels that did not return to the control range. There was no significant effect on ALT serum levels. All guinea pigs fed fireweed and dosed with chloroform died within 11 days. Oxalate crystals were found in kidneys of 1 guinea pig fed fireweed and 5 guinea pigs fed fireweed and receiving chloroform. No signs of photosensitization developed in any animals. The results suggest a potentiating effect of fireweed and chloroform.

Kochia scoparia (L.) Schrad (fireweed) is a hardy annual herb with simple, entire, lanceolate leaves belonging to the family Chenopodiaceae, the goosefoot family (Fig. 1). Fireweed is extremely drought resistant and is commonly found throughout South and North America. It is used as forage by many ranchers--being grazed in its immature stage prior to blooming, cut and ensiled before going to seed, cut and baled as hay for winter feed (Paulsen, 1946; South Dakota Experiment Station, 1947; Bell et al, 1952). Fireweed has been incriminated as causing hepatogenous photosensitization in cattle, sheep, and horses (Rottgardt, 1944; Clare, 1952; Sperry et al, 1968).

Toxicity develops at and subsequent to the plant's blooming stage (Oyuela and Gualdoni, 1941) and is particularly pronounced during prolonged summer droughts. Clinical signs of fireweed toxicity in domestic animals reported by Kansas veterinarians included photosensitization, blindness, hyperexcitability and jaundice[1]. The plant contains saponins (Greshoff, 1909) oxalates (Camp, 1963), alkaloids (Borkowski et al, 1965) and nitrates (Bradley et al, 1940). Rottgardt (1944) suggested that fireweed is non-toxic if cut and allowed to dry in the sun for a few days. No experimental feeding trials have been reported in the literature.

Because the reported toxicity due to fireweed included photosensitization and apparent liver damage, toxicity studies

were conducted in several species of animals by feeding the plant alone and then by administering it to guinea pigs with chloroform induced liver dysfunction.

METHODS

Preliminary Studies

Four New Zealand white rabbits were daily given an aqueous slurry of dried fireweed by stomach tube and exposed to ultraviolet light (320-400 nm) for 15 days (1.96 $J/cm^2/day$). No clinical signs or changes in blood chemistry and hematology were observed[2,3]. Another group of 2 rabbits, fed only freshly picked fireweed for 30 days and exposed to ultraviolet light (320-400 nm, 4.5 $J/cm^2/day$), had no clinical signs or blood parameter changes[4]. Two sheep grazing fireweed pasture for 30 days also had no clinical or hematologic effects[4].

Animals

Male Dunkin-Hartley albino guinea pigs[5] weighing 250-300 g were utilized. Animals were housed in polycarbonate shoebox cages (60 cm x 33 cm x 20 cm) with Sanicel[6] as litter. Upon arrival, all guinea pigs were examined, tattooed, and stabilized for at least five days. Feed and water was provided *ad lib*. Ascorbic acid (250 ppm) was added to the water supply. Room temperatures were maintained at 20 C and the relative humidity was 50%. Animals were divided into 8 treatment groups and maintained on experiment for 15 days.

Dosing

Nineteen guinea pigs were fed only fireweed picked fresh daily in the full flower stage. A sample of the plant fed was placed on file in the Kansas State University Herbarium. As controls, 17 animals were fed leafy alfalfa hay and 11 guinea pigs received standard laboratory guinea pig chow[7]. Chloroform was utilized to produce liver damage that was assessed by monitoring serum sorbitol dehydrogenase (EC 1.1.1.14; SD) and alanine aminotransferase (EC 2.6.1.2; ALT) (Galitzer and Oehme, 1978). Thirty guinea pigs received 300 or 400 mg chloroform/kg body weight in 0.5 ml corn oil ip (13 guinea pigs fed fireweed, 11 guinea pigs fed alfalfa hay and 6 guinea pigs fed chow). The other guinea pigs received 0.5 ml corn oil ip (6 guinea pigs fed fireweed, 6 guinea pigs fed alfalfa hay and 5 guinea pigs fed chow). The chloroform/corn oil or corn oil alone was given ip periodically for 5 injections (days 0, 3, 7, 10, and 13). Animals were weighed daily and observed for any toxic effects.

Photosensitization

Each animal was exposed daily to an artifical ultraviolet light source[8] emitting at wavelengths of 320-400 nm and maintained 12.9 cm from the guinea pigs. The irradiation delivered daily was 5.886 J/cm^2. Each guinea pig was examined for erythema and other signs of photosensitization prior to and after irradiation.

Serum Collection and Assay

A 2 ml blood sample was taken from each guinea pig via cardiac puncture periodically (days 1, 5, 9, 13, 16 or days 1, 7, 11, 15, 16). Serum was assayed for SD and ALT by spectrophotometric techniques[9,10]. Sorbitol dehydrogenase results were expressed as Sigma Units/ml (SU/ml)[11]; ALT values were reported in Sigma-Frankel Units/ml (SFU/ml)[12].

Histopathological Studies

All guinea pigs were euthanitized by decapitation on day 16 and immediately necropsied. Kidney tissue was fixed in neutral buffered formalin, and 7 μ thick sections were mounted and stained with hematoxylin-eosin. All sections were examined under polarized light for the presence of oxalate crystals.

Statistical Evaluation

Difference in ALT values between groups were evaluated by analysis of variance; analysis of SD was not possible due to large differences in variance values between samples and groups. Statistical evaluation of lethality differences and the presence or absence of oxalate crystals in kidneys was accomplished by Chi square analysis.

RESULTS

Guinea pigs fed fireweed and dosed with chloroform experienced 100% mortality by day 11 of feeding ($P < 0.01$). Seventeen percent mortality occurred in the control group fed chow and

dosed with chloroform. No mortalities occurred in the alfalfa hay and dosed with chloroform group. In the group fed fireweed alone, 25% mortalities occurred; no mortalities occurred in the alfalfa hay alone or the chow alone groups.

None of the guinea pigs in any of the groups developed signs of photosensitization during the experimental period.

The SD levels of the guinea pigs fed chow and receiving corn oil ranged from 2320 SU/ml to 5800 SU/ml during the 15-day study (Fig. 2). Those fed alfalfa hay and receiving corn oil had SD levels of 2561 SU/ml to 7830 SU/ml (Fig. 3). Fireweed-fed guinea pigs (which also received corn oil) had SD levels that ranged from 2513 SU/ml to 7830 SU/ml (Fig. 4).

Serum SD levels from all three groups dosed with chloroform were elevated (Fig. 2-4). Those guinea pigs maintained on chow experienced a reduction in SD levels following an initial elevation (Fig. 2). The alfalfa hay-fed and the fireweed-fed groups had elevated SD levels that did not approach normal values (Fig. 3, 4).

There were no significant differences in ALT levels between groups.

Oxalates were found in the kidneys of one guinea pig receiving only fireweed and in 5 guinea pigs fed fireweed and receiving chloroform. No oxalates were found in any other kdiney sections ($P < 0.05$).

DISCUSSION

The elevated SD levels that persisted in the alfalfa hay and fireweed-fed animals also dosed with chloroform suggests an increased toxic stress on the liver. The proposed toxin in fireweed could have acted in this capacity when the animals were in the induced state of hepatic dysfunction. This is supported by observations of field cases in Kansas. Only a few animals were usually affected in any herd and all cases occurred during a period of stress, i.e. greatly reduced feed and water. The effect in the alfalfa hay-fed group possibly is due to decreased protein in the diet. Korsrud et al (1976) found increases in SD in rats with carbon tetrachloride-induced liver damage and protein deficient diets. The chemical similarity of chloroform and carbon tetrachloride suggests that the effect of protein in the diet will cause SD increases in animals with chloroform-induced liver damage.

Finding oxalate crystals in the kidney sections of fireweed-fed guinea pigs supported the report of Camp (1963) that fireweed contained up to 10.24% oxalic acid (dry weight basis).

Albino guinea pigs and rabbits are routinely used for the laboratory investigation of plant-induced photosensitization. Their herbivore diet, small size, and skin sensitivity to light are distinct advantages. Quin et al (1935) determined that neither guinea pigs nor rabbits produce the large amounts of phylloerythrin that ruminants do in their digestive tracts.

Photosensitization may not have occurred in the guinea pigs used in this study because phylloerythrin was not generated in sufficient quantity to create the systemic levels needed for photosensitization of plant origin.

This study has demonstrated that if hepatic-stressed guinea pigs receive fireweed, a potentiated toxic response results in increased liver toxicity and a significant rise in mortality.

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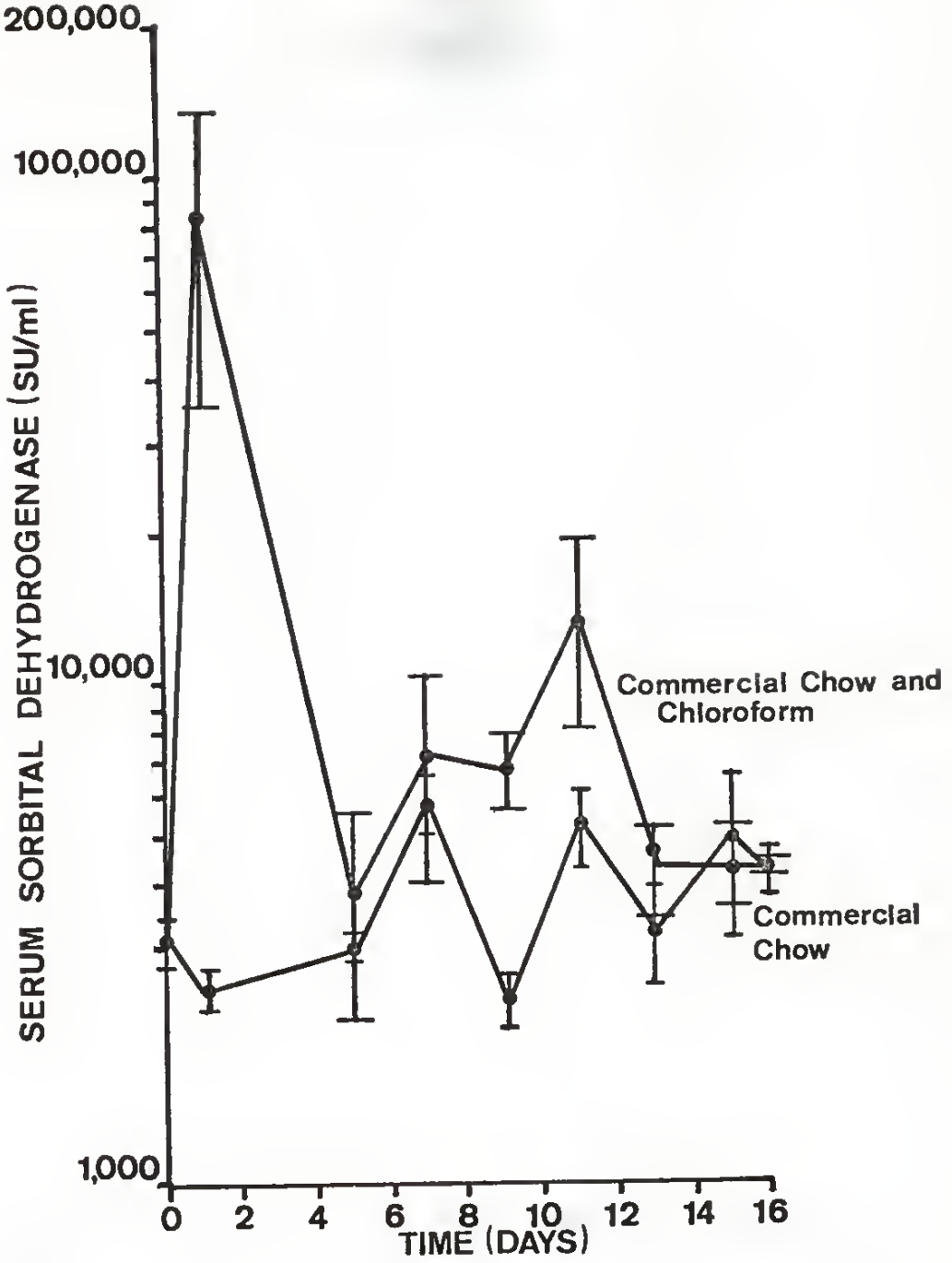
Figure 1. Kochia scoparia (L.) Schrad, the adult plant (University of Illinois Agricultural Experiment Station, 1973).

