Time.	Injection, %.	Dose, Cc.	Rise, Mm.
9.55	Propadrin 1	0.1	22
10.45	Ephedrine 1	0.1	18
11.30	Propadrin 1	0.1	12
12.30	Ephedrine 1	0.1	12
13.30	Propadrin 1	0.1	12
14.50	Ephedrine 1	0.1	12

After the first two injections the effects agree exactly. Even including them, the ephedrine effects average 14 mm., the propadrin 15 1/3, the results being within 10 per cent.

These protocols illustrate the close agreement in quantitative action and stability of propadrin and ephedrine.

SUMMARY AND CONCLUSIONS.

1. Of the four methods used for the quantitative physiologic assay of propadrin and ephedrine, namely, the effect on the pupil, the effect on the isolated uterus, the comparison of the pressor action with that of epinephrine and the comparison of the pressor action with that of a standard solution of the same drug, only the last was found to be suitable.

2. By the method described, assays can be made on one or two dogs with an accuracy of 20 or even of 10 per cent.

3. Propadrin is of approximately the same activity as ephedrine and is equally stable in solution.

A STUDY OF SENECIO RIDDELLII.*

BY F. S. BUKEY AND R. W. CUNNINGHAM.[1]

PART 1.

INTRODUCTION.

A number of Senecio such as *S. integerrimus, S. riddellii, S. vulgaris, S. ilicifolius, S. burchellii, S. latifolius, S. jacobœa, S. aureus, S. præcox, S. canicidia, S. albicaulis, S. grayanus, S. cervariæfolius* and many others, have been found in many parts of the world. *A ureus* is official in the N. F. V and has enjoyed some use as a drug. It was employed by the Indians as a vulnerary and later was used as an emmenagogue. Some of the above-mentioned species have been used as purgatives, emetics and internal hemostatics, although of doubtful value in most cases.

It has been found that some of these species are poisonous to animals. Debrierri (1) reports the poisonous effects of canicidia on dogs. Gilruth (2), (3), (4), (5), (6), (7), (8) proved the toxicity of *S. jacobœa* in producing cirrhosis of the liver (Winton disease) in horses of New Zealand. Chase (9), Robertson (10), Dixon (11) and Theiler (12), (13) succeeded in proving *S. latifolius* and *S. burchellii* to be the cause of hepatic cirrhosis or Malteno disease of South Africa. Stockman (14), Thompson (15), Craig and Kehoe (16), Stanley (17), Knowles (18), Leychon (19), Reeks (20) and Rutter (21) all report cases of poisoning by *S. jacobœa* among the

* Scientific Section, A. PH. A., Toronto meeting, 1932.

[1] The authors wish to express their thanks to Dr. L. Van Es of the department of Animal Pathology, University of Nebraska, for his coöperation and assistance in this study.

live stock of the British Isles. As a result of their work, the government made provisions for the extermination of the plant (22).

Wilmott and Robertson (23) in 1920 give an interesting report of a number of cases of hepatic cirrhosis in man. They attribute the disease to the presence of seeds and other parts of *S. burchellii* and *S. ilicifolius* in the wheat used for bread making. The disease required from three weeks to two years to run its course. Nearly always death resulted. The malady produced nausea, vomiting and severe abdominal pain, with occasionally diarrhea and passing of blood. Post mortem disclosed a discolored cirrhotic condition of the liver and in some cases ulcers of the digestive tract were noted.

Pictou disease of Canada, an enzootic liver disorder affecting cattle, was found to be caused by *S. jacobœa* (24), (25), (26), (27), (28), (29), (30).

German investigators (35), (36), (37), (38), (39), (40), (41), (42), (43), (44), (45) describe an enzootic liver disorder of horses called "Schweinsberger disease" leaving scarcely any doubt but that it is similar to enzootic disorders mentioned in the other parts of the world.

In 1912 and succeeding years, western Nebraska ranchers reported great losses among horses from a new disease in these parts which became known as "walking disease." Adjacent parts of Colorado and Wyoming reported similar losses. It has been reported from the Pacific Northwest (32). A similar disease known as "bottom disease" was once prevalent in the Missouri valley and was described by Schroeder (33) and Smith (34) in 1893. The area concerned in Nebraska is table land of sandy loam soil having an altitude of 4000 to 5000 feet. The climate is characterized by relatively low rainfall (17 inches per annum) and free wind movement. This being a stock-grazing section, these losses from "walking disease" became of great economic importance.

The disorder in this section affected horses chiefly, although a few cases were reported among cattle (Van Es (31)). The affected animal separates from the others and becomes sluggish and drowsy. Usually the appetite fails and may become perverted. The animal may chew at the mangers, or eat dirt. This habit, and the manner in which affected animals push themselves against objects, are likely responsible for the bruises and lacerations about the mouth and head.

