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DIVISION OF VETERINARY SCIENCE

In Cooperation with the Bureau of Animal Industry,
United States Department of Agriculture

Lechuguilla (*Agave Lecheguilla*) Poisoning in Sheep, Goats, and Laboratory Animals

AGRICULTURAL AND MECHANICAL COLLEGE OF TEXAS

T. O. WALTON, President

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A disease of sheep and goats known as "lechuguillaed," "goat fever," or "swellhead," occurs in regions wherever the lechuguilla plant is found. It is characterized by jaundice, liver and kidney lesions, and at times edematous swellings of the face and ears. It was produced experimentally by feeding the leaves of *Agave lecheguilla*. It occurs as a result of grazing the leaves of the plant during periods of unfavorable range conditions, and frequently causes serious losses.

Alcoholic extracts of the plant were found to contain two poisonous principles, one being a photodynamic agent which is activated by light of greater wave length than 5328 A. U., and so is toxic only when the animal is exposed to direct sunlight after feeding on the plant. The immediate result of the action of this agent is the production of edematous swellings of the face and ears. The second toxic principle has a destructive action on the liver and kidneys, is not activated by light, and has many of the properties of a saponin. Thus the disease, as it occurs in the field, seems to be the result of the combined action of these two toxic principles.

The disease is found only rarely in cattle and has not been found in horses. White rats were found to be suitable animals for the study of the action of both toxic principles. The disease was produced in rats by feeding extracts of the plant.

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LECHUGUILLA *(AGAVE LECHEGUILLA)* POISONING IN SHEEP, GOATS, AND LABORATORY ANIMALS

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For many years the well informed ranchers throughout the Southwest have recognized the toxicity of lechuguilla for sheep and goats. The disease may be referred to as "swellhead" but is most frequently designated as "goat fever," "lechuguilla fever," or "lechuguillaed." The analogous disease in cattle is rarely recognized, and frequently cattlemen deny losses from this source. The losses among cattle are unquestionably much lighter than among sheep and goats, but the fact remains that lechuguilla poisoning has been observed in cattle under range conditions.

Although the disease occurs most frequently during the spring months, it is one of the results of drouth and unfavorable range conditions and may, and frequently does, occur during any or all seasons of the year. Sporadic outbreaks are of common occurrence and become enzootic as range conditions become more unfavorable. The morbidity varies from five to thirty per cent with mortalities approaching the same percentages, since under ordinary range conditions the affected animals are left to shift for themselves and, as a result, recoveries are the exception rather than the rule. On ranches equipped with pastures in which the plant does not occur, outbreaks are readily checked by removal from infested to non-infested pastures, but it is not uncommon to find areas in west Texas in which hundreds of thousands of sheep and goats are grazed and in which few, if any, pastures are free of the plant. In such areas transfer from one pasture to another is of questionable value, and since eradication by grubbing the plant is impracticable, the only available recourse for the rancher is to move to another locality or to hope for improved climatic conditions and the appearance of a more palatable forage.

The disease is characterized by icterus, liver and kidney lesions, and at times the appearance of edematous swellings of the face and ears similar to the photodynamic diseases which are produced by grazing buckwheat, *Hypericum*, puncture plant *(Tribulus terrestris)*, etc.

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BOTANICAL DESCRIPTION OF AGAVE LECHEGUILLA*

Lechuguilla is known botanically as *Agave lecheguilla* Torr. In many localities of southwestern Texas it is a dominant feature of the vegetation. Lechuguilla is a conspicuous long-lived perennial from a thick fibrous-toothed crown bearing a cluster of thick, fleshy basal leaves and a tall flower stalk. Each plant requires a number of years, possibly ten or more, to reach maturity, when it produces a flower stalk, blooms, and fruits, and then dies. At this time of maturity one or more young plants have started growth from a rootstock of the parent plant and replace it to carry on the cycle of growth. As seen ordinarily the plant above ground consists of a crown bearing 10-30 thick, fleshy leaves, which are rigid, ascending, green or bluish, more or less concavo-convex in cross section, 2-4 cm. wide, and 40-60 cm. long. The leaf has a detachable horny border, scarcely 1 mm. wide, bearing gently recurved triangular teeth 20-40 mm. apart, and 3-7 mm. long, and terminate in a stout, subterete, dark-colored spine. When developed the flower stalk is woody, 2 meters or more in height and 2-3 cm. in diameter towards the base. The flowers are borne in a terminal panicle, which has very short branches, the inflorescence thus appearing spike-like. The flowers are relatively small, the perianth being tubular-funnelform and parted into 6 narrow, nearly equal, divisions. The six stamens are prominently exserted, and bear relatively long, narrow anthers, which are attached at or near their middle to the filament. The fruit is an oblong, leathery, three-celled capsule, which contains numerous flat, black seeds.

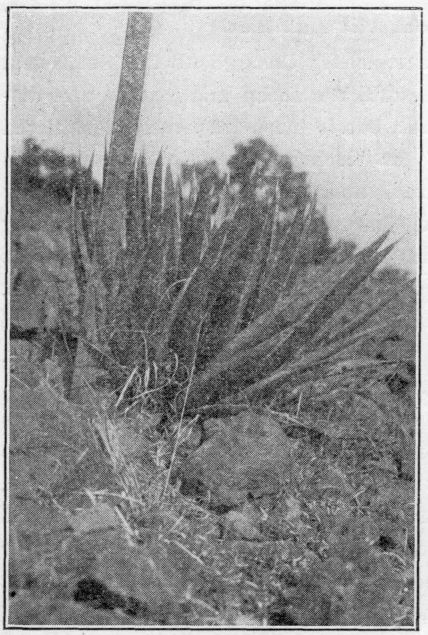

FIG. 1. *Agave lecheguilla* plant.

Lechuguilla occurs in Texas from the southern and western portions of the Edwards Plateau on west to the Franklin Mountains at El Paso, and in general from southeastern New Mexico on south into Mexico through Chihuahua to Tamaulipas and Zacatecas. It is found especially on low limestone hills or in dry elevated valleys, and is a characteristic feature of the vegetation bordering deep canyons lying west of the Pecos River

*Description by V. L. Cory, Range Botanist, Texas Agricultural Experiment Station.

gorge. It grows in dry, well-drained situations, where moisture conditions are not favorable for the growth of most other plants naturally occurring in these regions.

REVIEW OF LITERATURE*

Fagopyrism. *(Buckwheat poisoning.)* According to Merian (24), who reviewed the literature contributed prior to 1915, the first published report of buckwheat poisoning in farm animals was that by Hertwig in 1833. Hertwig observed that the occurrence of the disease in a herd of swine was associated with the eating of buckwheat and exposure to direct sunlight. Merian reviewed 23 reports of this or similar diseases in cattle, horses, sheep, goats, and swine. In some of the early reports photosensitization was evidently due to the eating of some other plant since there was no history of contact with buckwheat, but in the remainder of the reports the etiology was established by a history of grazing buckwheat pastures, or in the case of stabled animals, the feeding of the plant followed by exposure to bright sunlight. In the earliest reports two forms of the disease were observed. The first was an acute condition manifested by sudden attacks of convulsions and cerebral excitement with squealing, bellowing, etc. In many cases the appearance of the first symptoms was soon followed by paralysis and death. The second or chronic form consisted of pruritis, erythema, edematous swellings of the face and ears with necrosis and sloughing of the skin over the edematous areas. Licking and rubbing in addition to the sloughing resulted in large denuded areas. The disease was observed to be confined to white animals or to the white portions of spotted animals. Protection from direct exposure to sunlight was generally followed by uneventful recovery.

Practically all of our knowledge of the pathology of this disease was included in these early reports, little having been added in recent years. According to Merian the first experimental proof of the relationship of light to buckwheat poisoning was reported by Medding in 1887, who fed a cow on buckwheat and exposed her to bright sunlight after painting one side with coal tar. The characteristic reaction developed on the unpainted side, but the painted side was not affected.

Oehmke (25) fed the fruit of buckwheat *(Fagopyrum esculentum*, the species used by all investigators) to mice, guinea pigs, and rabbits, and upon exposure to direct sunlight observed manifestations similar to fagopyrism as found in farm animals. Alcoholic extracts of buckwheat were found to produce photosensitization, while the residual buckwheat was no longer capable of sensitizing animals to light. Fisher (10) found that the feeding of buckwheat to the same species of experimental animals rendered them sensitive to sunlight, the first evidence of sensitization appearing within 7 days. He also observed an enteritis in addition to the usual lesions

*Also presented in the Archives Pathology 23:399: 1937 as part of the review of photodynamic diseases of man and the lower animals.

of photosensitization. Merian produced photosensitization in rabbits and guinea pigs by feeding the foliage of buckwheat, and obtained like results by feeding *F. tartaricum* and *F. griseum*, with the sensitization occurring as early as the fourth day. Similar results were obtained by Lutz (20) and Lutz and Schmidt (21) by feeding the fruit of buckwheat to mice and the fruit and foliage of the plant to guinea pigs. Sensitization was demonstrated in some of the guinea pigs as early as the second day and persisted in some cases for 36 days after the feeding of the buckwheat had been discontinued. Sheard *et al.* (42) sensitized guinea pigs, goats, and swine by feeding the green foliage of the plant, but obtained no sensitization in rabbits, rats, or dogs by the same method. Guinea pigs were found to be the most susceptible to this form of poisoning.