Figure 2. Serum Sorbitol dehydrogenase (± SE) in guinea pigs dosed ip with 400 mg chloroform/kg or corn oil on days 0, 3, 7, 20 and 13 and on chow diet.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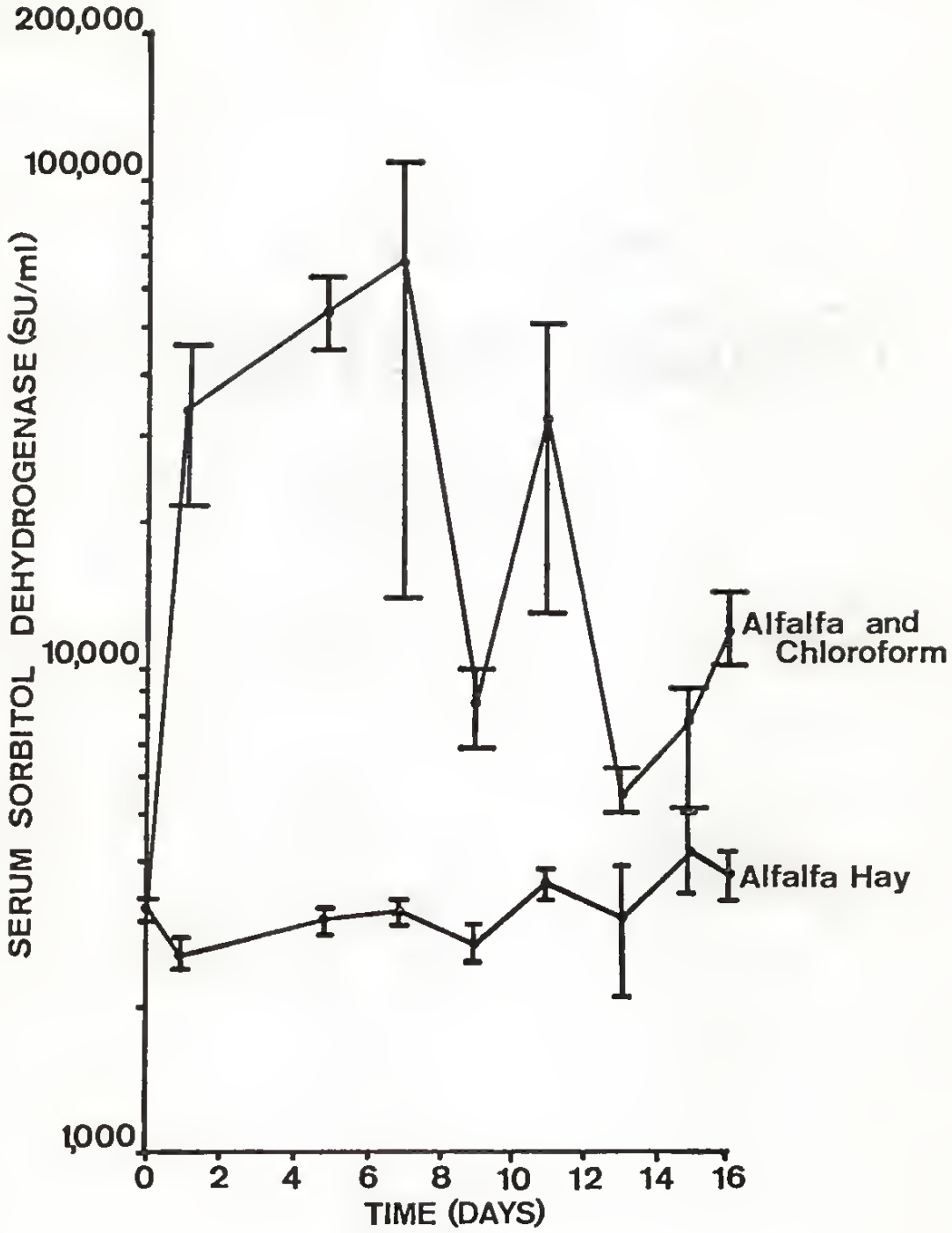
100,000
10,000
1,000
SERUM SORBITAL DEHYDROGENASE (SU/ml)
0
2
4
6
8
10
12
14
16
TIME (DAYS)
Commercial Chow and Chloroform
Commercial Chow

Figure 3. Serum sorbitol dehydrogenase levels (± SE) in guinea pigs dosed ip with 300 or 400 mg chloroform/kg or corn oil on days 0, 3, 7, 10 and 13 and on alfalfa hay diet.


200,000
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10,000
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SERUM SORBITOL DEHYDROGENASE (SU/ml)
0
2
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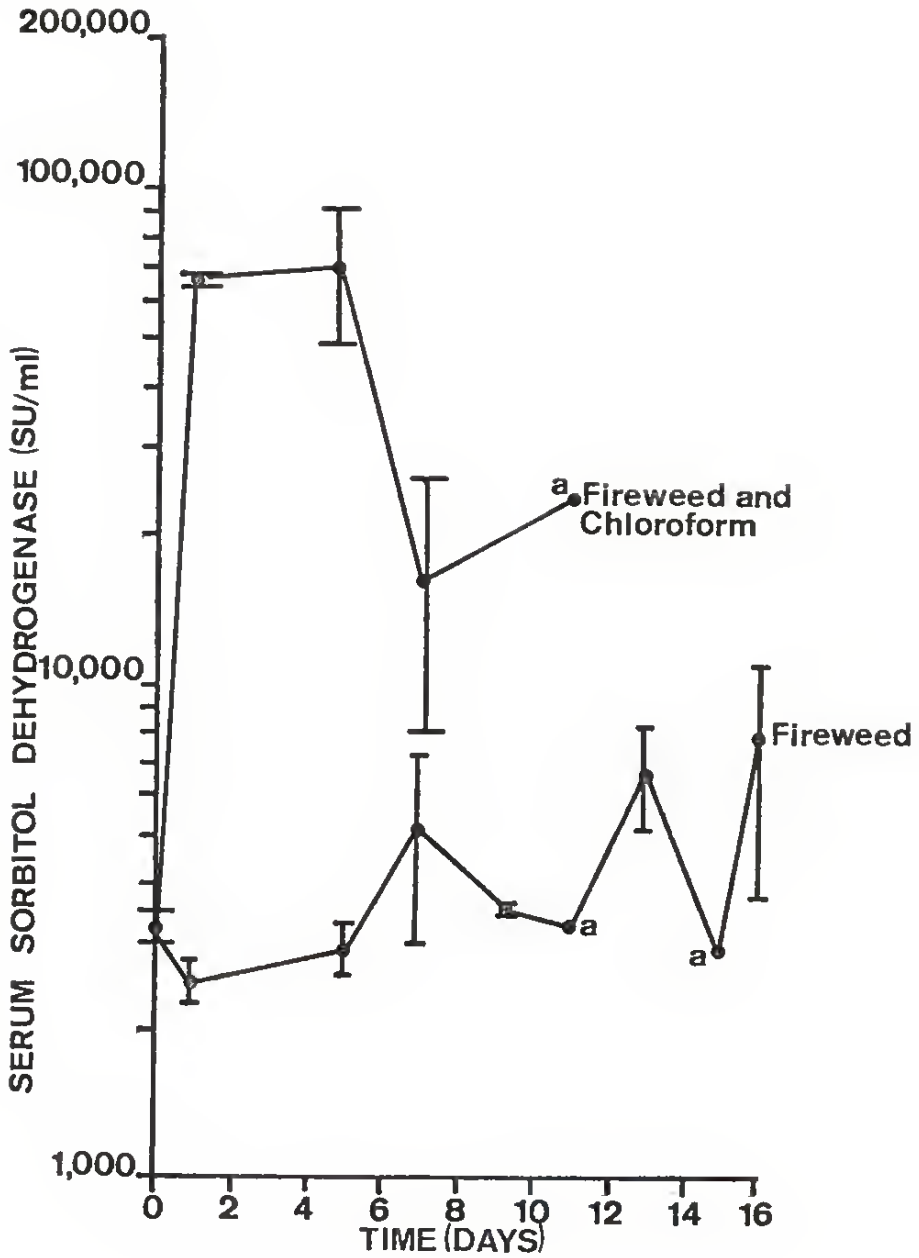
12
14
16
TIME (DAYS)
Alfalfa and Chloroform
Alfalfa Hay

Figure 4. Serum sorbitol dehydrogenase levels (± SE) in guinea pigs dosed ip with 300 or 400 mg chloroform/kg or corn oil on day 0, 3, 7, 10 and 13 and on fireweed diet. (^{a}n = 1 due to mortality)


200,000
100,000
10,000
1,000
SERUM SORBITOL DEHYDROGENASE (SU/ml)
0
2
4
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8
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12
14
16
TIME (DAYS)
a
Fireweed and
Chloroform
Fireweed
a
a

[1]Galitzer, S. J. (1976), unpublished data, Comparative Toxicology Laboratory, Kansas State University, Manhattan, KS.

[2]Nwude, N. (1975), unpublished data, Comparative Toxicology Laboratory, Kansas State University, Manhattan, KS.

[3]Galitzer, S. J. and Oehme, F. W. (1975), unpublished data, Comparative Toxicology Laboratory, Kansas State University, Manhattan, KS.

[4]Galitzer, S. J. and Oehme, F. W. (1976), unpublished data, Comparative Toxicology Laboratory, Kansas State University, Manhattan, KS.

[5]Ancare Corp., Manhasset, Long Island, NY.