In the more advanced stages, there are signs of abdominal pain, general depression and inability to coördinate movements. Standing and walking are difficult and are accompanied by staggering. The animal's fetlock joints knuckle over and he stands with feet apart. Nervous disorders are also exhibited in the tendency of the animal to wander aimlessly about, walking through fences or over the rim of a canyon, hence the name "walking disease." There is a marked loss of weight and animals are very weak in the later stages. The course of the disease averages about four weeks.

Autopsies performed by Dr. Van Es and his co-workers established the liver as the chief point of attack. This organ was discolored and usually presented a pale brownish to a blue-gray cast. The whole organ was mottled in appearance. The tissue was more firm than normal tissue and resisted the knife. Upon histologic examination he found a typical necrobiosis. Much functioning tissue had been destroyed resulting in scar tissue and enlargement of many cells in an effort of com-

pensatory hypertrophy. All stages of destruction and hypertrophy were noted. Nearly all cases exhibited conspicuous hepatic hemorrhages.

Liver disturbances of this nature are not uncommon in higher mammals. Among the intoxicants capable of injuring liver cells and setting up reparative processes are spoiled foods, toxins from certain infections, poisonous plants, phosphorus, arsenic and others.

The work of investigators in other parts of the world casts suspicion upon plants and more specifically upon the *Senecios*. "Walking disease" of Nebraska showed marked resemblance to other diseases caused by the *Senecios*. Van Es (31) reports that samples of diseased livers obtained from certain other countries were identical in the character of the lesions with those produced by the investigators in Nebraska.

As the etiologic relationship of *Senecio* to enzootic liver disorders was well established in other parts of the world, it was strongly suspected here. Two species are found in the region involved: *S. integerrimus* and *S. riddellii*. Van Es (31) reports that feeding experiments with the former showed no evidence of liver lesions as produced in "walking disease." However, such experiments carried out with *S. riddellii* gave positive results.

S. riddellii is a perennial with a long tap root, from the crown of which arises a branched, very leafy stem from 12 to 24 inches in height. The leaves are from 1 to 3 inches long, and are pinnately parted into 3 and 9 narrow filiform segments. Each branch ends in a corymb of 8 to 20 flower heads. The plant is typically an arid type.

Kelly and Lynn (46) report the presence of alkaloids in *S. aureus*. Grandval and Lajoux (47) working on *S. vulgaris* describe two alkaloids present which they call senecionine and senecine. Müller (48) isolated an alkaloid, fuchsisenecionine from *S. fuchsii* and another from *S. sylvaticus*. Watt (49), (50) in 1909 extracted two alkaloids, *senecifolins* and *senecifolidine* from *S. latifolius*. Cushny (51), (52), (53), (54) found that these alkaloids were toxic and experimentally produced symptoms comparable to those found in the field cases of poisoning among horses. He concludes that enzootic liver disease among horses reported from Africa, Canada and New Zealand was probably caused by these or similar toxic principles in some specie of Senecio.

It can be concluded from the foregoing discussion that several of the *Senecios* contain toxic principles. The work of Dr. Van Es has shown *riddellii* to be toxic to horses. The literature apparently contained no account of experimental work having been done upon the toxic principle of this species. This paper constitutes the first part of a study being carried out upon the isolation and identification of this substance, and the determination of its physical, chemical and pharmacological properties.

EXPERIMENTAL.

The material for this problem was gathered in Scotts Bluff county, Nebraska, during the latter part of June 1931. This was several weeks previous to the time of flowering. The plants were air dried and the leaves and stems separated from the roots before grinding. The material was ground in a disintegrator so that all of it would pass through a 40-mesh sieve.

The percolator used in extracting the material was made from a ten-gallon cream can. A hole was drilled in the bottom of the can and a three-eighths inch pipe and stop-cock were welded into this opening. This made an ideal percolator as the top might be closed during maceration, diminishing the solvent loss.

About twenty pounds of the ground leaf and stem material were packed in the percolator and macerated with ether for twenty-four hours. The ether was then drawn off and the process repeated. At the end of the third extraction most of the waxes and the color had been removed. The ether was reclaimed for other extractions. The residue was a deep green waxy substance. Without drying the material in the percolator, hot ninety-five per cent alcohol was added and the above procedure repeated until the plant was exhausted. This required from four to five extractions for completion. Eighty per cent of the alcohol was next removed from the extract under reduced pressure, keeping the temperature under 70° C. for several days. The concentrated extract was placed in an evaporating dish and, on exposure to the air for several days, brown crystals formed. Several crops of these were obtained on further standing. These crystals, on repeated recrystallization from hot absolute alcohol, gave a buff-colored powder which had a m. p. 156° C.