Lutz found that his animals were sensitive to light from either a mercury arc or from a 1500 watt electric light, but that the reactions to artificial light were less pronounced than those produced by solar irradiation. Sheard *et al.* observed no reaction upon irradiation by the mercury arc and but slight response to light from a carbon arc. By the use of filters the activating light was located between 5800 A.U. and the red end of the spectrum. Merian found that the activating light was absorbed by fresh solutions of either methylene blue or eosin, but that after bleaching of the dyes had occurred the specific light was no longer absorbed. He found artificial light to be unsatisfactory for the demonstration of sensitization in his animals.

Fischer and Lutz each obtained a flourescent substance from the foliage of buckwheat by extracting with alcohol. This product caused sensitization when injected into laboratory animals but none when fed. Both authors attached etiological significance to these results.

Bruce (4) produced a vesicular dermatitis by feeding another member of the Polygonaceal Lady's Thumb *(Polygonum persicaria)*, to a pig in the presence of direct sunlight, but obtained negative results upon feeding the same plant to a bull.

The observations of Bichlmaier (2) are rather surprising in view of the clinical and experimental evidence for poisoning by buckwheat. He claimed that buckwheat fields were commonly used as a pasture for calves, swine, and birds in Hungary and that no ill effects resulted from this practice. He fed the fruit and foliage of the plant to guinea pigs, sheep, and rabbits, and upon exposure to direct sunlight observed no evidence of a photosensitization. Hilz (17) accepted these results as positive evidence that the relation of buckwheat to fagopyrism had not been proved. Brandl and Schaertel (3) stated that they were unable to produce sensitization to light in experimental animals by feeding the fruit of buckwheat or by the injection of alcoholic extracts from the same fruit. The details of this part of their experimental work were not given.

Hypericism. *(St. Johnswort poisoning.)* It is difficult to determine when the effect of grazing *Hypericum* species first began to attract attention. The Arabian custom of painting horses with tobacco or henna to

protect them against the dermatitis resulting from the grazing of *Hypericum* has evidently been practiced for several centuries, although the frequent references in the literature upon this subject do not state how ancient the practice is. According to Marsh and Clawson (22) the earliest published report on the toxicity of *H. crispum* is that by Cirillo in 1787. Although Cirillo observed that the toxicity was confined to white sheep he evidently did not associate the toxicity with exposure to light. The significance of sunlight in this disease was recognized by Verheyen (45) in 1848. The principal manifestations are practically the same as those of fagopyrism and are generally associated with grazing the plant. However, Paugoué (26) and Henry (15) observed the disease in horses which had eaten hay containing *H. perforatum.*

Dodd (9) fed *H. perforatum* to sheep that were exposed to sunlight and observed edematous swellings of the face and ears on the thirteenth day. The light sensitive reaction was probably delayed by cloudy weather. The edema and dermatitis which followed were similar to the lesions he had observed in cattle, horses, and sheep under range conditions in Australia. Henry (16) also produced the disease in sheep by feeding the same plant, and by muzzling the sheep and allowing them to run in fields which were badly infested with the plant he proved that external contact with *H. perforatum* was followed by no ill effects. Marsh and Clawson produced a mild pruritis, dermatitis of the muzzle, and inflammation of brand scars in cattle by feeding *H. perforatum,* but these results were not the edematous swellings described by other investigators. After feeding the same plant to sheep they observed a mild dermatitis of the nose, face, and ears with slight edematous swellings about the nose. It is of interest to note that this mild evidence of photosensitization disappeared while the experimental feeding was still in progress. They also noted albuminous degeneration of the kidney and liver parenchyma in addition to the external lesions. A dermatitis confined to the lips of horses was considered by Richert (34) to be due to the eating of St. Johnswort, but since this lesion was observed in two colored animals, one brown and the other chestnut the diagnosis is subject to question. Seddon and White (41) found that feeding of *H. perforatum* to a black and white steer for three days was sufficient to render the white portions of the skin sensitive to light, and that the feeding of the plant for one day produced like results in guinea pigs. Quin (29) found that 200 gms. of *H. ethiopicum* was sufficient to produce photosensitization in a sheep within two days, and that feeding *H. leucoptychodes* resulted in similar photodynamic action, but he considered this plant less potent than the former species.

According to the results of Seddon and White the activating light for hypericism was not absorbed by eithe water or ordinary window glass. Working with sensitized guinea pigs they painted one ear of each animal with one of the three dyes, carbol-fuchsin, toluidin blue, or picric acid, leaving the other ear unpainted as a control. Following exposure to sunlight the unpainted ears and those painted with picric acid showed the usual reaction, but the ears painted with either carbol-fuchsin or

toluidin blue showed no reaction, thus presenting evidence that the activating light for hypericism is located in the same region as that for fagopyrism. With no apparent justification, Richert considered the infra-red to be the activating light in this condition.

Ray (33) isolated a fluorescent pigment from *H. crispum* with which he produced photosensitization of experimental animals but did not give his method of administration. Rogers (36) found that the fluid extract of *H. perforatum* produced photosensitization in sheep and rabbits but like Ray did not publish his method of administration. Hausmann (12) and Hausmann and Zaribnicky (14) isolated a pigment from the same plant which produced hemolysis of erythrocytes in the presence of light. Cerny (6) and Melas-Joannides (23) also isolated a fluorescent pigment from Hypericum, but both authors failed to demonstrate the photodynamic properties of their extacts.

Trifoliosis. Schindelka (38) and Lutz cited several reports in which the grazing of *Trifolium* was associated with the appearance of lesions similar to those of a photosensitization. Hausmann and Glueck (13) reported upon the occurrence of a dermatitis in cattle which were grazing on a field of *T. hybridum*. In one black and white cow the dermatitis appeared on all the white spots but in most of the cases the lesions were confined to the udders and teats and consisted of edema and vesicular and pustular eruptions. In view of the nature and location of the dermatitis in this outbreak the etiology is subject to question. The affected animals recovered without removal from the pasture. Bruce fed red clover to one pig exposed to sunlight and observed an erythema but no other evidence of a photosensitization. From field observations it is, therefore evident that the grazing of clover may be followed by a dermatitis which has all the appearance of a true photosensitization. However, experimental proof of the photodynamic properties of the various species of *Trifolium* has not been presented.

An investigation of the "aphis disease" of New South Wales led Dodd (8) to believe that it was caused by grazing on trefoil *(Medicago denticulata)*. He therefore fed guinea pigs on the fresh, green plant and upon exposing them to sunlight observed a photosensitization on the seventh day of feeding. The reactions were of the usual type consisting of pruritis, edema of the face and ears, followed by necrosis, sloughing and healing beneath a scab. In his early investigations he had observed the same lesions in cattle, horses, and sheep which were grazing extensively on this plant. Bull and Macindoe (5) failed to confirm Dodd's results and attributed the failure to the fact that they fed a more mature plant than was fed by Dodd. In field cases of the disease they observed that the edema was confined to the skin rather than being subcutaneous as is generally reported in the diseases of the lower animals which are the result of photosensitization.

Sudan Grass. Howarth (18) observed an outbreak of dermatitis in a band of sheep after they had been grazed on a pasture of Sudan grass

for ten days. The pathology consisted of pruritis, edematous swellings of the lips, eyelids and ears, followed by an oozing of serum from the edematous regions, superficial necrosis, and healing beneath a brownish-red scab. Black faced rams were not affected. Removal of the animals to an adjacent alfalfa field was followed by complete recovery with no new cases developing after the change of pasture. A second flock was observed in which there was an erythema followed by shedding of the wool, but the nature of the pasture in this case was not reported.

Oat and Wheat Pasture. Schmidt (39) reported the occurrence of edematous swellings of the head and ears of sheep and goats which had been grazed on green oat and wheat fields for at least ten days. A marked keratitis was also observed in some of the affected animals. In his unpublished notes* on the investigation of this condition, he recorded the occurrence of inspissated bile and in many cases the mucous membrane of the gall bladder was covered with small glistening crystals.

Geeldikkop (Tribulosis). The "geeldikkop" of South Africa, an important disease of sheep and goats, presents the usual lesions of a photosensitization but it differs from fagopyrism, hypericism, etc., in that the dermatitis is accompanied by an icterus of hepatic origin. In an early investigation of the cause of "geeldikkop," Theiler (43) collected *Tribulus terrestris* (sometimes called sand bur) from various locations and fed it to sheep. In this series of experiments 56 sheep were employed, but the disease was reproduced in only 12 animals. Feeding periods of 10 to 16 days were required to produce the positive results. Quin (27) continued the investigation by grazing sheep in paddocks containing nothing but *T. terrestris* and reproduced the condition in 8 out of 9 animals employed in one experiment. The sensitization occurred after grazing periods of three to six days. In subsequent experiments conducted after "geeldikkop" had disappeared from the ranges, he obtained negative results in sheep by grazing and feeding the plant. It is a matter of record that these investigators have obtained more negative than positive results in their experimentation with this plant, but their positive results are sufficiently clear-cut to establish *T. terrestris* as one of the etiological agents of "geeldikkop." From their investigations it appears that the photodynamic principle is not a constant constituent of this plant. Quin (28) produced fatal results by drenching sheep with water extracts of this plant, but the pathology he observed was not the pathology of "tribulosis."

Quin (30) fed a shrub, *Lippia rehmanni*, and obtained a photosensitization in three days. The lesions produced by this plant were similar but not as severe as the lesions observed in range cases of "geeldikkop." He obtained similar results by drenching sheep with alcoholic extracts of the plant. In the same publication he reported that on feeding *Lippia pretoriensis* to sheep he observed a photosensitization on the third day. The sensitization disappeared four days later in spite of the continued feeding of the plant.

*Personal communication.