[6]Sanicel[R], Paxton Processing Co., Inc., Paxton, IL.

[7]Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, MO.

[8]Blak-Ray X-30 Ultraviolet Lamp; light source = 30 watt tube, 36 in. long, self-filtering, long wave. Arthur H. Thomas Co., Philadelphia, PA.

[9]Bulletin 50-UV, Sigma Chemical Co., St. Louis, MO.

[10]Bulletin 505, Sigma Chemical Co., St. Louis, MO.

[11]SU/ml can be converted to milli-International Units by dividing the data by 60.

[12]SFU/ml can be converted to International Units by multiplying the data by 48.

INDEX TERMS: Toxicity, *Kochia scoparia*, Fireweed, Guinea Pigs, Photosensitization, Liver Enzymes, Sorbitol Dehydrogenase, Alanine Aminotransferase, Chloroform, Oxalates.

APPENDIX A

EXPERIMENTAL ANIMAL DATA

Table 1. Enzyme Levels in Normal Guinea Pigs (ALT = alanine aminotransferase, SD = sorbitol dehydrogenase, AR = arginase)

ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)	ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)
26	4	1,067	-	21	1,230
-	4	1,879	-	3	1,740
-	-	1,624	10	-	1,508
28	-	3,016	-	-	2,053
14	20	2,181	-	-	1,276
-	-	1,114	-	2	1,369
-	-	2,262	-	3	15,950
-	0	1,589	-	0	2,204
-	0	2,494	-	-	2,030
8	2	1,473	-	0	1,566
28	0	1,508	-	-	2,088
16	8	1,740	-	0	1,914
-	-	3,248	-	0	3,364
-	-	2,436	-	-	4,408
-	-	3,074	-	-	3,225
-	-	4,756	-	-	5,846
-	-	4,686	-	-	2,366
-	-	3,132	-	-	2,552
-	-	2,691	-	-	2,436
-	0	2,320	-	-	4,640
-	-	3,712	-	-	3,642
18	132	5,916	-	0	4,524

Table 1 (continued)

ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)	ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)
–	4	2,598	17	2	2,853
–	3	3,086	–	140	8,507
–	–	2,552	–	0	3,132
0	13	2,842	12	6	2,726
–	0	3,248	25	18	2,146
–	0	2,958	18	–	1,450
14	–	2,900	20	–	3,422
8	–	2,552	–	–	4,234
10	–	3,712	12	–	2,262
–	–	3,016	14	–	3,190
14	–	2,726	–	–	3,770
32	–	3,596	–	–	7,250
–	–	2,030	–	–	2,320
8	–	3,248	–	–	2,552
–	–	2,726	10	–	2,668
–	–	2,262	–	–	3,944
–	–	3,074	–	–	6,090
–	–	2,320	–	–	3,132
–	–	3,364	–	–	2,900
–	–	2,610	–	–	3,190
–	–	2,146	–	–	1,740
–	–	2,900	–	9	3,074
–	–	3,074	–	–	3,944

Table 1 (continued)

ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)	ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)
-	-	3,045	-	141	3,190
19	4	3,408	14	0	2,320
7	0	2,146	35	0	4,235
22	0	5,162	6	2	2,436
0	3	2,436	7	14	2,900
7	9	4,060	6	3	2,370
10	4	3,190	6	8	2,552
6	0	2,062	25	3	3,306
19	3	4,060	0	3	2,552
7	0	3,538	16	3	5,510
14	0	3,190	2	12	1,276
22	6	3,944	2	2	2,030
10	4	3,480	10	35	3,422
4	0	2,436	6	4	2,784
-	-	4,640	7	11	3,074
2	3	1,740	54	-	2,900
28	8	3,480	22	5	2,610
22	5	4,640	28	3	4,350
44	-	1,740	32	-	2,900
54	2	2,900	18	9	3,770
38	0	2,030	12	6	2,610
32	3	1,740	18	5	2,320
22	6	3,190	38	-	2,320

Table 1 (continued)

ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)	ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)
28	36	4,930	22	78	6,090
34	29	6,670	26	14	5,510
54	-	3,190	18	-	6,670
18	-	4,350	22	17	3,190
16	9	-			

Table 2. Enzyme Levels in Guinea Pigs After Exposure to 300 mg Chloroform/kg (ALT = alanine aminotransferase, AR = arginase, SD = sorbitol dehydrogenase)

Time After Dosing (hr)	ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)
0	-	-	2,146
0	-	-	1,740
0	-	-	2,900
0	-	-	3,248
0	-	-	3,074
0	-	-	3,074
0	-	-	3,944
0	-	-	3,045
0	-	-	3,190
16	-	99	87,000
16	8	45	16,820
24	-	-	316,100
24	-	6	3,364
48	-	-	46,400
48	-	-	81,200
72	-	6	2,668
72	24	6	8,700
96	-	-	4,930
96	12	17	2,900

Table 3. Enzyme Levels in Guinea Pigs After Exposure to Chloroform Every 48 hr (ALT = alanine aminotransferase, AR = Arginase, SD = sorbitol dehydrogenase)

Dose (mg/kg)	Doses (no.)	ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)
10	1	-	24	4,756
10	1	-	-	2,552
10	2	24	12	3,306
10	2	23	45	4,350
10	3	18	22	2,320
10	3	12	68	6,380
30	1	-	-	2,900
30	1	-	-	2,436
30	2	18	22	2,552
30	2	0	0	4,060
30	3	-	0	2,900
30	3	1	8	1,914
50	1	-	-	3,190
50	1	-	-	2,900
50	2	10	2	3,770
50	2	14	-	2,320
50	3	8	5	2,668
50	3	0	2	3,480
250	1	26	-	2,030
250	1	42	-	5,800
250	2	60	-	226,200
250	2	32	2	2,320
250	3	28	2	4,176
300	1	42	2	7,540
300	1	16	2	1,452
300	2	10	2	17,400
300	2	16	0	26,100
300	3	12	3	4,060
300	3	6	0	4,060

Table 3 (continued)

Dose (mg/kg)	Doses (no.)	ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)
400	2	24	0	5,800
400	2	18	6	55,360
400	3	16	0	9,860
400	3	8	0	9,860
500	1	40	-	9,860
500	1	28	0	44,660
500	2	118	-	440,800
500	3	26	-	67,280
500	3	8	0	10,440
500	3	18	0	7,540
750	1	34	-	48,720
750	1	-	72	167,040
750	1	38	8	29,000
750	1	-	3	23,200
750	2	125	-	247,080

Table 4. Enzyme Levels in Guinea Pigs After Exposure to Chloroform Every 72 hr (ALT = alanine aminotransferase, AR = arginase, SD = sorbitol dehydrogenase)

Dose (mg/kg)	Doses (no.)	ALT (SFU/ml)	AR (IU/L)	SD (SU/ml)
300	2	-	9	3,490
300	2	-	100	2,940
300	2	10	2	5,220
300	3	-	-	2,780
300	3	4	45	7,540
300	3	6	3	2,900
400	2	34	0	10,440
400	2	6	3	7,540
400	3	0	0	5,800
400	3	42	2	17,400
75[a]	1	-	36	3,770
75[a]	1	-	-	1,740
75[a]	2	-	8	4,640
75[a]	2	-	9	2,610
150[a]	1	-	25	3,190
150[a]	1	-	6	3,490
150[a]	1	-	5	2,030
150[a]	2	-	0	3,480
150[a]	2	-	1	3,480
150[a]	2	-	-	3,480
150[a]	2	-	0	2,610
150[a]	2	-	10	2,940
150[a]	3	-	3	2,610
150[a]	3	-	-	3,770

[a] 300 mg/kg given initially.

Table 5. Hematologic Parameters in 4 Rabbits Force Fed Dried Kochia scoparia (Fireweed) (PCV = packed cell volume, WBC = leucocyte count, SD = sorbitol dehydrogenase, ALT = alanine aminotransferase, GOT = aspartate aminotransferase, BUN = blood urea nitrogen, SAP = serum alkaline phosphatase, TPN = total protein)

Day	PCV (%)	WBC (cells/mm^3)	SD (SU/ml)	ALT (SFU/ml)	GOT (SFU/ml)	BUN (mg%)	SAP (SU/ml)	TPN (g%)
Rabbit no. 1001 - fed water								
-10	-	5,170	812	75	68	29	-	5
-7	-	9,790	418	68	57	16	-	4
-3	26	13,530	812	96	71	27	3	6
0	32	11,330	696	86	36	11	3	6
4	40	6,710	754	102	40	15	3	6
7	38	2,530	-	82	28	18	3	6
11	33	8,250	464	105	75	3	2	6
14	36	8,580	696	101	60	5	3	7
Rabbit no. 1002 - fed guinea pig chow								
-10	42	5,940	696	83	69	21	-	6
-7	38	14,190	580	72	73	16	-	6
-3	33	14,850	986	80	87	19	5	6
0	41	15,290	870	80	67	11	4	5
4	36	7,040	754	108	106	11	3	6
7	40	3,410	-	96	83	32	3	7
11	40	10,780	928	96	60	16	2	6
14	-	3,080	522	64	48	16	2	6
Rabbit no. 1003 - fed fireweed								
-10	-	8,580	696	80	72	27	-	6
-7	-	15,730	-	100	124	23	-	6
-3	40	13,530	1,044	115	72	19	-	5
0	34	9,460	1,044	100	50	19	2	5
4	33	6,490	638	114	48	18	2	6
7	30	3,530	-	88	51	12	1	6
11	28	15,510	696	90	47	18	1	6
14	-	17,600	522	104	86	21	3	7
Rabbit no. 1004 - fed fireweed								
-10	36	9,130	116	88	82	17	-	6
-7	40	9,240	673	65	76	21	-	6
-3	36	9,350	696	77	82	19	5	5
0	38	7,040	1,044	80	67	10	-	6
4	39	6,600	928	94	66	14	4	6
7	42	5,830	-	65	35	0	4	6
11	30	8,360	928	84	55	7	5	6
14	-	8,030	464	36	42	12	4	6

Table 5 (continued)

Day	PCV (%)	WBC (cells/mm^3)	SD (SU/ml)	ALT (SFU/ml)	GOT (SFU/ml)	BUN (mg%)	SAP (SU/ml)	TPN (g%)
Rabbit no. 1005 - fed fireweed								
-10	38	9,900	290	99	87	19	-	5
-7	36	8,140	522	60	66	16	-	6
-3	36	7,590	986	89	86	19	5	6
0	36	6,710	580	86	60	11	5	6
4	35	4,950	1,160	96	62	8	5	6
7	39	3,740	-	113	57	8	4	6
11	38	7,040	754	113	60	5	4	6
14	38	7,700	-	93	60	9	5	6
Rabbit no. 1006 - fed fireweed								
-10	-	8,250	348	97	73	21	-	5
-7	38	3,410	423	77	76	28	-	6
-3	39	5,170	464	112	86	26	3	6
0	40	3,520	522	93	55	17	3	6
4	37	7,040	696	124	62	10	3	6
7	38	4,950	-	106	48	8	2	6
11	35	9,130	870	117	67	10	2	6
14	36	7,260	464	102	57	10	2	6