The residue from the crystallization was a black tarry mass which became solid in a few days. This material was found to be insoluble in both water and alcohol in which it was formerly quite soluble. Further work is in progress on this material to determine its composition.

Through the coöperation of Dr. Van Es of the department of Animal Pathology, University of Nebraska, the toxicity of the substance melting at 156° C. was determined.

Two horses received daily an amount of the crystals approximately equivalent to 4 pounds of green plant. This quantity was comparable to the amount of green plant which gave positive results in previous experimentation by Dr. Van Es (31) in determining the cause of walking disease. Each horse received a total of 16 doses.

The substance of Dr. Van Es's report follows:

About ten days after beginning the feeding, the first horse showed a failing appetite. A gradual loss in weight was noted and in fifteen days' time the animal was manifestly ill. He refused feed and occasionally showed evidence of abdominal pain. He became stupid and died early on the twentieth day.

The second horse exhibited similar symptoms. This subject showed diarrhea and also presented symptoms of cerebral origin. On the twentieth day the animal staggered when induced to move, and had great difficulty in standing. Abdominal pain was evidenced. The subject died on the evening of the twentieth day of the experiment.

Post mortem examination upon both animals showed excessive abdominal fluid and great changes in the livers and kidneys. The liver was greatly enlarged and showed a mottled surface. There was evidence of hyperemia and hepatic hemorrhages.

Histologic examination of the livers of both animals indicate necrobiosis with the beginning of interstitial thickening. Necrosis was especially noted in one case. The kidneys showed severe nephrosis.

Dr. Van Es concludes as follows:

"The dry alcohol extract of *Senecio riddellii* was toxic to the two horses fed with it and caused their deaths. The intoxication produced, for so far as this can be measured by patho-anatomic observation of the livers, was more severe than that usually observed in field cases of 'walking disease.' We attributed this to the greater concentration of the intoxicant. The symptoms of the two horses are comparable to those seen in the field disorder and whatever differences observed were traceable to the manner in which the animals were kept.

"Renal involvements rarely seen in the field cases were, no doubt, caused by the excessive amount of the intoxicant used in the experimental horses. In our experiment with green plants the same phenomena could be observed (31).

"There is warrant for conclusion that the intoxicant factor in the dry extract is of the same character as that which causes the 'walking disease' of Northwestern Nebraska."

Due to the small quantity of the toxic substance present in *S. riddellii* it was necessary to extract large amounts, to satisfy the needs, in this type of animal experimentation. During the summer of 1931, more than 1200 pounds of the plants were collected. This represented a little over 300 pounds of dry material. In July 1932, an additional 1200 pounds of plants were collected. Most of the extract of the first collection was used for animal experimentation. With the toxic principle in a fairly pure state, work is in progress to determine its composition. Further study is being made as to the quantity of toxic principle present in the plant at various seasons of the year. It is thought by some that the young plants in the early spring are the most toxic. However, this point has not been definitely proved. Our next paper will constitute the chemical study of *S. riddellii*.

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A COMPARATIVE STUDY OF TWO XANTHINE DIURETICS.*

THEOPHYLLINE SODIUM ACETATE AND THEOBROMINE SODIUM SALICYLATE.

BY A. RICHARD BLISS, JR.,[1] AND ROBERT W. MORRISON.[2]

INTRODUCTION.

No reliable quantitative clinical experiments on the comparative diuretic effects of the various Xanthine derivatives in graduated doses have been reported in the literature, and, with the exception of one quantitative report in German and the reports of the authors of this paper, there are no published reports concerning the actions produced in laboratory animals by graduated doses of the newer Theobromine and Theophylline derivatives. This investigation was prompted by numerous inquiries concerning the diuretic powers of these agents, and this paper is the second of a series of reports concerning comparative studies of Theobromine and its derivatives "Theocalcin" (Theobromine Calcium Salicylate) and "Diuretin" (Theobromine Sodium Salicylate), and Theophylline and its derivatives "Phyllicin" (Theophylline Calcium Salicylate) and "Theocin Soluble" (Theophylline Sodium Acetate).

Although Xanthine possesses very little diuretic power, certain derivatives containing the Xanthine nucleus are among the most powerful of the diuretics. Of the dimethylxanthines Theophylline and Theobromine are the most active, while Caffeine is the most active of the trimethylxanthines.

Because of its pronounced central effects, Caffeine has been more or less supplanted as a diuretic by Theophylline and Theobromine which have not its strong central effects, and which are credited generally with more powerful diuretic actions. Some writers consider Theobromine to be more reliable in action and less prone to

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* Scientific Section, A. PH. A., Toronto meeting, 1932.

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