Quin (31) produced a condition similar in all respects to "tribulosis" by ligating the common bile-duct of sheep and goats. In a continuation of this work Rimington and Quin (35) and Quin, Rimington and Roets (32) showed that the sensitization was due to the presence of phylloerythrin in the blood stream of animals treated in this manner, and concluded that the sensitization in "geeldikkop" was due to the action of this pigment. However, it remains to be shown that the feeding of *T. terrestris* to normal sheep will produce a phylloerythrinemina and subsequent photosensitization.

Bighead. The disease of sheep known as "bighead" in Utah and other Western states also presents the picture of a light-sensitization and in addition an icterus similar to that of "tribulosis." Clawson and Huffman (7) feed sheep on *Tetradymia glabrata* and *T. canescens* and observed a degeneration of the liver with a subcutaneous edema about the face. The lesions thus observed were similar to field cases of "bighead," except that the reaction to light exposure was less marked than is generally observed under range conditions. However, the extent of the photosensitization is reported as being quite variable in the natural occurrence of the disease. From the results of this investigation it appears probable that the "bighead" described by Frederick (11) was due to the grazing of one or both of these species of plants.

Swellhead or Goat Fever. The disease in sheep and goats referred to as "swellhead" or "goat fever" in the Southwest is similar in most respects to "geeldikkop" and "bighead," but in addition to the usual lesions of the two latter diseases there is a marked destruction of the kidney parenchyma. By feeding lechuguilla *(Agave lecheguilla)* to sheep and goats, Jungherr (19) killed 20 out of 24 animals, the lesions consisting of icterus, hepatopathy, and a "turkey egg" kidney. Two animals developed an edematous swelling about the head 24 hours before death, though the relation of light to the swelling was not shown. From his field observations he concluded that this disease was another example of photosensitization. Tunnicliff (44) fed the fruit of sacahuiste *(Nolina texana)* to one sheep and observed an edematous swelling about the head.

THE NATURAL OCCURRENCE OF LECHUGUILLA POISONING

Symptoms

Evidence of intoxication is marked by a listless attitude with little or no effort made to keep up with the remainder of the flock. A yellow, inspissated excretion adheres to the internal canthus of the eye and may extend for some distance along the side of the face. The nostrils are more or less occluded with a yellow, tenacious discharge. There is a progesssive decrease in the consumption of both water and food with complete loss of appetite for a few days before death. The sclera, skin, and visible mucous membranes show a pronounced icterus. A purplish discoloration may occur beneath the coronary band. The urine is clear amber but

occasionally port wine in color. The animals become progessively weaker and emaciated, a short period of coma preceding death. Scratching or rubbing of affected regions similar to that observed in other photodynamic diseases is not a prominent manifestation in this condition.

Pathology

A subcutaneous, edematous swelling of the face and ears and in some cases extending down into the intermandibular space is of frequent occurrence, especially during the late spring, summer, and early fall months. It is reported by ranchmen as also occurring during the winter months but has not yet been observed by the author during this season. The edematous swellings, or the reaction to light exposure, is of more frequent occurrence at the beginning than in later stages of an outbreak. Rupture of the skin of the ears with drainage of the edema through the rupture is of common occurrence. Reabsorption or drainage of the edema is accompanied by necrosis of the skin covering the involved regions. Mummification followed by sloughing of a part or all of an ear frequently occurs in the cases which eventually terminate in recovery. A less frequent manifestation is a wrinkled, rigid, and opaque cornea. Upon autopsy, with the exception of the pronounced icterus, the only constant and significant changes are found in the liver and kidneys. The liver is a light brownish-yellow in color, and from its gross appearance it is evident that considerable retrograde change has occurred. The capsule is frequently thickened though there is some doubt as to the genesis of this condition since it does not appear probable that sufficient time could have elapsed for the development of a definite cirrhosis. The bile is generally normal in appearance but occasionally the content of the gall-bladder has a doughy consistency. Incision of the liver and compression along the edge of the cut surface results in the expression of numerous plugs of thickened bile from the severed bile-ducts. The kidneys are swollen and greenish-black in appearance; it is not uncommon to find these organs fully twice their normal size. Upon stripping away the capsule, the greenish-black background is observed to be studded with numerous gray specks slightly smaller than the head of a pin. Upon incising the organ urine drips from the cut surface and the tubules may be sufficiently distended as to be identified with the unaided eye.

In differential leucoytic counts, polymorphonuclears have been found to comprise as high as 90 per cent of the leucocytes, thus evidently accounting for the leucocytosis which is invariably present. There is a marked decrease in the number of erythrocytes in some cases but not in others. The non-protein nitrogen content of the blood is greatly increased, values of over 100 mg. per 100 cc. of blood being frequently encountered. Albuminuria is of constant occurrence.

The microscopic pathology, as in the case of the gross, is of two distinct types depending upon the etiology as will be shown later. The lesions located in the skin and adjacent connective tissue about the head are the

result of photosensitization. Those located in the liver and kidneys are produced by a hepoto-nephro-toxin. The primary lesions which occur in the deeper layers of the corium and adjacent connective tissue and consist of an intracellular edema of the capillary endothelium, accompanied by a marked, edematous infiltration of the surrounding tissue, are the result of the photosensitization. As the disease progresses leucocytic and reticulo-endothelial accumulations, together with regeneration of the endothelium, give the walls of the capillaries a striking, rich cellular appearance. In the more superficial blood-vessels the injury to the endothelium and the subsequent cellular reaction is much less pronounced than it is in the deeper layers of the corium and underlying connective tissue. There is a moderate infiltration of wandering cells in the edematous areas, which becomes predominately poly-morphonuclear in type as necrosis of the skin develops. Discrete hemmorrhages are frequently observed, but thrombosis is of rare occurrence. Necrosis of the skin, which is accompanied by an inflammatory exudate, occurs as early as the third day and by the fifth day becomes quite extensive. The sebaceous glands, especially in the skin of the ears, frequently show a necrosis with liberation of the lipoid material far in advance of a similar change in the surrounding tissue. Necrosis of these glands is accompanied by a marked polymorphonuclear leucocytic infiltration. It is of interest to note that the reaction to light exposure on the ears is practically confined to the outer surface, the inner surface showing little or no change.

The parenchymatous tissue of the liver shows a marked degenerative fatty infiltration, local necrosis, and an abundance of bile pigment deposited in the degenerated cells. Bile ducts of all sizes show numerous casts composed of a nucleus of cholesterol crystals surrounded by an amorphus, brownish staining material. In the kidneys there is a pronounced destruction of both the tubular and glomerular epithelium. Degenerative fatty infiltration is also observed, the necrobiotic changes being more pronounced in the kidneys than in the liver. Numerous large casts composed of degenerated cells and precipitated albumin prevent the flow of urine and cause an enormous distention of the tubules above the casts. Albuminous deposits are of frequent occurrence within the glomeruli.

EXPERIMENTAL PROCEDURE

With the few exceptions noted, angora goats were employed; various breeds of sheep were used, the absence of pigmented skin being the only precaution observed. Unless otherwise stated the sheep and goats were fed ground, green leaves of the plant. Part of the animals were forced to eat the plant by being deprived of all other food; for the remainder of the animals the plant was force-fed by means of a balling gum, alfalfa hay being allowed ad libitum. Exposure to light in open pens was continuous for sheep and goats from the hours of 9 A. M. to about 5 P. M. For rats the exposures were of two to three hours duration between the hours of 10 A. M. and 2 P. M., depending on weather conditions and the amount

of exposure which could be tolerated by non-sensitized controls. The control sheep and goats were maintained in a building in diffuse light. All rats were maintained under similar conditions when not actually exposed to sunlight, a preliminary test having shown that the sensitization remained unaltered in the presence of diffuse light. Both water and alcoholic extracts of the plant were employed in the investigation. Since alcohol proved to be the better solvent for the extraction of the two toxic principles found, the work herein reported on rats was confined to the use of the alcoholic extract. All extracts were administered by stomach tube on the basis of dry solids. The water extract was obtained by soaking the ground, green leaves in distilled water at room temperature for 12 to 14 hours. The extract was filtered and brought to the desired concentration by boiling. In the case of the alcoholic extract the dry, ground leaves were extracted in boiling 96 per cent ethyl alcohol for two to three hours, the extract decanted and the process repeated twice. The combined extracts were filtered while hot and again upon cooling to room temperature. The alcohol was evaporated, the residue taken up in distilled water and administered as an aqueous solution. The light filters consisted of gelatine films in which the desired dye had been incorporated, the films being protected between two plates of ordinary window glass. Some of the rats were used in more than one experiment.