Table 6. Sorbitol Dehydrogenase (SD) and Blood Urea Nitrogen (BUN) Values in 2 Rabbits Fed Fresh, Green *Kochia scoparia* (Fireweed) Daily for 32 Days

Day	Rabbit no. 1011 SD (SU/ml)	Rabbit no. 1011 BUN (mg%)	Rabbit no. 1012 SD (SU/ml)	Rabbit no. 1012 BUN (mg%)
-3	-	14	290	14
0	580	14	638	12
4	232	23	406	17
7	406	17	348	20
11	638	25	464	25
14	638	30	348	30
18	754	25	464	30
21	928	20	704	30
25	986	25	812	45
27	1,044	25	812	35
29	1,450	25	986	35
32	1,392	20	1,218	30

Table 7. Hematologic Parameters in 2 Sheep Which Grazed Green Kochia scoparia (Fireweed) Pasture for 30 Days (SD = sorbitol dehydrogenase, BUN = blood urea nitrogen, PCV = packed cell volume, WBC = leucocyte count)

Day	Sheep no. 2001				Sheep no. 2002			
	SD (SU/ml)	BUN (mg%)	PCV (%)	WBC (cells/mm^3)	SD (SU/ml)	BUN (mg%)	PCV (%)	WBC (cells/mm^3)
-3	174	8	45	17,400	174	13		11,900
0	116	28	48	12,800	290	35		9,100
5	300	19	49	11,200	522	19		9,900
10	1,102	24	57	12,900	522	27		10,700
15	522	27	46	9,100	232	35		12,000
20	1,102	30	41	8,400	348	41		7,600
26	522	20	43	8,000	812	27		8,700
30	232	29	35	9,800	406	39		10,100

Table 8. Enzyme Levels in Guinea Pigs Fed Kochia scoparia (Fireweed), Alfalfa Hay, or Chow ad lib (ALT = alanine aminotransferase, SD = sorbitol dehydrogenase, AR = arginase)

Day	ALT (SFU/ml)	SD (SU/ml)	AR (IU/L)	Comments
Guinea Pig no. 3076		Fireweed + 0 mg Chloroform/kg ip		
1	46	2,030	-	
5	14	2,320	-	
9	38	3,610	5	
13	80	3,480	5	
15	-	-	-	Death due to cardiac puncture
Guinea Pig no. 3077		Fireweed + 0 mg Chloroform/kg ip		
1	32	2,610	-	
5	34	3,480	-	
9	-	3,770	-	
13	72	8,410	36	
16	38	15,950	306	Euthanitized
Guinea Pig no. 3078		Fireweed + 0 mg Chloroform/kg ip		
1	28	1,450	-	
5	22	3,190	-	
9	34	4,350	24	
13	54	8,120	-	
16	52	5,220	32	Euthanitized
Guinea Pig no. 3080		Fireweed + 0 mg Chloroform/kg ip		
1	125	3,190	-	
7	18	3,190	5	
11	-	-	-	Death due to chloroform intoxication
Guinea Pig no. 3081		Fireweed + 0 mg Chloroform/kg ip		
1	44	2,610	-	
7	40	2,900	5	
11	22	3,480	8	
15	44	2,900	12	
16	28	2,320	17	Euthanitized; oxalates found in kidneys
Guinea Pig no. 3082		Fireweed + 0 mg Chloroform/kg ip		
1	76	3,190	-	
7	40	9,280	45	

Table 8 (continued)

Day	ALT (SFU/ml)	SD (SU/ml)	AR (IU/L)	Comments
Guinea Pig no. 3083		Alfalfa Hay + 0 mg Chloroform/kg ip		
1	46	3,190	-	
5	12	2,900	-	
9	26	2,610	17	
13	58	2,320	6	
16	18	3,190	36	Euthanitized
Guinea Pig no. 3084		Alfalfa Hay + 0 mg Chloroform/kg ip		
1	40	2,030	-	
5	34	2,900	-	
9	14	2,350	9	
13	14	2,030	20	
16	16	2,320	6	Euthanitized
Guinea Pig no. 3085		Alfalfa Hay + 0 mg Chloroform/kg ip		
1	64	2,320	-	
5	32	3,480	-	
9	38	3,190	-	
13	60	4,930	6	
16	70	5,800	9	Euthanitized
Guinea Pig no. 3086		Alfalfa Hay + 0 mg Chloroform/kg ip		
1	46	2,610	-	
7	26	3,480	5	
11	32	3,770	12	
15	34	5,800	48	
16	34	4,640	14	Euthanitized
Guinea Pig no. 3087		Alfalfa Hay + 0 mg Chloroform/kg ip		
1	64	2,610	-	
7	34	3,190	3	
11	18	3,190	27	
15	58	3,190	15	
16	22	2,610	5	Euthanitized
Guinea Pig no. 3088		Alfalfa Hay + 0 mg Chloroform/kg ip		
1	58	2,610	-	
7	22	2,900	3	
11	10	3,770	17	
15	34	3,770	21	
16	22	4,350	14	Euthanitized

Table 8 (continued)

Day	ALT (SFU/ml)	SD (SU/ml)	AR (IU/L)	Comments
Guinea Pig no. 3089		Chow + 0 mg Chloroform/kg ip		
1	54	2,900	-	
5	28	3,480	-	
9	22	2,610	5	
13	22	4,640	5	
16	28	4,350	3	Euthanitized
Guinea Pig no. 3090		Chow + 0 mg Chloroform/kg ip		
1	44	1,740	-	
5	32	2,900	-	
9	-	2,610	-	
13	54	2,900	2	
16	18	3,770	9	Euthanitized
Guinea Pig no. 3091		Chow + 0 mg Chloroform/kg ip		
1	38	2,030	-	
5	12	2,610	-	
9	32	1,740	3	
13	18	2,320	5	
16	22	3,190	6	Euthanitized
Guinea Pig no. 3092		Chow + 0 mg Chloroform/kg ip		
1	38	2,320	-	
7	28	4,930	36	
11	22	6,090	78	
15	34	6,670	29	
16	26	5,510	14	Euthanitized
Guinea Pig no. 3095		Chow + 0 mg Chloroform/kg ip		
1	54	3,190	-	
7	18	6,670	-	
11	18	4,350	-	
15	22	3,190	17	
16	16	-	9	Euthanitized

Table 8 (continued)

Day	ALT (SFU/ml)	SD (SU/ml)	AR (IU/L)	Comments
Guinea Pig no. 3119		Fireweed + 300 mg Chloroform/kg ip		
1	52	68,150	234	
5	52	45,400	810	Death due to cardiac puncture
Guinea Pig no. 3120		Fireweed + 300 mg Chloroform/kg ip		
1	46	10,150	50	
4	-	-	-	Death due to CHC13 intoxication; oxalates found in kidneys
Guinea Pig no. 3121		Fireweed + 300 mg Chloroform/kg ip		
1	34	7,055	24	
5	-	-	-	Death due to chloroform intoxication
Guinea Pig no. 3122		Fireweed + 300 mg Chloroform/kg ip		
1	38	5,600	2	
5	-	-	-	Death due to CHC13 intoxication; oxalates found in kidneys
Guinea Pig no. 3123		Fireweed + 300 mg Chloroform/kg ip		
1	28	13,050	38	
5	-	-	-	Death due to chloroform intoxication
Guinea Pig no. 3124		Fireweed + 300 mg Chloroform/kg ip		
1	60	4,350	8	
5	-	-	-	Death due to chloroform intoxication; oxalates found in kidneys
Guinea Pig no. 3096		Fireweed + 400 mg Chloroform/kg ip		
1	64	5,800	-	
5	44	50,750	-	
9	-	-	-	Death due to chloroform intoxication

Table 8 (continued)

Day	ALT (SFU/ml)	SD (SU/ml)	AR (IU/L)	Comments
Guinea Pig no. 3098		Fireweed + 400 mg Chloroform/kg ip		
1	64	500,200	-	
3	-	-	-	Death due to cardiac puncture
Guinea Pig no. 3099		Fireweed + 400 mg Chloroform/kg ip		
1	125	72,500	-	
5	38	113,000	-	
6	-	-	-	Death due to chloroform intoxication
Guinea Pig no. 3100		Fireweed + 400 mg Chloroform/kg ip		
1	80	29,020	-	
7	-	40,600	0	Death due to chloroform intoxication; oxalates found in kidneys
Guinea Pig no. 3102		Fireweed + 400 mg Chloroform/kg ip		
1	50	15,950	-	
7	54	19,140	-	
9	-	-	-	Death due to chloroform intoxication; oxalates found in kidneys
Guinea Pig no. 3103		Fireweed + 400 mg Chloroform/kg ip		
1	76	108,800	-	
7	26	3,770	6	
11	-	-	-	Death due to chloroform intoxication
Guinea Pig no. 3105		Fireweed + 400 mg Chloroform/kg ip		
1	58	11,920	-	
7	26	3,480	-	
11	40	23,650	174	
12	-	-	-	Death due to chloroform intoxication

Table 8 (continued)

Day	ALT (SFU/ml)	SD (SU/ml)	AR (IU/L)	Comments
Guinea Pig no. 3126		Alfalfa Hay + 300 mg Chloroform/kg ip		
1	42	23,350	109	
5	64	43,500	339	
9	28	10,440		
13	42	7,250	90	
16	114	23,200	-	Euthanitized
Guinea Pig no. 3127		Alfalfa Hay + 300 mg Chloroform/kg ip		
1	52	5,700	9	
5	70	43,500	5	
9	34	4,060	0	
13	26	5,800	3	
16	52	17,400	180	Euthanitized
Guinea Pig no. 3128		Alfalfa Hay + 300 mg Chloroform/kg ip		
1	34	13,050	84	
7	26	21,700	9	
11	64	108,800		
15	38	9,280	5	
16	42	11,600		Euthanitized
Guinea Pig no. 3130		Alfalfa Hay + 300 mg Chloroform/kg ip		
1	60	137,800	216	
7	18	17,400	20	
11	46	6,380	2	
15	22	4,060	0	
16	42	5,800	-	Euthanitized
Guinea Pig no. 3131		Alfalfa Hay + 300 mg Chloroform/kg ip		
1	46	18,850	87	
7	34	275,500	910	
11	34	13,050	3	
15	18	6,670	5	
16	46	13,250	-	Euthanitized
Guinea Pig no. 3106		Alfalfa Hay + 400 mg Chloroform/kg ip		
1	96	27,550	-	
5	44	50,750	-	
9	44	7,250	2	
13	-	5,800	6	
16	38	27,550	20	Euthanitized

Table 8 (continued)

Day	ALT (SFU/ml)	SD (SU/ml)	AR (IU/L)	Comments
Guinea Pig no. 3107		Alfalfa Hay + 400 mg Chloroform/kg ip		
1	90	43,500	-	
5	60	79,750	-	
9	34	7,250	-	
13	26	4,350	2	
16	42	5,510	6	Euthanitized
Guinea Pig no. 3108		Alfalfa Hay + 400 mg Chloroform/kg ip		
1	90	34,800	-	
9	84	13,050	41	
13	40	4,350	2	
16	28	4,350	9	Euthanitized
Guinea Pig no. 3109		Alfalfa Hay + 400 mg Chloroform/kg ip		
1	72	17,400	-	
7	28	11,600	9	
11	20	5,800	9	
15	22	4,350	15	
16	22	4,350	14	Euthanitized
Guinea Pig no. 3110		Alfalfa Hay + 400 mg Chloroform/kg ip		
1	90	20,300	-	
7	28	7,200	17	
11	44	26,820	-	
15	28	14,500	6	
16	42	7,250	0	Euthanitized
Guinea Pig no. 3112		Chow + 400 mg Chloroform/kg ip		
1	68	8,700	-	
7	26	13,050	2	
11	28	18,850	186	
15	34	5,800	8	
16	28	4,350	12	Euthanitized
Guinea Pig no. 3113		Chow + 400 mg Chloroform/kg ip		
1	38	5,800	-	
5	18	2,900	-	
9	20	4,350	8	
13	18	2,900	0	Death due to cardiac puncture

Table 8 (continued)

Day	ALT (SFU/ml)	SD (SU/ml)	AR (IU/L)	Comments
Guinea Pig no. 3114		Chow + 400 mg Chloroform/kg ip		
1	54	1,450	-	
5	18	7,250	-	
9	26	7,250	3	
13	34	4,350	2	
16	28	4,250	5	Euthanitized
Guinea Pig no. 3115		Chow + 400 mg Chloroform/kg ip		
1	112	18,850	-	
5	22	1,450	-	
9	32	8,700	-	
13	28	5,700	5	
16	26	4,250	0	Euthanitized
Guinea Pig no. 3116		Chow + 400 mg Chloroform/kg ip		
1	100	324,500	-	
3	-	-	-	Death due to chloroform intoxication
Guinea Pig no. 3117		Chow + 400 mg Chloroform/kg ip		
1	125	121,200	-	
7	14	2,610	3	
11	14	2,900	-	
15	26	2,900	8	Euthanitized
Guinea Pig no. 3118		Chow + 400 mg Chloroform/kg ip		
1	125	108,800	-	
7	20	6,090	-	
11	28	18,820	-	
15	28	4,350	3	
16	42	4,350	6	Euthanitized

APPENDIX B

PHOTOSENSITIZATION: A LITERATURE REVIEW

One hundred fifty million kilometers from earth, thermonuclear reactions within the sun generate energy which is converted to heat on its surface. The sunlight earth receives is the radiant energy emitted by this heat (1). Sunlight is perceived as a complete range of wavelengths (from very long radiowaves to very short X-rays); the short wavelengths are the most beneficial and also the most dangerous to life on earth.

Fortunately, the ozone layer which surrounds the earth protects living systems from the harmful effects of this radiation. Ozone absorbs all wavelengths shorter than 290 nm, therefore approximately 99% of the solar energy reaching earth is in the visible and longer wavelengths. Less than 1% is in the ultraviolet spectrum of 290 to 400 nm (2). Exposure to these ultraviolet wavelengths can produce skin lesions.

"Photosensitization" refers to a change in the integrity of unpigmented skin in the presence of a "photodynamic agent" and after exposure to sunlight. The disease is produced by any single wavelength or combination of wavelengths in the 280 to 790 nm spectrum. "Sunburn" is the result of shorter wavelengths of energy (290 to 320 nm) penetrating the skin and causing changes within viable cells. Ordinary window glass effectively blocks this part of the spectrum.

Photosensitization has been studied since the turn of the century. In 1900, Raab showed that paramecia mixed with certain dyes rapidly died if the mixture was exposed to light. In 1905, Tappeiner described the effect that light had on a system to which a light-sensitizing substance (i.e., a photodynamic agent) had been introduced. He introduced the term "photodynamic action" to describe this effect (3).

During the same year, Busck hypothesized that certain plants contained photodynamic agents, since photosensitization seemed to follow ingestion of these plants. Proof of this hypothesis did not come until Ray, in 1914, isolated a fluorescent red pigment from Hypericum crispum, and Horsely, in 1934, produced photosensitization after po administration of a chlorophyll-free extract of the fluorescent red pigment from Hypericum perforatum (3).

Each photodynamic agent is activated by the energy of a specific wavelength or combination of wavelengths (4). Sensitivity to sunlight and subsequent skin changes result from the photodynamic agent within the integument. The unanswered question is: What is the photodynamic agent in each instance of photosensitization?

Various drugs, chemicals and plants have been incriminated as causing photosensitization. There are relatively few plants positively identified as the cause of photosensitization and fewer still in which the specific photodynamic agent has been identified (Table 1).

There is no general structure which describes the photodynamic agents, but all photosensitizers fluoresce when activated by the appropriate wavelength. The fluorescence phenomenon occurs when a photon of light collides with a photodynamic agent creating an unstable, energized molecule. With the return of the molecule to ground state, energy is emitted in the form of fluorescence.

Transfer of electrons may also occur by collision of the unstable, high energy molecules with other molecules in the cells. Collision with an energized molecule creates free-radical chain reactions. A free-radical is an atom or group of atoms which contain an odd or unpaired electron. This makes the free-radical reactive because there is a tendency to pair the odd electron. Collision with another molecule and subsequent transfer of electrons satisfies the original molecule but creates a new free-radical. This chain reaction continues until two free-radicals collide, bond together, and terminate the reaction. The amino acids, histidine, trytophane, and tyrosine are oxidized by these chain reactions, thereby weakening cell structure.

Two mechanisms are proposed to produce photosensitization; both create increased membrane permeability. These two mechanisms are dependent upon where the photodynamic agent binds to the cell. The dye, rose bengal, and plant furocoumarins attach to the cell surface and change the permeability of the cell membrane. This causes a loss of cellular potassium which

produces cytoplasmic extrusion (Fig. 1). Other chemicals, such as anthracene and porphyrin, concentrate within the lysosomes and destroy the integrity of the lysosomal membrane, causing increased permeability of lysosomal membranes and seepage of lytic enzymes into the cell (Fig. 2).

Photosensitization occurs when the photodynamic agent reaches the skin via the systemic circulation. This occurs as a result of the administration of a photodynamic agent or in the presence of liver or kidney dysfunction in which a normally present photodynamic agent is not excreted. Some plant-induced photosensitizations are due to a photodynamic pigment and a hepatotoxin. The precise mechanisms of many cases of photosensitization are not known.

Regardless of the cellular mechanism or the photodynamic agent, the clinical signs in the affected animal are the same. The animal becomes restless, shakes its head and ears, rubs or scratches affected areas, and seeks shelter from the sun. Early skin changes resemble the triple response of histamine release: First erythema appears, followed by swelling and serum leakage under the skin. In severe cases in sheep, the ears have a drooping appearance with the tips curled. Intense itching permits secondary bacterial intrusion. In a few days this leads to necrosis and sloughing of the affected area.

All areas exposed to light and lacking pigment can be affected. In sheep these areas are the eyes, eyelids, face and coronets. In cattle, the teats, udder, escutcheon,

muzzle and other non-pigmented areas are involved. In severe photosensitization cases the animal may become comatosed and will die within a few hours. Deaths are usually attributed to starvation, liver damage, or secondary effects, such as infection (5).

TYPE I, PRIMARY PHOTOSENSITIZATION

Primary photosensitization occurs from the introduction of a photodynamic agent directly into the systemic blood circulation. It is either a chemical not part of the normal diet which is absorbed from the digestive tract and incompletely excreted by the liver or a chemical introduced by parenteral administration, as when rose bengal is used as a liver function test. Whatever the route, the photodynamic agent reaches the systemic circulation unchanged.

Hypericism (St. John's wort poisoning) and fagopyrism (buckwheat poisoning) are well-known examples of type I photosensitization. The photodynamic agent involved in hypericism is hypericin, a derivative of naphthodianthrone (Fig. 3) (6). This red fluorescent pigment is found in all growth stages of Hypericum spp. Affected cattle, goats, sheep and horses display convulsive reactions when exposed to cold water and light (7).

The photodynamic agent in fagopyrism is also a derivative of naphthodianthrone-fagopyrin. It is the red fluorescent

pigment found in Fagopyrum sagittatum. Cattle, sheep, goats, horses and swine are affected (6).

Plants in the Umbelliferae (carrot) family are common photosensitizers for man and animals. Cymopterus watsonii (spring parsley) has caused photosensitization in sheep, cattle and chickens (8). Ammi majus has produced avian cases of type I photosensitization. The photodynamic agent in this and the Rutacae (rue) family is the furocoumarin (psoralen) group of chemicals. Five and 8-methoxypsoralen have been identified as the active photodynamic agents in A. majus (Fig. 4) (9). Other plants suspected of type I photosensitization are Erodium cicutarium, E. moschatum, and the legumes (Trifolium pratense, T. hybridum, Medicago denticulata, M. sativa, Brassica rapa, Vicia sp.) (3).

Phenothiazine and phenothiazine-derivative drugs are common causes of photosensitization in cattle, sheep, swine and pheasants. The photodynamic agent is the metabolite phenothiazine sulphoxide (10). Caution should be exercised when this drug is used.

TYPE II, PHOTOSENSITIZATION DUE TO ABERRANT PIGMENT SYNTHESIS

The photodynamic pigment in type II photosensitization results from defective porphyrin metabolism. Porphyrins are the metabolic by-product of the biosynthesis of heme. Delta-aminolevulinic acid (ALA) is formed by reaction of succinyl

coenzyme A and glycine. The condensation of two ALA molecules produces porphobilinogen; further synthesis, through intermediate steps, produces uroporphyrinogen III and coproporphyrinogen III which ultimately form protoporphyrin IX, the precursor to heme. The reaction to produce uroporphyrinogen III requires the presence of two enzymes, uroporphyrinogen I synthetase and uroporphyrinogen III cosynthetase. Without uroporphyrinogen III cosynthetase, the reaction generates uroporphyrinogen I and coproporphyrinogen I, which are not heme precursors (Fig. 5). In type II photosensitization excessive amounts of photodynamic uroporphyrins and coproporphyrins are generated from porphyrinogens in the bone marrow due to the uroporphyrinogen III cosynthetase deficiency (11).

This condition, commonly called "pink tooth" in cattle, is similar to the human congenital disease, erythropoietic porphyria. Other animals which develop a similar porphyria but without the photosensitization are swine, cats and the fox squirrel (Table 2). Affected animals display brown to red pigmentation of the teeth, bones and urine and accounts for the name of the disease in cattle.