AN EXCLUSIVE RATION OF LECHUGUILLA

Of the 16 goats which were forced to eat lechuguilla by withholding all other food, 13 were fed in open pens and the remaining three were maintained in diffuse light for the duration of the experiment. A weighed quantity of fresh, green leaves was supplied each morning, at which time the unconsumed amounts from the previous day were weighed and then discarded. The results of this experiment are summarized in Table 1. One lot of animals was fed during December, a second lot during February and March, and the third lot during May. Without exception these animals developed the usual icterus, liver, and kidney lesions of lechuguilla poisoning but no evidence of a photosensitization. The failure to react to sunlight exposure was not due to unfavorable weather conditions as the sunlight was not obstructed by cloudy weather during any part of the various feeding periods. Starvation may have contributed to the mortality of 75 per cent which occurred among these animals, since the death rate was much higher than it was when the plant was force-fed by balling gum and alfalfa hay provided ad libitum as shown later in Tables 2 and 3. For an average feeding period of 18 days the average consumption of the plant was 8.8 pounds with a recovery of 25 per cent whereas force feeding was followed by a 45 per cent recovery although an average of 10.6 pounds was fed for an average feeding period of 15 days. The percentage of recovery in the latter case would probably have been higher if not for the fact that three of the animals were killed for microscopic study when the appearance of these animals at the time of slaughter indicated possible recovery. Since the icterus, liver, and kidney

Table 1. Effect of an exclusive ration of lechuguilla on goats

Goat No.	Weight Lbs.	Pounds consumed	Dates—inclusive	Results
Ex	posed to di	rect sunlight	for the duration of the	experiment
35	85	12	12/4 —12/20/32	No ps., icterus; recovered
42	55	11	12/4 —12/20	No ps., icterus; died 12/27
43	55	11	12/4 —12/20	No ps., icterus; died 1/3/33
63	40		2/28—3/10/33	No ps., icterus; died 3/25
64	55	18	2/28—3/10	No ps., icterus; died 3/17
65	50		2/28—3/10	No ps., icterus; died 3/14
37	70	14	5/6 —5/29	No ps., icterus; recovered
44	63	12	5/6 —5/26	No ps., icterus; died 5/27
56	65	13	5/6 —5/29	No ps., icterus; died 6/1
49*	58	9	5/6 —5/23	No ps., icterus; died 5/27
52*	70	8	5/6 —5/24	No ps., icterus; died 5/25
53*	75	10	5/6 —5/30	No ps., icterus; recovered
54*	65	4.4	5/6 —5/14	No ps., icterus; died 5/16
		Fed	in diffuse light	
60	45		2/28—3/10/33	No ps., icterus; recovered
61	53	20	2/28—3/10	No ps., icterus; died 3/18
62	45		2/28—3/10	No ps., icterus; died 3/25

*Brown or brown and black Spanish goats.
Ps. =photosensitization.
Goats 60 to 62 and 63 to 65 were fed as a lot and not as individuals.

lesions occurred irrespective of light condition or the color of the animals (four of the goats were brown, or brown and black), it is evident that this part of the clinical picture is not a photosensitization.

FORCE FEEDING OF LECHUGUILLA

Eleven animals were force-fed lechuguilla and exposed to sunlight for the duration of the experiments, while six animals were fed in a like manner but maintained in diffuse light for the same length of time. As is indicated in Tables 2 and 3 these experiments were conducted at different seasons of the year. The first experiment was conducted in August, the second in September, the third in October, and the fourth during November and December. Of the five animals fed in the presence of sunlight during August, three reacted with the complete picture including a pronounced photosensitization. No evidence of toxic effects was obtained in the two remaining animals (S37, G72) in spite of the fact that they were fed a pound a day for 24 days, after a preliminary feeding period of 7 days, during which time they received .25 and .5 pounds per day respectively. A definite resistance to both the toxic saponin and the photodynamic agent was probably produced in these animals as the amounts of the plant which were fed were in excess of that required to produce serious illness or death in all other experiments. However, the resistance was of short duration as the typical liver and kidney lesions were later produced in both animals by the daily feeding of one pound doses for six and seven days respectively. The liver and kidney degeneration was obtained in the one control (No. S30, Table 3) but no evidence of a photosensitization. Sensitization was not produced by feeding the plant during September, possibly because of climatic

conditions as cloudy weather prevailed throughout much of the period required for the experiment. However, as shown by results of another experiment, sufficient light energy was available during this time to produce the reaction in one sheep which had been fed the hydrolyzed extract of the plant. In view of the results of later experiments the failure to produce the photosensitization of lechuguilla poisoning during the month of October is difficult to explain. Sheep 57 and goat 94 were supposedly susceptible animals but in the case of sheep 37 the seeming resistance to the photosensitization, which had been produced in the previous experiment, may have persisted up to this time. However, the resistance to the hepato-nephro-toxin had disappeared. The amounts of the plant which were fed in this experiment had been found sufficient to produce sensitization in other experiments, and even though bright clear days prevailed for the duration of the experiment, the only lesions produced were those of the toxic saponin.

The failure to produce a photosensitization during October was first attributed to insufficient light energy, as rats which were exposed at the same time failed to react, although they had been fed an extract which

Table 2. Goats and sheep force-fed lechuguilla and exposed to direct sunlight for the duration of the experiment; alfalfa hay ad libitum

Exp. No.	Animal	Weight	Daily dose	Dates—inclusive	Results
		lbs.	lbs.	1936	
I	G72	85	.5	August 10–16	No ill effects
			1.0	August 17 to Sept. 9	No ill effects
	S37	65	.25	August 10–16	No ill effects
			1.0	August 17 to Sept. 9	No ill effects
	G93	50	.25	August 10–16	No ill effects
			.5	August 17 to 25	Ps. August 25, icterus; died Sept. 4
	S58	70	.5	August 10–21	Ps. August 21, icterus; killed August 24
	S55	75	1.0	August 17–25	Ps. August 25, icterus; killed August 29
II	G92	60	1.0	September 9–19	No ps., icterus; recovered
	G72	85	1.0	September 5–11	No ps., icterus; recovered
III	S37	62	1.0	October 5–10	No ps., icterus; recovered
	S47	50	1.0	October 5–10	No ps., icterus; died October 20
	G94	90	1.0	October 5–10	No ps., icterus; died October 20
IV	G96	60	.25	November 9–11	No ill effects
			.5	November 14–16	Loss of appetite
			1.0	November 17–21	Ps. November 22, icterus; died November 24
	G97	80	.25	November 9–13	No ill effects
			.5	November 14–16	Loss of appetite
			1.0	November 17–21	Ps. November 22, icterus; died November 26
	S64	70	.25	November 9–14	No ill effects
			.5	November 15–16	Slight loss of appetite
			1.0	November 17–21	Ps. November 24, icterus; recovered
	S63*	65	.25	November 9–14	No ill effects
			.5	November 15–16	No ill effects
			1.0	November 17–21	No ps., icterus; recovered

*Not exposed to sunlight from November 22–30. Exposed on December 1.
Ps. =photosensitization.
G and S before the animal number stand for goat and sheep respectively.

constantly produced photosensitization during the summer months. Therefore goats 96 and 97 and sheep 63 and 64 were placed on experiment for the purpose of again producing a resistance to the toxic saponin similar to that which had apparently been produced in the two animals during August. The feeding of lechuguilla was started on November 9, the animals maintained in a shed with an open southern exposure and no special attention paid to direct sunlight exposure since it was assumed that the reaction to light exposure would not occur. November 11, 12 and 13 were clear bright days but during the week from the 14th to 21st the weather was almost continuously cloudy, with November 19, 20, and 21 so foggy that no possibility existed of a direct sunlight exposure on these days. By the 21st it was obvious that no resistance to the toxic saponin had developed and the feeding was discontinued. Weather conditions improved on the 22nd and a bright sunlight exposure occurred for about three hours during the middle of the day, although the animals could have avoided the exposure had they so desired. On the 23rd the two goats exhibited a marked edematous swelling of the face and ears, but the two sheep showed no reaction on this day. The edematous swellings began to recede by the evening of the 24th and were entirely reabsorbed in the case of goat 97 at the time of its death on the 26th. On the morning of the 23rd, which was again cloudy, sheep 63 was placed in a building and not exposed to direct sunlight until December 1. On November 24, sheep 64 was exposed to bright sunlight from 8 A. M. to 5 P. M., at which time she was showing a moderate swelling about the face and ears. This reaction was intensified by a second exposure on the following day. Up to December 1 sheep 63 had shown no evidence of a similar reaction; she was, therefore, exposed to bright sunlight on December 1, 2, and 3, but no reaction was obtained. This experiment was repeated with goats 98, 99, and sheep 65 and 66, except that the animals were maintained in diffuse light for the duration of the feeding period, November 24 to December 9. On the latter date goat 99 and sheep 66 were exposed to bright sunlight from 10 A. M. until 3 P. M. Both animals showed a moderate swelling of the face and ears on the following day, which was intensified and

FIG. 2. Sheep 57A showing the edematous swelling of the face and ears.

Table 3. Goats and sheep force-fed lechuguilla in diffuse light; alfalfa hay ad libitum

Exp. No.	Animal	Weight	Daily dose	Dates—inclusive	Results
		lbs.	lbs.	1936	
I	S30	72	.5	August 10–25	No ps., icterus; recovered
II	S54	90	.5	Sept. 9–Oct. 3	No ps., icterus; recovered
III	G84	90	1.0	Oct. 5–20	No ps., icterus; died October 26
	S62	120	1.0	Oct. 5–20	No ps., icterus; died November 1
IV	G98	75	.25	November 24–28	No ill effects
			.5	November 29 to December 1	No ill effects
			1.0	December 2–6	No ps., icterus; recovered
	G99*	80	.25	November 24–28	No ill effects
			.5	November 29–Dec. 1	No ill effects
			1.0	December 2–6	Ps. November 9, icterus; killed December 14
	S65	60	.25	November 25–30	No ill effects
			.5	December 1–2	No ill effects
			1.0	December 3–6	No ps., icterus; recovered
	S66*	70	.25	November 25–30	No ill effects
			.5	December 1–2	No ill effects
			1.0	December 3–6	Ps. November 9, icterus; died December 13

*Exposed to bright sunlight December 9 and 10.
Ps. =photosensitization.

accompanied by marked pruritis following a second light exposure on December 10. Marked evidence of the action of the hepato-nephro-toxin was observed in the two remaining animals but no evidence of a photosensitization. Sensitized rats exposed for the same length of time as the sheep and goat showed an erythema and moderate lacrymation but no edematous reaction.

THE FEEDING OF EXTRACTS OF LECHUGUILLA

The water extract of lechuguilla was given by the mouth to six goats and two sheep with the light exposures conducted in the usual manner and bright sunlight prevailing for the duration of the experiments. Controls were not employed as this was deemed unnecessary until a successful production of photosensitization had been obtained. As is indicated in Table 4, six of the eight animals developed the constant lesions of lechuguilla poisoning (icterus, hepatitis, and nephritis) but no evidence of a photosensitization. In addition to the usual lesions, goat 85 developed a slight edematous swelling of the ears but no reaction about the face and no pruritis. One animal died ten hours after receiving a single dose of the extract but no evidence of an acute photosensitization was observed in this case. With a mortality of seven of the eight animals, ample proof of the toxicity of the extract was obtained, but evidence of a photosensitization was questionable and confined to the one goat.

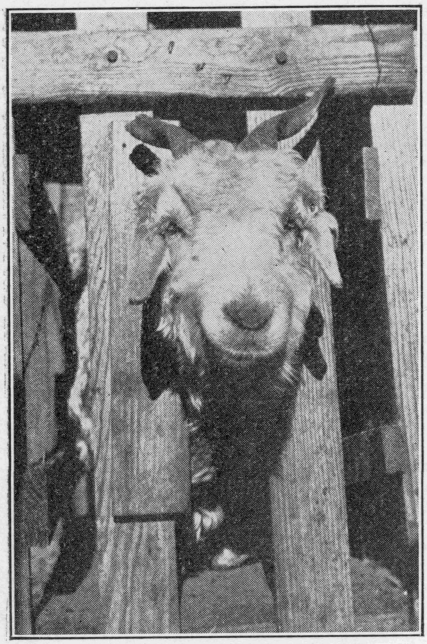

FIG. 3. Goat 99 showing the edematous swelling of the face and ears.

In working with rats, which will be discussed later, it was found that the hepato-nephro-toxin in alcoholic extracts could be hydrolyzed without destruction of the photodynamic agent. Such an extract was, therefore, hydrolyzed with .37 per cent hydrochloric acid, neutralized with sodium hydroxide and given to four sheep through a stomach tube as is indicated in Table 4. Sheep 57A and 60 were exposed to direct sunlight, whereas sheep 59 and 62 were kept in a building for one week after receiving the last dose of the extract. The two former animals reacted with pruritis and edematous swellings of the face and ears but the other two showed no evidence of a photosensitization. Since a moderate icterus developed in sheep 57A it was evident that four hours' hydrolysis was not sufficient to destroy the hepato-nephro-toxin. For the other three sheep the hydrolysis was, therefore, extended to eight hours. Administration of these hydrolyzed products produced no evidence of a toxic action on the liver and kidneys. Two doses of the original extract resulted in the death of sheep 61 during the night of the second day. Since exposure to sunlight

Table 4. Effect of administering extracts of lechuguilla to goats and sheep by mouth

Animal	Weight Lbs.	Daily dose	Dates—inclusive	Results
		Water extract—direct sunlight exposure		
G70	75	200 Gms.	12/26—12/29/33	No ps., icterus, died 1/6/34
G78	80	225 Gms.	12/28—12/30/33	No ps., icterus, died 1/18/34
G72	75	250 Gms.	1/19—1/24/34	No ps., icterus, recovered
S39	90	275 Gms.	1/24—1/26/34	No ps., icterus, died 2/4/34
S42	85	250 Gms.	1/31—2/2/34	No ps., icterus, died 2/6/34
G81	68	250 Gms.	8/16—8/17/34	No ps., icterus, died 8/27/34
G84	67	266 Gms.	8/21/34	Died 6 P. M. 8/21/34
G85	60	250 Gms.	8/22—8/23/34	Ps. 9/1/34, icterus, died 9/3/34
		Hydrolyzed alcoholic extract—direct sunlight exposure		
S57A	50	50 Gms.	7/20—7/23/36	Ps. 7/23/36, slight icterus, killed
S60	40	46 Gms.	9/ 5—9/7/36	Ps. 9/10/36, no icterus
		Hydrolyxed alcoholic extract—in diffused light		
S59	35	46 Gms.	9/ 5—9/7/36	No ill effects (non-toxic)
S62	38	46 Gms.	9/ 5—9/7/36	No ill effects (non-toxic)
		Original alcoholic extract—direct sunlight exposure		
S61	40	46 Gms.	9/5 —9/6/36	No ps., no icterus, died 9/6/36

Ps. =photosensitization, i. e., edematous swelling of face and ears.

in this case was followed by no symptoms suggestive of a photosensitization, it was assumed that the animal died of an acute intoxication similar to that obtained in rats. Death in this case was too early for the development of the liver and kidney lesions.

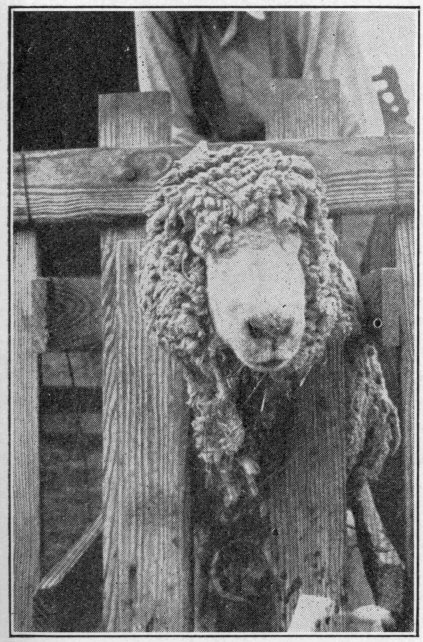

FIG. 4. Sheep 55 showing the edematous swelling of the face.

SYMPTOMS AND PATHOLOGY OF EXPERIMENTAL CASES

There is little to add to the symptoms and pathology as the experimental cases were reproductions of many cases which had been observed in field outbreaks of the disease. Rubbing of the head during the development of the photodynamic reaction was a prominent symptom, thus indicating that this symptom is probably much more prevalent under range conditions than is generally observed. There was no evidence of an acute reaction similar to that which is frequently observed in fagopyrism. With one exception, goat 99, the swellings of the face and ears attained a maximum degree by the end of the second day, began to recede by the third day, and

were reabsorbed by the fourth or fifth day. Rupture of the skin of the ears as a result of excessive edema was not observed, indicating that a less severe reaction was obtained in the experimental production of the disease than is frequently observed on the ranges. Temperatures of 106° to 107° were noted during the development of the edematous reaction but at all other times the temperature was normal, or subnormal towards the end in case of a fatal termination. In field outbreaks of the disease high body temperatures are the exception rather than the rule. A high temperature was never observed in animals which failed to react to light exposure.

The most significant blood changes, the study of which is summarized in Table 5, consisted of a leucocytosis with an increase in the percentage of polymorphonuclear leucocytes and a marked increase of the non-protein

Table 5. Blood studies before and during the production of lechuguilla poisoning

Goat Number		Dates					
		2/28/33	3/7/33	3/11/33	3/14/33	3/17/33	
60	R.B.C.	22		22		22	
	Whites	19,500		19,600		21,000	
	Polys	32		66		52	
	N.P.N.	38 mg.	96	84	62	48	
61	R.B.C.	17		18		20	
	Whites	8,400		8,200		16,000	
	Polys	41		60		69	
	N.P.N.	33	60	107	82	65	
62	R.B.C.	18		23		23	
	Whites	11,600		11,200		17,400	
	Polys	38				69	
	N.P.N.	33	60	107	82	65	
63	R.B.C.	20		19		23	
	Whites	6,600		9,000		27,000	
	Polys	23		72		72	
	N.P.N.	33	88	107	120	139	
64	R.B.C.	18		22		23	
	Whites	10,600		10,200		14,000	
	Polys	34		60		52	
	N.P.N.	32	33	90	96	62	
65	R.B.C.	18		18		—	
	Whites	14,000		13,000		—	
	Polys	16		70		—	
	N.P.N.	32	50	120	170	—	
		12/1/32	12/12/32	12/19/32	12/22/32	12/27/32	1/12/33
35	R.B.C.	20	19	17	—	17	3.7
	Whites	10,600	11,600	17,000	—	—	16,800
	Polys	32	36	72	—	—	63
	N.P.N.	38	36	33	83	108	87
42	R.B.C.	18	11	—	9	6	—
	Whites	7,200	12,800	—	18,400	1',600	—
	Polys	30	70	—	73	78	—
	N.P.N.	32	36	168	280	275	—
43	R.B.C.	23	22	20	—	5	—
	Whites	9,200	14,600	18,800	—	20,000	—
	Polys	30	70	73	—	78	—
	N.P.N.	32	42	84	—	—	—

R.B.C. = (Red Blood Corpuscles) expressed in millions per cmm. of blood.
Whites = (leucocytes) in thousands.
Polys = (polymorphonuclear leucocytes) percentage.
N.P.N. = (non-protein nitrogen) expressed in mg. per 100 cc. of blood.

nitrogen. Evidence of kidney degeneration was reflected by an increased non-protein nitrogen content of the blood as early as the eighth day. By the twelfth day values of over 100 mg. per 100 cc. of blood were of common occurrence. In such cases the odor of urine was readily detected

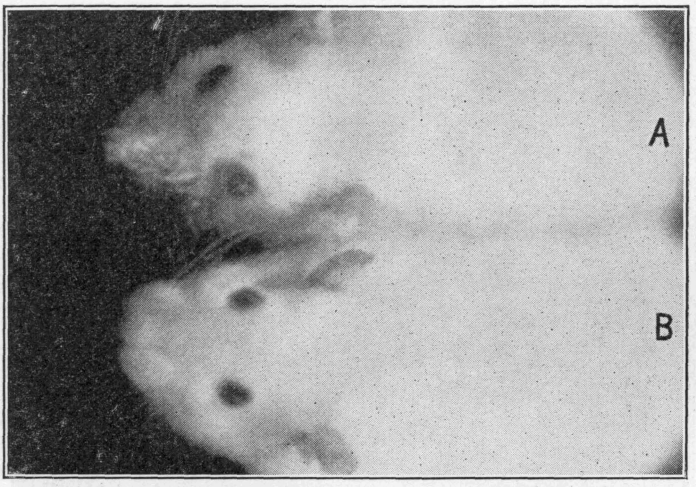

FIG. 5. A, rat sensitized with lechuguilla extract 26 hours after receiving the first sunlight exposure. B, normal control exposed at the same time as rat A.

during the micro-Kjeldahl digestion of the protein free, blood filtrate. No explanation was available for the marked decrease of erythrocytes which occurred in some cases and not in others.