TYPE III, HEPATOGENOUS PHOTOSENSITIZATION

Type III photosensitization is the result of primary hepatic dysfunction, and can be caused by a plant or synthetic toxin, a congenital condition, or an infectious agent. Regardless of the etiology, a derrangement of bile excretion occurs.

The photodynamic agent, phylloerythrin (Fig. 6), is normally formed in the gastrointestinal tract of herbivores by bacterial degradation of chlorophyll. The amount of chlorophyll in the diet and the degree of digestive bacterial fermentation are critical factors determining the amount of phylloerythrin produced and absorbed in the portal circulation. Phylloerythrin is normally excreted in the bile, and any liver dysfunction which alters bile excretion may permit phylloerythrin to accumulate in the systemic circulation.

The specific toxin responsible for the hepatic dysfunction is often unknown. In plant poisoning, many factors must be present to produce this type of photosensitization. The climate, specifically temperature and amount of precipitation, is an important precursor to photosensitization. Often the plants are toxic only when growing in a period of drought followed by rain and another period of hot, dry weather. Although a great number of plants have been associated with this type of photosensitization, most are unproven as responsible etiological agents.

Geeldikkop, also called yellow thickhead, is a type III photosensitization occurring in South Africa. The disease derives its name from the icterus that is present and the edema of the face and head. A characteristic lesion is the presence of cholesterol-like crystalloid material in the hepatocytes, Kupffer cells, and bile ducts (12).

Trihulus terrestris is an important plant which induces the disease. The wilted plant appears most toxic, the agent heing either a toxic plant constituent or a mycotoxin produced hy a fungus growing on the plant (12).

Facial eczema affects animals in New Zealand and South Africa. Clinical cases occur in animals grazing rye grass and white clover pastures. Liver pathology consists of characteristic interlohular cirrhosis and bile-duct proliferation (13). The saprophytic fungus, Pithomyces chartarum, is the responsihle agent and produces the mycotoxin sporidesmin (14).

Other mycotoxins have heen suggested as causes of photosensitization. Periconia minutissima has been incriminated in cases induced hy the ingestion of frosted common hermuda grass (15). Penicillium viridicatum produced experimental photosensitization in mice fed rice cultures (16).

Hepatotoxins have heen identified in some type III photosensitizing plants. Two such plants produce signs similar to Geeldikkop: Lantana camara contains lantadene A and B (17), and Lippia rehmanni contains icterogenin (18). Thlapsi arvense, penny-cress mustard, contains allyl isothiocyanate (oil of mustard) (19).

Photosensitization may he due to more than one constituent of the plant. Alecrim (Holocalyx glaziovii) contains hydrocyanic acid and an unknown hepatotoxin. Photosensitization occurs when cattle consume green shoots of the plant (20).

Nolina texana (sacahuiste), Panicum spp. and Tetradymia spp. produce hepatogenous photosensitization, but no toxins have been isolated (21, 3). Bighead is a photosensitization that occurs following the ingestion of Tetradymia sp. Animals must also graze Artemisia sp. before bighead will occur (22). A compilation of plants which produce photosensitization is printed in Table 1.

Congenital photosensitization in Southdown sheep and the Dubin-Johnson syndrome in Corriedale sheep are type III photosensitizations (23, 24). Affected sheep have an inherited derrangement of the phylloerythrin-excreting mechanism in the liver. The disease in Corriedale sheep is an excellent model to study the human disease.

Hepatogenous photosensitization can be due to viruses, such as Rift Valley Fever in cattle. Affected animals display photosensitization secondary to the infectious hepatitis (3).

Liver lesions may be a secondary effect of photosensitization, or more than one type of photosensitization may exist in the same animal. Waterbloom, or blue-green algae (Microcystis flos-aquae), causes photosensitization and hepatic dysfunction. The algae contains an alkaloid and a photodynamic pigment, phycocyanin (25). It is possible that both agents are responsible for the photosensitization.

Although the lesions of all types of photosensitization are identical, the etiologies are diversified. Differentiation

of the specific types is dependent on identifying the photodynamic agent and the degree of hepatic involvement. A complete history of the circumstances is imperative for the determination of the type of photosensitization involved.

Animals may have to be predisposed to the insult of the photodynamic agent, as in bighead disease. All factors (weather, diet, health and organ status) must be considered before conclusions can be reached of the etiology of the photosensitization.

Table 1. Plants Known to Produce Photosensitization (compiled from available sources)

Plant	Type of Photosensitization Produced	Toxic Principle	Reference
Agave lechuguilla Torr.	I or III	unknown	26
Ammi majus	I	psoralens	27
Ammi visnaga	I	psoralens	27
Asaemia axillaris	III	unknown	28
Avena sativa	III	unknown	3
Brachiaria brizantha	III	unknown	3
Brassica rapa	I*	unknown	3
Chloris truncata	III	unknown	3
Cucumis trigonis	III	unknown	3
Cymopterus watsonii	I	psoralens	8
Echinochola crus-galli var. frumentacea	III	unknown	3
Enterolobium gummiferum	III	unknown	29
Erodium cicuturium	uncertain	unknown	3
Euphorbia maculata	uncertain	unknown	37
Fagopyrum spp.	I	fagopyrin	37
Heliotropium	III	unknown	30
Holocalyx glaziovii	III	HCN + unknown hepatotoxin	20
Hydrocotyle sp.	uncertain	unknown	3
Hypericum sp.	I	hypericin	37
Kochia scoparia	III	unknown	31
Lantana camara	III	lantadene	17
Lasiospermum bipinnatum	III	unknown	28

Table 1 (continued)

Plant	Type of Photosensitization Produced	Toxic Principle	Reference
Lippia sp.	III	icterogenin	3
Medicago denticulata	I[a]	unknown	3
M. sativa	I[a]	unknown	3
Mentha satureiodes	III	unknown	3
Microcystis sp.	I and III	Phycocyanin + an alkaloid	3
Mimosa pudica	uncertain	unknown	3
Muehlenbeckia cunninghamii	III	unknown	3
Myoporum laetum	III	unknown	3
Nolina texana	III	unknown	21
Panicum sp.	III	unknown	3
Penicillium viridicatum	III	unknown	16
Phomopsis leptostromiformis	III	unknown	32
Pithomyces chartarum	III	sporidesmin	13
Setaria italica	uncertain	unknown	3
Sorghum vulgare	uncertain	unknown	3
Stackhousia monogyna	uncertain	unknown	3
Stryphnodendrum obovatum	uncertain	unknown	33
Swainsonia affinis	uncertain	unknown	3
Terminalia oblongata	uncertain	unknown	3
Tetradymia sp.	III	unknown	22
Thlapsi arvense	III	allyl isothiocyanata	34
Trema aspera	uncertain	unknown	3

Table 1 (continued)

Plant	Type of Photosensitization Produced	Toxic Principle	Reference
Tribulus terrestris	III	unknown	3
Trifolium pratense	I[a]	unknown	3
T. hybridum	I[a]	unknown	3
Vicia sp.	I[a]	unknown	3
Zinnia pauciflora	uncertain	unknown	3

[a] The absence of liver lesions may indicate type I photosensitization.

Table 2. Comparative Aspects of Erythropoietic Porphyrias (26)

Species	Probable Autosomal Heredity	Photosensitivity	Anemia	Major Red-cell Porphyrins
Human	recessive	present	usually	uroporphyrin
Bovine	recessive	present	usually	protoporphyrin uroporphyrin
Fox squirrel	unknown	absent	absent	uroporphyrin
Feline	dominant	absent	absent	protoporphyrin coproporphyrin
Porcine[a]	dominant	absent	unknown	unknown

[a] Porcine porphyria has not been shown to be erythropoietic in origin.

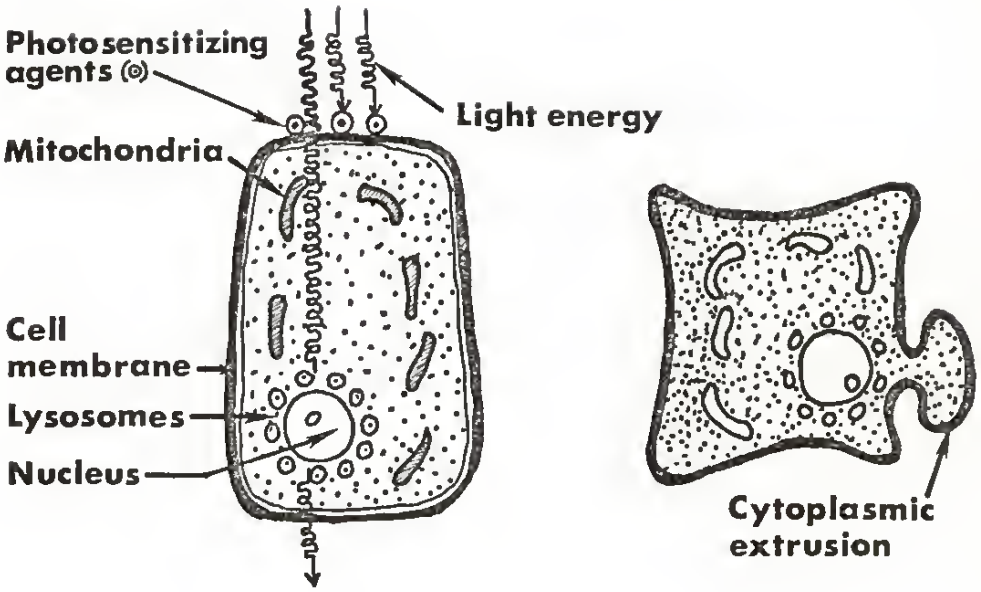

Figure 1. Binding of photodynamic agents to the cell membrane as a theoretical cellular mechanism of action (27).

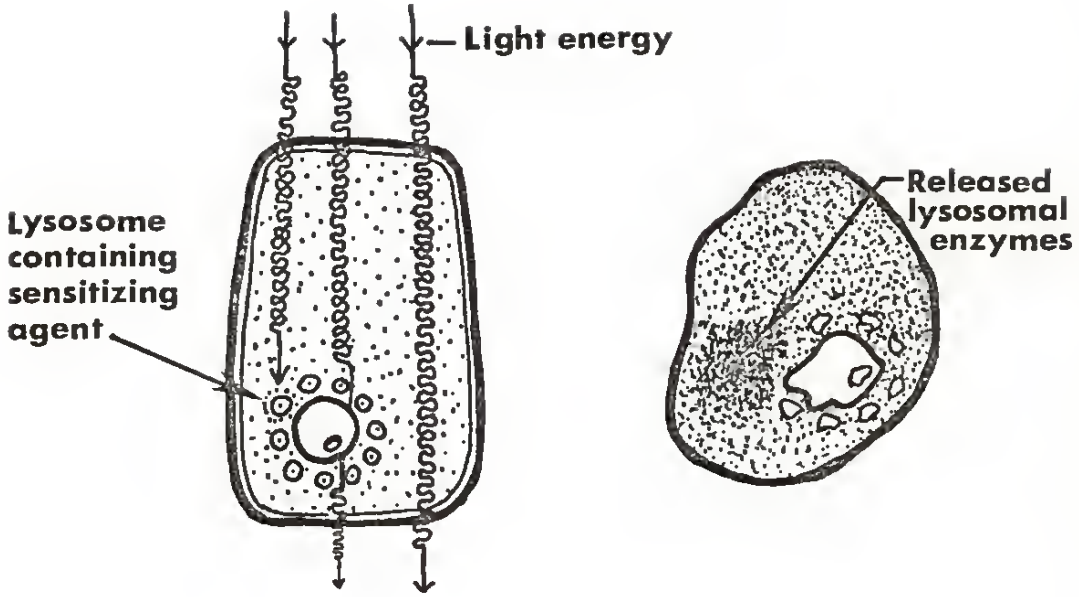

Figure 2. Concentration of photodynamic agents within lysosomes as a theoretical cellular mechanism of action (27).