IDENTIFICATION OF THE HEPATO-NEPHRO-TOXIN

The investigation included rats, rabbits, and guinea pigs as experimental animals, all of which proved to be suspectible to this toxic principle. Although more than 150 of the laboratory animals were employed, in order to conserve space the work herein reported is confined to a presentation of the data on a sufficient number of rats to illustrate the results.* The toxic principle was found to be extractable with water, 70%, 96%, absolute ethyl and methyl alcohol, but not with ether, chloroform, or acetone. The alcoholic extracts were found to be about equal in toxicity and much more toxic than the water extract. The toxicity of the 96 per cent alcoholic extract is summarized in Table 6. The repeated administration of .2 gm. doses of this product had no ill effects; ten .35 gm. doses produced fatal results. The minimum toxic dose was .7 gm. and the minimum lethal dose 1 gm.

*A detailed report on this part of the investigation is presented in the complete thesis. See footnote, page 1.

According to the solubility the toxic principle was assumed to be a saponin. Proceeding upon this hypothesis it should be possible to hydrolyze it with dilute hydrochloric acid, precipitate it with either barium hydroxide or basic lead acetate, and according to the suggestion of Autenrieth (1), use cholesterol as an antidote. Therefore with these objectives an aqueous

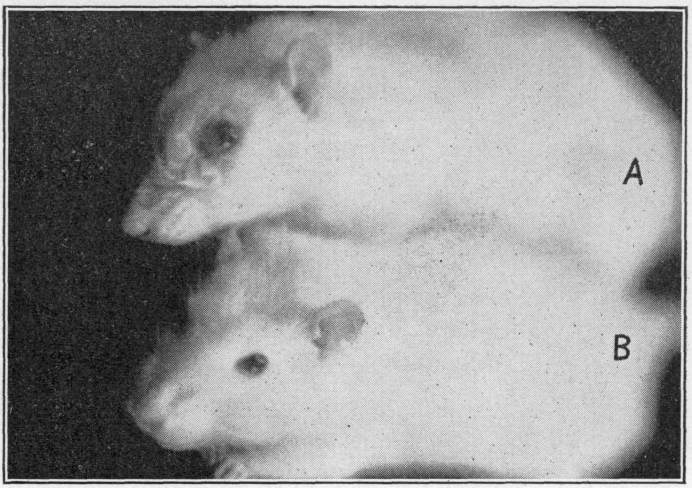

FIG. 6. A side view of rats A and B of fig. 5.

solution of the alcoholic extract was divided into four parts, treated according to the various methods and the derivities thereof fed to rats as indicated in Table 6. The hydrolyzed product, after neutralization with sodium hydroxide, was fed in doses equivalent to one minimum lethal dose of the original extract for 8 to 12 consecutive days without ill effects. After the precipitation of the toxic principle the excess barium was precipitated with carbon dioxide and the excess lead with hydrogen sulfide; the precipitated metals filtered off and the filtrates concentrated by boiling. One gram doses of both filtrates were found to be non-toxic. A saturated alcoholic solution of cholesterol was added to the extract at the rate of .2 gm. of cholesterol to each minimum lethal dose of the extract. The alcohol was evaporated and the residue concentrated until three cubic centimeters were equivalent to one minimum lethal dose of the original extract. The product was given to five rats, each of which received the equivalent of one minimum lethal dose for 5 to 8 consecutive days without ill effects. The saponin which had been precipitated with the barium was later recovered and its toxicity proved by administration to rat 74. A similar recovery from the lead precipitate was not obtained, probably due to adsorption of the saponin by the lead sulfide during the precipitation of the lead. The susceptibility of rats 16, 30, 69 and 70 was later tested

Table 6. The toxicity and detoxication of alcoholic extracts of lechuguilla

Rat	Original Extract Daily	Detoxication of original extract by			Combined with cholesterol	Results
		Hydrolysis with .37% HCl	Precipitation with			
			BaOH	$Pb(C_2H_3O_2)_2$		
	Dose	Doses	Doses	Doses	Doses	
73	.2 gm.					40 doses non-toxic
59	.35 gm.					10 doses fatal
58	.7 gm.					1 dose toxic
45	1.0 gm.					1 dose M.L.D.
69		12 M.L.D.				No ill effects
70		8 M.L.D.				No ill effects
76		8 M.L.D.				No ill effects
77		8 M.L.D.				No ill effects
72			4 M.L.D.			No ill effects
74			4 M.L.D.			No ill effects
16				4 M.L.D.	5 M.L.D.	No ill effects
36				4 M.L.D.	5 M.L.D.	No ill effects
75					8 M.L.D.	No ill effects
17					6 M.L.D.	No ill effects
30					6 M.L.D.	No ill effects

M.L.D. = Minimum Lethal Dose, refers to the equivalent of the original extract, one minimum lethal dose being administered daily to each animal.

by the administration of .7 gm. of the original extract, and in every case toxic effects were obtained.

PURIFICATION OF THE SAPONIN

An excess of magnesium oxide was added to an aqueous solution of the extract, the mixture dried and re-extracted with boiling 96 per cent ethyl alcohol. An equal volume of ether was added to the filtered extract and the precipitated saponin redissolved in alcohol and reprecipitated. The second precipitate was found to be non-toxic for rats in one gram doses, but the filtrate after evaporation of the ether and alcohol was proved to be more toxic than the original extract. To the aqueous solution of this product was added a saturated alcoholic solution of cholesterol at the rate of .5 gm. of cholesterol to each minimum lethal dose. The alcohol was evaporated, the precipitate filtered, washed several times with water, once with 96 per cent alcohol and then dried for several days in a desiccator. The dried precipitate was dissolved in a small volume of pyridine and ten volumes of ether added to the solution. The white precipitated saponin was washed several times with ether, with equal volumes of ether and alcohol, and then dried for several hours at 90° centigrade in order to remove all but traces of the pyridine. The recovered saponin was suspended in a small amount of water and fed to rats, with results which are summarized in Table 7. Each of two rats was given .2 gm. per day for three consecutive days; one died on the fourth and the other on the sixth day. A third rat showed marked toxic effects after receiving .3 gm. at one dose. A marked concentration of the toxic principle was therefore obtained.

Thus, in recovering a saponin from its cholesterol compound, according

to the method of Schoenheimer and Dam (39) a product was obtained which was much more toxic than the original extract. The solubility of the purified product in water was much less than that of the original extract. The addition of a small amount of water to the dry powder produced a jelly but, upon further addition of water, a milky solution was obtained. Heat did not increase this solubility. Shaking the aqueous solution produced a foam. In physiological salt solution it hemolyzed

Table 7. The toxicity of the purified saponin recovered from the cholesterol compound

Rat	Daily Dose	Number of doses	Results
73	.2 gm.	3	Sick on third day, died on the fourth
75	.2 gm.	3	Sick on third day, died on the sixth
80	.3 gm.	1	Sick on second day, recovered

erythrocytes in dilutions of 1-2500. It was hydrolyzed by hydrochloric acid; its hydrolyzed products reduced Fehling's solution and were non-toxic. Upon the addition of concentrated sulfuric acid the color reactions were red, later changing to a blue-green, which according to some authors is not quite characteristic for saponins. Neither the saponin nor its hydrolyzed products were photodynamic.

Other Methods of Purification

The precipitation of the alcoholic extract with ether resulted in a precipitate and a filtrate, both of which were toxic. As elsewhere stated, redissolving and reprecipitation of the first precipitate resulted in a non-toxic precipitate and a toxic filtrate. It was observed that if the filtrates, either first or second, were allowed to stand for three or four days a second precipitation would occur. This precipitate was freely soluble in boiling alcohol but much of it was crystallized out upon cooling. By repeated recrystallization from 70 per cent alcohol a saponin was obtained which had the same properties as that obtained from the cholesterol compound.

A second and still more simplified method of purification was obtained. The alcoholic extract was freely soluble in distilled water but in chloroform water a flocculent precipitate was formed. After repeated washing of this precipitate with chloroform water doses of 15, 20 and 25 grams on three consecutive days were found sufficient to produce serious illness accompanied by icterus, liver, and kidney lesions in one sheep. This precipitate, when recrystallized from 70 per cent alcohol was found to have all the properties of the saponin obtained by the previous method.

THE PHOTODYNAMIC ACTION OF ALCOHOLIC EXTRACTS FOR RATS*

The results with a few of the rats which were employed in this part of the investigation are summarized in Table 8. As is indicated in this table doses of the crude extract ranging from .025 to .1 gm. were effective sensitizing doses, but the photodynamic action decreased as the dosage approached toxic limits (toxicity due to the saponin), with practically no sensitization obtained with a dose of .7 gm. With the toxic action of the saponin eliminated, either by hydrolysis or by combination with cholesterol as previously stated, doses equivalent to one minimum lethal dose of the original extract could be given with safety, and when sensitized with the large doses of the modified extract, rats reacted to light exposures in the usual manner. The reactions obtained by these methods of sensitization were perhaps a little more pronounced than those obtained with small doses of the unaltered extract. However, the extent of the reaction was never proportionate to the excess amount of the photodynamic agent administered, which in the case of one gram doses was 40 times greater than the minimum sensitizing dose.

Table 8. The photodynamic action of alcoholic extract before and after hydrolysis or neutralization of the toxic saponin

Rat	Crude Extract	Saponin of crude extract modified by		Reaction to sunlight exposure
		Hydrolysis with HCl	Neutralization with cholesterol	
	Dose	Dose	Dose	
104	.025 gm.			Pronounced
92	.1 gm.			Pronounced
70	.3 gm.			Moderate
76	.4 gm.			Slight
72A	.5 gm.			Slight
84	.6 gm.			Doubtful
87	.7 gm.			Doubtful
96		.025 gm.		Pronounced
104A		.125 gm.		Pronounced
107		.48 gm.		Pronounced
105		.5 gm.		Pronounced
98		1.0 gm.		Pronounced
24			.05 gm.	Pronounced
26			.1 gm.	Pronounced
27			.5 gm.	Pronounced
16			1.2 gm.	Pronounced
36			1.2 gm.	Pronounced

RESISTANCE

The exposure of sensitized rats to direct sunlight was followed by the development of resistance, regardless of the reaction produced by the exposure. The development of resistance was first encountered in a group of 12 rats which were placed on experiment on March 1. Between that date and March 21 they received a total of six hours' exposure, on March 10, 14, and 17, with no visible reaction following the exposures except a pronounced erythema and slight lacrymation. On March 21 this group

*A detailed report of this work is presented in the complete thesis.

was exposed at the same time as a second group which had been given comparable doses of the same extract but had not been exposed prior to this date. On the following day, March 22, the second group exhibited a well developed swelling of the face, but in the group of 12 the erythema of the preceding day had disappeared leaving no evidence of a reaction. The size of the dose was, therefore, increased for this group, but repeated light exposures failed to produce an edematous reaction. During this time, light exposures of recently sensitized animals were followed by the typical edematous swellings without exception. Resistance was also observed in another group of rats following a single light exposure. In this group the first exposure was followed by the appearance of the edema, but because of weather conditions a second exposure was delayed for 72 hours. By this time the edema had been completely reabsorbed and a similar change could not be reproduced in spite of the continued administration of the extract and light exposures. Under favorable conditions the maximum reactions were obtained by exposures for three or four consecutive days. Under such conditions reabsorption of the edema began by the end of the third day and was generally complete by the end of the fourth or fifth day and could not be reproduced. With resistance once established the disease could not be reproduced until sufficient time had elapsed for loss of the resistance. The duration of the resistance did not exceed ten weeks, and in most cass susceptibility had been re-established within seven weeks from the time of discontinuation of the sensitization and light exposures.

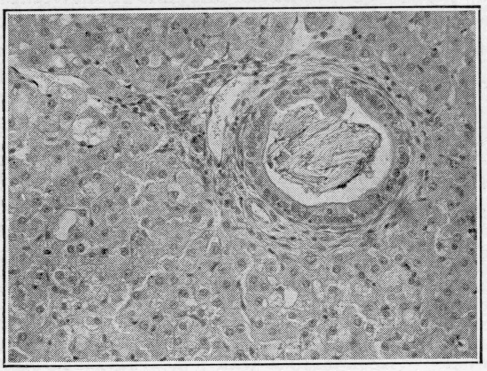

FIG. 7. A section of liver from a case of lechuguilla poisoning, showing some of the degeneration of the liver cells and an obstructed bile-duct.

THE ACTIVATING LIGHT FOR THE PHOTODYNAMIC ASPECT OF LECHUGUILLA POISONING

In determining the region of the activating light one sensitized rat was placed beneath each filter.* In this experiment, which is summarized in Table 9, the light exposures were continued for thirty minutes after the appearance of profuse lacrymation, previous observations having shown that this was sufficient time to produce the edematous reaction if the light injury was sufficient to produce an injury to the blood-vessels. The first exposure was followed by the complete reaction in the rats exposed beneath

*Rats sensitized with buckwheat were also exposed at the same time and found to react the same as those sensitized with lechuguilla extract.

the acridine yellow and the ordinary window glass, but those exposed to the red, blue, and green light were not affected. On the following day the two rats which had been exposed beneath the green filter B and the methylene blue were exposed beneath the acridine yellow and window glass respectively. The typical reaction developed in both animals. Exposures of the remaining animals to green light were again negative. On the third day the two remaining animals were exposed to the visible spectruma and acridine yellow respectively, both of which reacted but much more mildly than the other animals on the preceding days. In repetitions of this experiment consistent negative results were obtained with red, blue, and green light; and in addition to acridine yellow, positive results were also obtained with methyl orange and picric acid filters. The milder reaction obtained in the

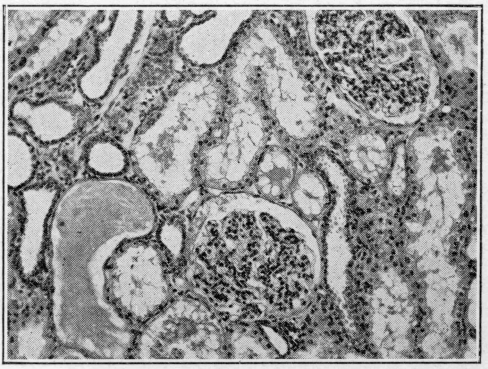

FIG. 8. A section of kidney from a case of lechuguilla poisoning, showing the degeneration of the epithelium, cast formation and distention of the tubules.

Table 9. Exposure of sensitized rats to filtered sunlight

Rate	Red A	Acridine Yellow	Green B	Methyl Green B	Methylene Blue	Window Glass	Date Exposed
							1936
93	—						May 6
92		+					May 6
89			—				May 6
88				—			May 6
90					—		May 6
91						+	May 6
89		+					May 7
88			—				May 7
93				—			May 7
90						+	May 7
88		±					May 8
93						±	May 8

+ =Edematous swelling of the face.
± =Slight edematous reaction.
— =No reaction.
Red A and Green B Wratten filters made by Eastman Kodak Company.

rats exposed on the third day was suggestive of a partial resistance produced by the exposures on the previous days to either the red, blue, or green light. Another lot of sensitized rats were, therefore, exposed on three consecutive days to red, blue, and green light in the order named, and followed on the fourth day by exposure to yellow light. The reaction obtained was the same as that which was constantly observed in resistant animals. It was evident that the red, blue, and green filters permitted the passage of sufficient light for the development of resistance without visible lesions. By increasing the duration of the exposure until non-sensitized controls were showing marked evidence of distress, it was found that a reaction consisting of lacrymation and later the development of a barely perceptible swelling of the face could be produced with both red and green light but not with the blue. Thus the red and green filters did not absorb all the activating light when the exposure was in excess of that required to produce the complete reaction with yellow or unfiltered light.

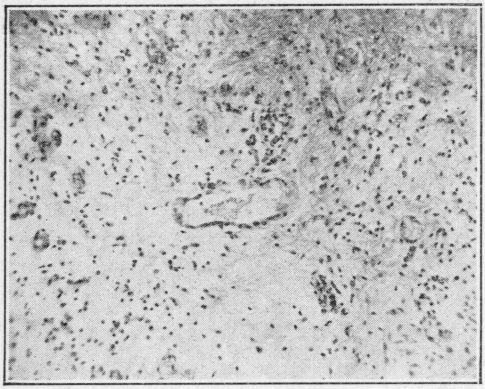

FIG. 9. The corium and subcutaneous connective tissue of a rat sensitized with lechuguilla and exposed to sunlight, showing edema of the endothelium and surrounding tissue and the cellular reaction in the edematous areas.

The spectral absorption of the filters listed in Table 9 is presented in Fig. 10. According to the spectral absorption of acridine yellow the activating light is of greater wave length than 5328 A. U., as this filter absorbed practically all light of shorter wave length. In considering the absorption of the other filters in the red-green end of the spectrum, the absorption of the red filter began a little above the sodium lines and that of the green filter B began a little below this region; consequently a narrow band was left between these two filters in the region of the sodium lines in which the amount of light energy was

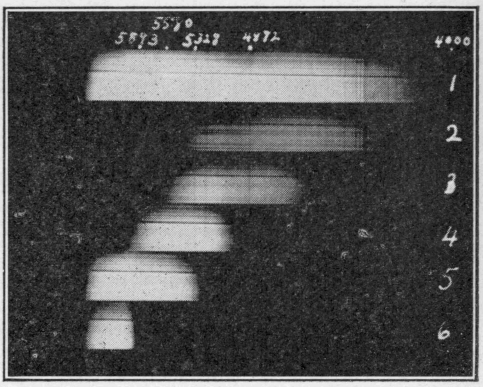

FIG. 10. 1, Solar spectrum; 2, Methylene blue filter; 3, Methyl Green B; 4, Wratten Green B; 5, Acridine Yellow; 6, Wratten Red A.

greatly reduced by the green filter and practically excluded by the red. There was little or no absorption of the light in this region by the acridine yellow. Since the reactions obtained beneath the acridine yellow filter were equal to those produced by unfiltered sunlight, the activating light for lechuguilla poisoning appears to be located in the region of 5893 A. U., provided that the action of a single band of light is all that is required.