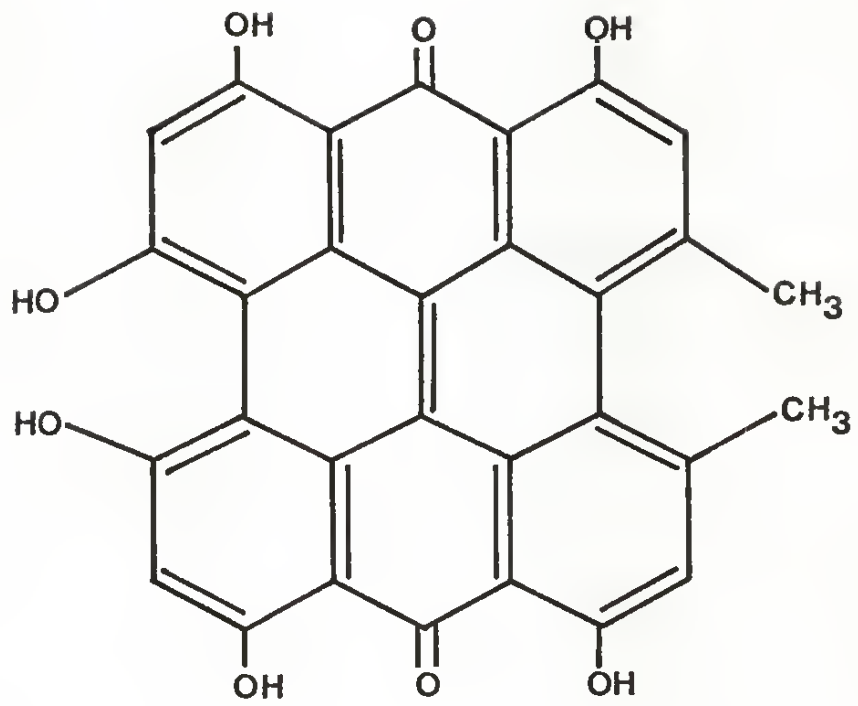

Figure 3. Chemical structure of hypericin, the photodynamic agent found in Hypericum spp. (37).

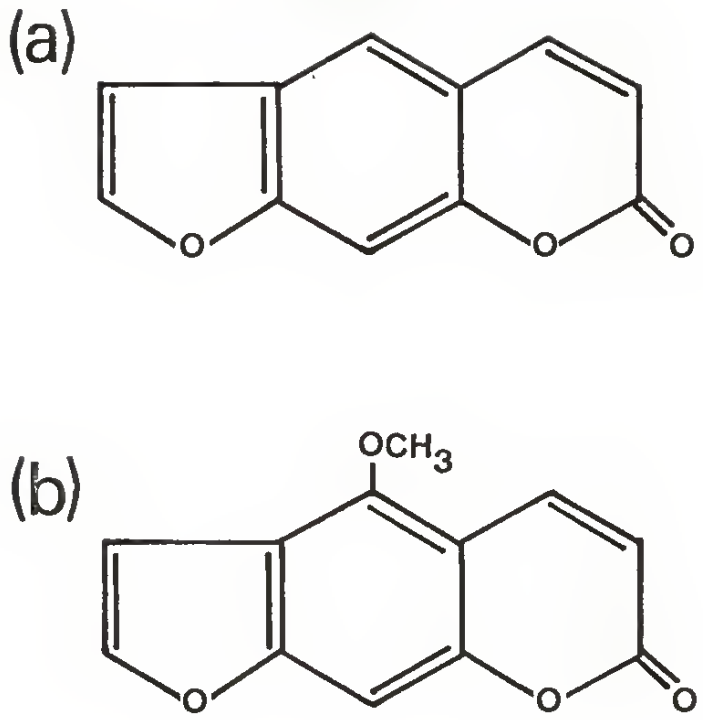

Figure 4. Furocoumarins, the photodynamic agents in Ammi majus: (a) chemical structure of psoralen; (b) chemical structure of 5-methoxypsoralen (bergapten) (28).

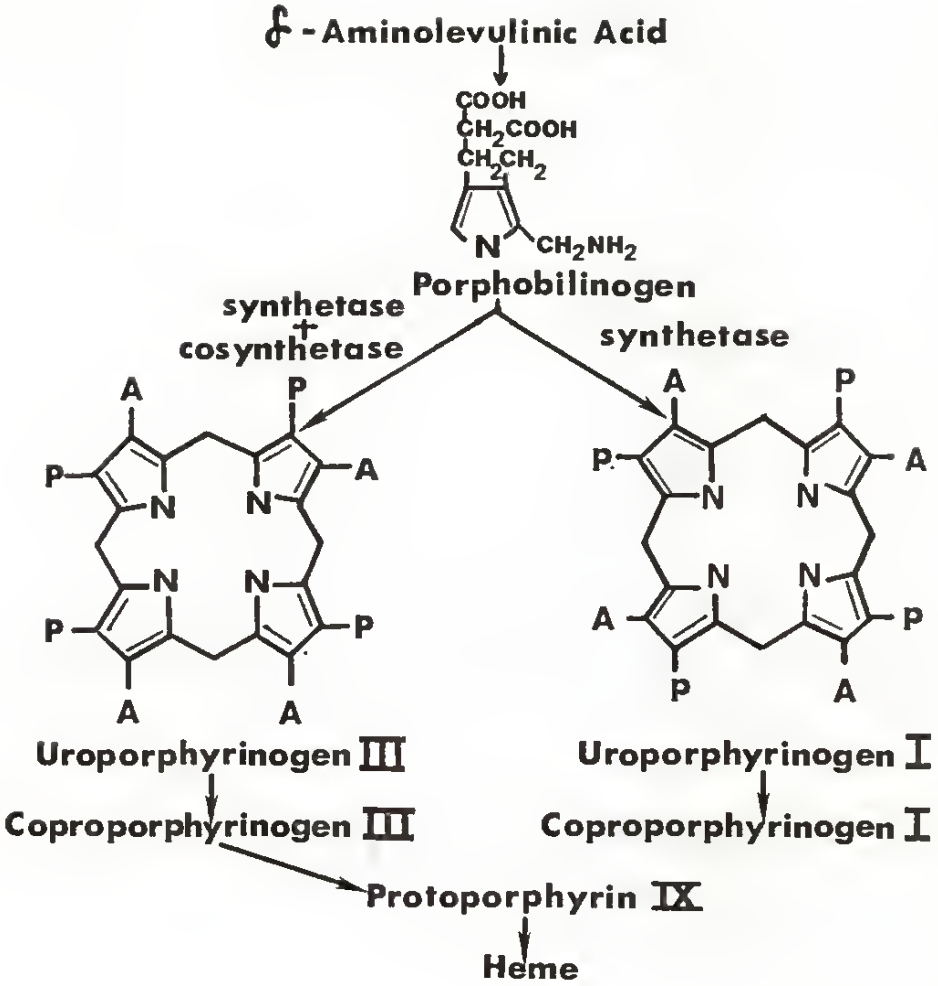

Figure 5. Metabolic pathway of the biosynthesis of heme (26).

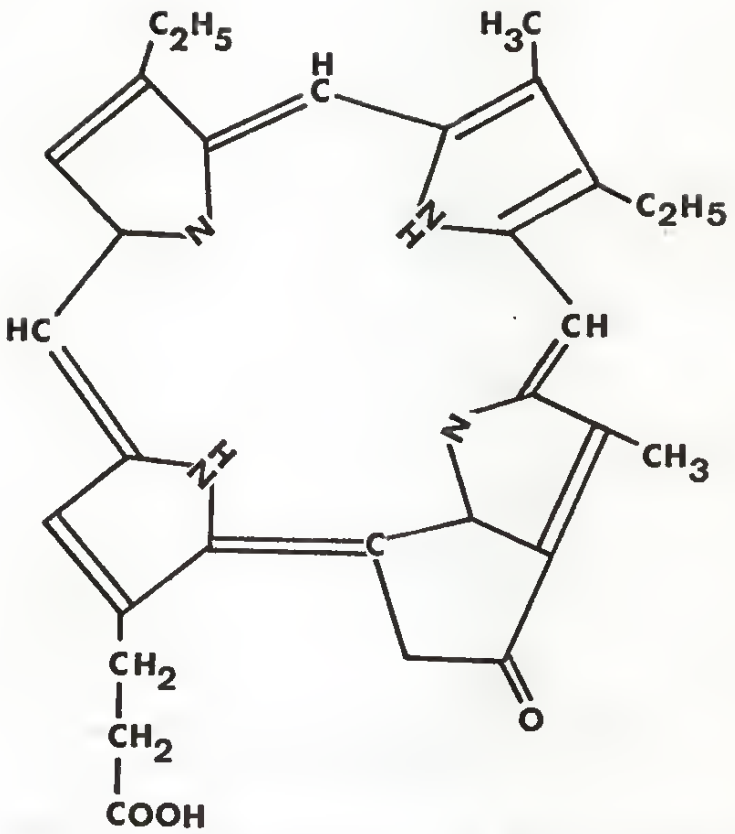

Figure 6. Chemical structure of phylloerythrin, the photodynamic agent producing type III photosensitization (37).

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APPENDIX C

KOCHIA SCOPARIA (L.) SCHRAD: A LITERATURE REVIEW

Kochia scoparia (L.) Schrad has the common names fireweed, kochia, fireball, belvedere, burning bush, summer cypress, and Mexican fireweed. It is commonly called morenita and alfalfa de los pobres in South America.

The plant is a hardy weed belonging to the family Chenopodiaceae (the Goosefoot family). Others in this family are beets, spinach, wormseed, lamb's-quarter, and Russian thistle (Table 1). Fireweed is an annual herb with simple, entire, lanceolate leaves. The size of the leaves of the mature plant varies from 0.5 to 3 inches (1.8 to 7.6 cm) in length. The green flowers are sessile in dense axillary clusters; the fruits are achene-like with a calyx of five horizontal wings (Fig. 1). In the fall many of the green plants turn purple-red and remain that color until they die soon after the first frost.