LIGHT ENERGY

While no determinations were made as to the amount of light energy required to produce a complete reaction, it is evident from the results of many experiments that this is an important factor in the occurrence of the disease. The experimental conditions were made as nearly constant as possible by exposure to sunlight during the same time of the day, weather conditions permitting. However, the degree of the reactions varied according to the season of the year in spite of the fact that the rats were from the same source and that in many cases identical methods were employed for the production of sensitization. The first edematous reaction was obtained in Ann Arbor, Michigan, on March 21, 1936. Prior to this date one lot of rats had failed to react to light exposures on three different dates. The amounts of lechuguilla extract which had been fed these rats were later proved sufficient to sensitize animals, without exception, during the late spring and summer months. The edematous reactions which were produced during the latter part of March and through April were not sufficiently extensive to be detected for at least 12 hours after the exposure, and in many cases were not detected until the morning of the following day. During this period two to two and one-half hours was sufficient exposure to complete the reaction; additional exposure would neither increase the extent of the reaction nor hasten its appearance. By the middle of May an exposure of two hours was sufficient to produce a visible swelling of the face by 5 P. M. of the same day; by 10 P. M. the maximum swelling for a single light exposure had occurred. As stated already, the first reaction was invariably intensified by an exposure on the following day. Furthermore, the reactions obtained during the summer were more extensive than those obtained during the early spring months. With the scene of operation shifted to Alpine, Texas, with the same methods of sensitization, and with some of the same rats, the reactions produced during July and August were identical to those obtained in Ann Arbor in May and June. However, by the latter part of September the speed and extent of the reactions had decreased until it was the same as that observed in Michigan in March and April. By the middle of October three hours' exposure produced a marked erythema and slight lacrymation but no injury to the blood vessels. Similar results were also obtained as a result of five hours' exposure on December 9 and 10, an exposure which was found sufficient in the case of a sensitized goat and sheep to produce marked edema of the face and ears.

DISCUSSION

The photosensitization of the lower animals which is produced by the ingestion of certain plants is a complicated reaction in all events but especially so in the case of ruminants when associated with hepatopathy. With obstacles to overcome such as variable light conditions, resistance, individual susceptibility, and the desensitizing action of the toxic saponin, as was demonstrated in the work with rats, it is not surprising that some difficulty should be experienced in reproducing the photodynamic aspect of lechuguilla poisoning. The surprising feature is the number of such cases that occur under natural conditions. From the results of the investigation it is evident that certain definite conditions must be fulfilled before a visible photosensitization can be produced. The nature of these requirements is not fully understood at this time and constitutes a problem for future investigation. One of the requirements would appear to be that sensitization and exposure to adequate light energy must be concurrent, or very nearly so, in order to obtain the injury to the blood vessels. If the sensitization is slow in developing or if considerable exposure to direct sunlight has occurred during the development of the sensitization, the tendency is to produce resistance rather than the disease.

The fact that edematous swellings in the natural occurrence of lechuguilla poisoning are not always observed may be due to the early reabsorption of the edematous fluid or to the development of resistance without injury to the blood vessels. The fact that the photodynamic aspect of the disease is of more frequent ocurrence between March and October than it is for the remainder of the year suggests that light conditions during the winter months are more favorable for the production of resistance than for the development of a visible reaction.

The toxic saponin was found to be non-photodynamic but it is possible that an indirect photosensitization may occur in lechuguilla poisoning since the toxic saponin produces an obstructive jaundice. Quin (31) experimentally produced obstructive jaundice leading to photosensitization by ligation of the bile ducts of sheep and goats. This photosensitization was shown by Rimington and Quin (35) to be due to the appearance of phylloerythrin in the blood as a result of the obstructive jaundice. Two sources of sensitization are therefore possible, the primary sensitization produced by the photodynamic agent in the plant and a secondary condition produced by phylloerythrinemia as a result of the hepatopathy. Assuming that such a condition prevails, resistance to sensitization by one agent would be expected to include resistance to the other, since in the work with rats, resistance to the photodynamic action of lechuguilla was found to include resistance to the photodynamic action of eosin, and vice versa. It should be mentioned that the photodynamic aspect of this disease is of minor importance as the mortalities are due to the toxic action of the saponin, especially in relation to the kidneys.

Lechuguilla contains a principle which is not dependent upon hepatopathy for its photodynamic action, but a chemical identification of this principle was not attempted. The extracts had a brownish-red color after removal

or destruction of the saponin and upon exposure to ultraviolet light exhibited a greenish-yellow fluorescence. In their investigation of a similar disease Rimington and Quin concluded that the photodynamic agent in "geeldikkop" was phylloerythrin, but the action produced by this agent is dependent upon hepatopathy. They listed four spectral absorption bands for phylloerythrin (6352, 5950-5898, 5580, 5266-5123 A. U.) and gave special attention to the band in the region of 5580 A. U. In comparing the absorption bands of this agent with the location of the activating light in lechuguilla poisoning, the possibility of phylloerythrin being the photodynamic agent in this disease can not be excluded. The activating light in this condition being of greater wave length than 5328 A. U., the spectral absorption of phylloerythrin below this wave length is excluded. Light in the region of 5580 A. U. was freely passed by the green filter B, the same being true in the region of 6352 A. U. in the case of the red filter, but both of these filters absorbed most of the activating light. This leaves for consideration the spectral absorption of phylloerythrin in the region of 5950-5899 A. U., the region which appears to be the most essential, from the present investigation, if the photosensitization of lechuguilla poisoning is produced by a single band of light and if phylloerythrin is the photodynamic agent. However, the present investigation did not exclude the possibility that the activating light is a combination of two or more widely separated bands. Since acridine yellow permitted the passage of light above 5328 A. U., the light passed by this filter included the three spectral absorption bands of phylloerythrin of 5580 A. U. or greater. The fact that reactions equal to those of unfiltered light were obtained by filtration with acridine yellow may be due to the action of two or more specific absorption bands, whereas the milder reactions obtained by an over exposure beneath the red and green filters may be due to the action of a single band of specific light. As the activating light for fagopyrism was found to be the same as for lechuguilla poisoning and since hepatopathy is not a part of the clinical picture of fagopyrism, it would appear that the photodynamic agent in these two diseases is the same and probably not phylloerythrin.

The toxic saponin in lechuguilla does not exactly conform to Rosenthaler's (37) classification of saponins which was based on solubility. He divided the saponins into four groups: (1) soluble in water and 60 per cent alcohol, insoluble in strong alcohol; (2) soluble in water and strong alcohol; (3) soluble in water and methyl alcohol; (4) insoluble in water but soluble in 70 per cent alcohol. On the basis of extraction the saponin in this case satisfies the requirements of all four groups. The fact that the water extract was less toxic than the alcoholic extract would appear to indicate that it was more soluble in alcohol than in water. However, the water extract may have contained a number of extractives which were insoluble in alcohol, thus reducing the percentage of the toxic principle in the extract. The solubility of the saponin in question appears to have been influenced by the presence of other extractives, as the crude alcoholic extract was freely soluble in water, but in the more or less purified state it was sparingly soluble in this solvent. The purified product was sparingly

soluble in strong alcohol, and freely soluble in 70 per cent ethyl alcohol and in methyl alcohol. From these results it appears that there must be considerable overlapping of the solubility of the members of the four groups, or that the purified product was a combination or two or more saponins. The methods employed in this investigation would not exclude this possibility, but the evidence at hand favors the classification of the saponin in either group three or four.

The isolation of fluorescent pigments and the production of photosensitization by the injection of these pigments, or other plant extractives, into laboratory animals does constitute significant evidence of a relationship of the extractives to the natural ocurrence of a photodynamic disease. Several investigators have isolated photodynamic pigments from plates which do not produce photosensitization when ingested by the lower animals. In the natural occurrence of the photodynamic diseases of the lower animals the normal portal of entry is through the digestive tract, and in order to show that an extractive from any given plant is related to the natural occurrence of the disease it must be administered by this route and not by subcutaneous or intraperitoneal injection.

Johns, Chernoff, and Viehoever (46) isolated a saponin from lechuguilla which they found to be toxic for fish. However, from a study of their report it is evident that this is not the toxic saponin responsible for lechuguilla poisoning of domestic animals. Among other reasons for this conclusion is the fact that the saponin which they isolated was not precipitated by either lead acetate, barium hydroxide or cholesterol.

SUMMARY

The disease of sheep and goats known as "lechuguillaed," "swellhead," and "goat fever" is described. This condition was produced by feeding the leaves of the lechuguilla plant and extracts thereof. The complete clinical picture of lechuguilla poisoning consists of the combined action of two toxic principles, one a photodynamic agent, the other a hepato-nephro-toxin. The photodynamic agent was not identified, but the hepato-nephro-toxin was found to have many of the properties of a saponin and was not photodynamic. Mortalities from this disease are principally due to the action of the toxic saponin, especially in relation to the kidney, and not to the photosensitization.

The toxic principles were extracted from the plant with both water and alcohol, but of the two solvents alcohol proved to be the most satisfactory for the extractions. Hydrolysis of the extract destroyed the toxic saponin but not the photodynamic agent.

Rats were found to be susceptible to both toxic principles and in some respects the most satisfactory and economical of the experimental animals. Small doses of the alcoholic extract had a greater photodynamic action on rats than large, toxic or sub-toxic doses. Destruction or neutralization of the toxic saponin resulted in photodynamic action from large doses of the extract. Resistance to photosensitization was observed.

The activating light for the photodynamic action of lechuguilla poisoning

was found to be of greater wave length than 5328 A. U. Evidence is presented which suggests that light in the region of the sodium lines is the most important. Greater light energy was required to produce the lesions of photosensitization in rats than in sheep or goats. This condition could not be produced in rats earlier than March 21 nor after October 15, but was reproduced in sheep and goats as late as December 10.

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