Fireweed is extremely drought resistant and is found in cropland, rangeland, and dry pastures. It was introduced to South America from central Europe about 1921. Molfino (1) traced the plant's origin in South America to Bahia Blanca, Argentina. Today Kochia scoparia is found throughout South America and the United States and in parts of Canada. The vast spread of the weed is attributed to the hardiness of the plant and to its small seeds which are easily disseminated by the wind.

Fireweed is used as forage by many ranchers. It can be grazed in its immature stage prior to hlooming or cut and ensiled before going to seed. Many ranchers cut, dry and hale the hay to use as winter feed. Paulsen (2), Bell et al (3), and the South Dakota Experiment Station (4) showed that fireweed has a high forage value because of its high digestible crude protein and ash content. It is inexpensive and easy to grow, and in nutritional quality is close to alfalfa. Apparently the plant has no toxic properties if immature, ensiled or dried (5). However, after twenty years of animal poisonings and human allergic reactions Argentina declared fireweed "a plague" and proposed to eliminate the weed (1).

Oyuela and Gualdoni (6), in Argentina, determined that toxicity develops at and subsequent to the blooming stage. The toxic properties are particularly pronounced during prolonged summer droughts. Poisonings commonly occur following a heavy rain after a lengthy drought.

The toxic principle has not been determined, hut saponins, oxalates, alkaloids, and nitrates have been suggested. Greshoff (7) was the first to report that fireweed contains saponins. Paulsen (2) proposed that toxicity was due to the saponin content, which seemed high during the last vegetative stages of the plant (the flower or fruit maturation stage). He further determined that the saponin level was greatest in the leaves and the seed covers, lesser in the stem, and least in the seeds. A triterpenic acid, sapogenin, was detected in the

fruits, and the saponin content of the fruits was 0.3% (8). Some samples of fruit contained 10% oil, while others had a waxy white product. These same authors did not detect any alkaloids or cyanogenetic glycosides in fireweed. However, alkaloids were detected by Borkowski et al (9). They were able to separate crude extracts of Kochia scoparia into a chloroform fraction containing three alkaloid compounds and a methanol fraction with one alkaloid. Bradley et al (10) reported that 5 specimens of fireweed contained 0.4-4.0% nitrate as potassium nitrate. Camp (11) recorded oxalic acid levels as high as 10.24% (dry weight basis) in fireweed.

Fireweed poisoning was first reported in South America in 1938 by Calvo in the Province of Cordoba, Argentina. The disease, Fiebre Negra (black fever), produced photosensitization in cattle. Because of the long drought that year, field conditions were poor and the only forage available was fireweed. Disseminated jaundice was found in every case, with itching and ophthalmic and auricular lesions. Veterinarians in other areas of Argentina reported similar cases with identical lesions. Desquamation of the tongue and petechiae on the gastrointestinal mucosa were also observed, together with an increase in liver size and a change in liver consistency.

During the long drought of 1942-1943 in Argentina, many cases of photosensitization in sheep, cattle, and horses were seen; many of these were attributed to Kochia scoparia (5). The cattle also had progressive signs of ataxia, incoordination, muscular spasms leading to recumbency, and death (12).

In a preliminary study, rabbits were given aqueous solutions of dried fireweed by stomach tube (Nwude, 1974, unpublished data, Kansas State University). Doses from 1 g/kg to 4 g/kg were administered daily for 15 days with no ill effects. No abnormal lesions were found on necropsy.

A survey of 5 Kansas veterinarians observing suspected fireweed poisoning cases indicated that affected animals were seen yearly (see following Case Histories). The described clinical signs included photosensitization, ulcerations of the mouth and digestive tract, enlarged fatty livers, and brain edema. Death often occurred. All the cases appeared during late summer, usually after the first rain following a drought. Occasional individuals reported fireweed hay poisoning. Feeding trials have not been performed to examine Kochia scoparia effects in Kansas.

CASE HISTORIES

Dr. Lammai, Dodge City, Kansas.

Dr. Lammai reported 5-20 cases each year, with an occasional death. The cases occurred from July to September during the drought season. Affected animals grazed the fairly lush fireweed since most edible plants had withered. Affected cattle were usually young steers weighing 300-650 pounds (136-295 kg). They appeared as "poor doers" with the animals stumbling and exhibiting general ataxia, which the veterinarians attributed to sore feet. They all had excessive lacrimation,

and the Herefords in particular had peeling of the mucosa of the nose and crustiness around the eyes. The primary lesions were a severe necrotic and ulcerative appearance of the oral mucosal surfaces, e.g. the palate, dental pad, gingiva, and tongue, and a mild to severe diarrhea. Some Herefords developed photosensitivity and others had icterus. Their behavior changed and they became intractable. Almost all the affected cattle recovered (90-100%), but if a post mortem examination was performed, irritation of the digestive tract and an enlarged fatty liver were usually seen.

Dr. Weiss, Garden City, Kansas

Dr. Weiss reported a few cases each year, typically in late summer. The cattle were usually in a stubble wheat pasture and grazed weeds, primarily fireweed. The affected cattle weighed about 400 pounds (182 kg). They wandered away from the herd and walked in circles or along the fences, exhibiting a central nervous system syndrome. Although the cattle appeared blind, both eyes looked normal. About a 25% mortality occurred; at necropsy only edema of the brain was noted.

Dr. Coddington, Larned, Kansas.

Dr. Coddington reported fireweed poisoning during August and September, when the plant was mature and flowering. Any breed of light colored cattle was affected after grazing in a wheat stubble field. Clinical signs were typical photosensitization--sloughing of the skin covering the nose, vulva,

teats, and other light colored areas. A 20% mortality was reported.

Dr. Rueter, Lakin, Kansas.

Dr. Rueter observed poisonings from July to September, and they were usually preceded by a rain one or two weeks previous. At that time plants were mature, 2 to 4 feet (61-122 cm) high, dark green, and going to seed. There was a higher incidence of poisoning when the plants were growing on dry alkaline soil with high levels of sulfates. Affected cattle developed labored breathing and some became hyper-excitable. This progressed to sternal recumbency and death. Although the prognosis was poor, those cattle that recovered displayed photosensitization. Impacted rumens were found on necropsy, with livers cirrhotic, fatty, swollen and engorged with blood. An inflamed abomasum was seen in some instances.

Dr. Fairbairn, Garden City, Kansas.

Dr. Fairbairn reported fireweed poisoning during the summer, both from pasture and from stored hay containing fireweed. Cases developed during the dry season, and sometimes after a late rain when the plant was mature and lush and growing on alkaline soil. Young cattle of any breed were affected. Although the eyes appeared normal, the animals acted blind; they were cautious when walking and progressed in later stages to head pressing. Mycotic stomatitis and photosensitivity were seen. Only edema of the brain was seen on necropsy.

Dr. Hurlburt, Riley, Kansas.

Dr. Hurlburt reported one case of fireweed poisoning which occurred in the fall. A flock of six-week-old chickens were fed broiler mash top dressed with green forage. The birds normally received lamb's-quarter seeds as top dressing for their feed, but this time they accidentally were given fireweed seeds stripped from lush, full blooming plants. Within 24 hours four of the chicks became lethargic and anorectic. All affected birds recovered except one, which was necropsied. Post mortem examination was remarkable except that the bird's crop was packed with mucous-covered fireweed and lamb's quarter seeds.

Table 1. Some Poisonous Plants of the Family Chenopodiacae.

Poisonous Plant	Toxic Principle
Atriplex spp. (saltbush)	selenium
Beta vulgaris (beet)	nitrate, oxalates
Chenopodium ambrosioides (wormseed)	oil of chenopodium (ascaridol)
Chenopodium album (lamb's quarter)	nitrate
Eurotia lanata	selenium
Halogeton glomeratus (halogeton)	oxalates
Kochia scoparia (fireweed)	saponin
Salsola pestifer (Russian thistle)	mechanical injury, nitrate
Sarcobatus vermiculatus (greasewood)	oxalates
Suckleya suckleyana (poison suckleya)	hydrocyanic acid

Figure 1. Kochia scoparia (L.) Schrad, the adult plant (13)

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INVESTIGATIONS ON THE TOXICITY OF *KOCHIA SCOPARIA* (L.) SCHRAD (FIREWEED)

by

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The annual weed Kochia scoparia (L.) Schrad (fireweed) is considered responsible for poisoning grazing animals in North and South America. The clinical signs produced are photosensitization accompanied by jaundice. Cases reported in Kansas included gastrointestinal irritation and blindness, in addition to photosensitization. Fireweed is not always toxic, as evidenced by its use as forage in the western states. This variability in toxicity could be due to disease-stressed animals responding adversely to fireweed, especially since liver dysfunction is apparent in many clinical instances. Studies were performed to determine if hepatic-stressed animals were adversely affected by fireweed consumption.

To develop an animal model to study hepatogenous photosensitization caused by fireweed, male guinea pigs were given 10-750 mg chloroform/kg body weight ip in 1, 2 or 3 successive doses at 48 or 72 hr intervals, and serum sorbitol dehydrogenase (EC 1.1.1.14; SD) and alanine aminotransferase (EC 2.6.1.2; ALT) levels were studied as indicators of liver damage. Increases of SD (3-139 times normal) and ALT (3 times normal) occurred at doses of 250 mg chloroform/kg and greater. Enzyme levels were greater and mortality was higher with dosing every 48 hr. SD levels peaked 24 hr after dosing and returned to normal by 96 hr. Two successive doses of chloroform every 48 hr resulted in greater SD increases than 1 dose or 3 successive doses every 48 hr. ALT increases were not as dramatic as SD increases. Dosing guinea pigs with

250-400 mg chloroform/kg every 72 hr produced hepatotoxicity, as evidenced by increased SD levels without overt toxicity and mortality.

Using the animal model, the toxicity of fireweed was investigated. Dunkin-Hartley albino guinea pigs were fed fireweed, alfalfa hay or guinea pig chow and exposed to ultraviolet light daily for 15 days. Guinea pigs were also dosed with chloroform (300 or 400 mg/kg ip) to create the liver dysfunction. SD and ALT were measured to evaluate liver damage. Kidneys were examined for oxalate crystals. Guinea pigs receiving chow and chloroform had elevated SD levels which returned to normal after 72 hr. Fireweed-fed animals also receiving chloroform had SD levels that did not return to the control range. There was no significant effect on ALT serum levels. All guinea pigs fed fireweed and dosed with chloroform died within 11 days. Oxalate crystals were found in kidneys of 1 guinea pig fed fireweed and 5 guinea pigs fed fireweed and receiving chloroform. No signs of photosensitiziation developed in any animals.

The results suggest a potentiating effect of fireweed in animals with liver damage. Field toxicity to fireweed may thus be dependent on pre-existing liver dysfunction. This is supported by observations of field cases in Kansas, in which only a few animals in any herd were affected and all cases occurred during periods of stress with greatly reduced available feed or water.