A STUDY OF THE CAUSE OF TREMBLES (IN DOMESTIC ANIMALS) AND MILKSICKNESS (IN MAN).*

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As early as 1820, there appeared in medical literature descriptions of the disease called milksickness. For nearly a hundred years these descriptions continued to appear. Their number runs into the hundreds. However, the writers of these articles confined their attention to the description of the symptoms of the disease. It is only within the past few years that any work has been done to determine the cause of the disease.

The early settlers of Indiana, Ohio, Illinois, Kentucky, North Carolina and adjoining States were severely affected by the malady. As the lands were brought under cultivation the disease became less prevalent, but the outbreaks of it continue in certain parts of these States. It has been a limiting factor in the production of beef and dairy products in some of the above States even as late as five years ago. As late as 1923, domestic animals have died from it in Indiana.

It has now been fairly well established that the so-called milksickness (in man) and trembles (in domestic animals) is due to white snakeroot, *Eupatorium urticæfolium*, which is synonymous with *Eupatorium ageratoides*. (For confirmation of this see *Bulletin* No. 270, Purdue University Agricultural Experiment Station, and *Technical Bulletin* 15, North Carolina Agricultural Experiment Station.) Feeding experiments have been conducted by the Purdue University Agricultural Experiment Station staff and animals have died with the typical symptoms of the disease. Therefore there is no doubt in the minds of the workers here regarding the cause of this disease.

Little work has been done to determine the toxic principle of the plant. Dr. E. L. Moseley[2] studied this problem and from feeding experiments with rabbits and concluded that aluminum phosphate caused this disease. Later Moseley[3] (1917) concluded that the resin of white snakeroot caused the disease. Many feeding experiments have been conducted, but, beyond the work of Moseley, little has been done to determine the toxic principle of this plant. This study was begun for the sole purpose of determining if possible the toxic principle or principles. The Purdue Experiment Station Staff was interested in feeding experiments, but the one question that concerned us was "What is the toxic principle?" and all our experiments were directed towards this part of the problem.

The material for this work was supplied to us by the workers in the Purdue Experiment Station. It consisted of the whole plant above the ground including stems, leaves, and flowers. The plants were collected in the autumn before frost (from the middle to the end of September), while the plants were in bloom and before seeds were formed. They were collected on a tract of land about two miles northwest of Purdue University known as "McCormic Woods." These

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[1] From the laboratories of the Purdue University School of Pharmacy.

[2] E. L. Moseley, "The Cause of Trembles and Milksickness," *Med. Rec. (N. Y.)*, 75, No. 20, 1909.

[3] Moseley, "Milksickness and Trembles Caused by Resin of White Snakeroot," *Ibid.*, 92, No. 10, 1917.

woods are rather dense and the soil is rich and plentifully supplied with leaf mould. The wood abounds in large oaks, beeches, walnut and hickory trees. It has not been pastured for several years because it is known that poisonous plants grow there.

Dr. E. G. Campbell, botanist for the Purdue University School of Agriculture, carefully identified these plants as *Eupatorium urticæfolium*. We considered this necessary because there are known to be about forty species of this genus in the United States and *urticæfolium* is the only one that has been proved to be poisonous to grazing animals.

EXPERIMENTAL PART.

First Experiment.—A considerable quantity of the whole dried plant (except the roots) was reduced to a fine powder. One hundred-gram portions of this material were moistened with six different solvents noted below, allowed to macerate for twenty-four hours, and than packed in ordinary drug percolaters and percolated slowly with the selected menstrum as follows:

(*a*) U. S. P. diluted alcohol (50%)
(*b*) U. S. P. alcohol (95%)
(*c*) 70% alcohol
(*d*) 1/2% aqueous solution of hydrochloric acid
(*e*) Equal parts of ether and chloroform
(*f*) Diluted alcohol containing 1/2% hydrochloric acid

(*a*) The fluid extract from (*a*) was evaporated to dryness on a water-bath. Considerable residue was obtained. This was taken up in 100 cc. of water and this aqueous extract was given to a rabbit by means of a stomach tube. No symptoms were shown in the rabbit.

(*b*) The fluid extract from (*b*) was evaporated on a water-bath and considerable residue was obtained. An attempt was made to dissolve this residue in 100 cc. of water. This was a failure, due, perhaps, to the fact that it contained so much resin. The aqueous extract was given to a rabbit by stomach tube and the resinous residue by capsule. No symptoms were shown in the rabbit except a slight loss in weight.

(*c*) The fluid extract from (*c*) was evaporated to dryness on a water-bath, taken up in 100 cc. of water and given to a rabbit by stomach tube. No results were observed except a slight loss of weight.

(*d*) The fluid extract from (*d*) was evaporated nearly to dryness on a water-bath. Considerable residue was obtained. This was taken up in 100 cc. of water and given to a rabbit by stomach tube. The rabbit died in four (4) hours; but no symptoms of trembles were detected. Postmortem showed severe irritation of the stomach walls and corrosion. Upon investigation we found that all of the hydrochloric acid had not been evaporated and that death was caused by the high acid content of the aqueous extract.

(*e*) The fluid extract from (*e*) was evaporated on a water-bath and a large amount of resinous residue was obtained. This was insoluble in water, therefore it was taken up in capsules and given to a rabbit. No symptoms were apparent.

(*f*) The fluid extract from (*f*) was evaporated to dryness on a water-bath. A large amount of residue was obtained. This was taken up in 200 cc. of water, carefully neutralized by sodium bicarbonate, and given to a rabbit by stomach tube. No effects were apparent except that the animal was very thirsty.

In all of these experiments the temperatures of the animals were taken at regular intervals and analyses of the urines were made. Therefore, if toxic principles were present in the residues, they would have been detected. We concluded from this series of experiments that we either had not extracted the toxic principle (which we now know is not true) or that the heat of the water-bath was sufficient to decompose the toxic principle. Later experiments have proved that the heat of the water-bath destroys the toxic principle. Workers at the Purdue Experiment Station have verified this by feeding experiments and we believe that it is definitely determined that the temperature of a water-bath for a few hours is sufficient to decompose the toxic principle.

At this point a consultation was held with the workers on feeding experiments in the Purdue Experiment Station. It was decided to continue research along this line, but not to use heat on our extracts and to increase the amount of drug given to each rabbit.

Second Experiment.—Five hundred (500) grams of the leaves and flower buds were ground to a fine powder. The powder was moistened with water containing $^1/_{10}$ of 1% of hydrochloric acid, allowed to macerate 24 hours, and packed in a percolater and slowly percolated with this menstruum.

We began giving this fluid extract to a rabbit by stomach tube. After receiving this fluid extract from approximately 150 grams of the drug, the rabbit died. The following data were secured:

Weight of rabbit—2225 grams. Respiration, heart, and temperature—normal at the beginning of the experiment.

Feb. 13, rabbit was given 100 cc. of above-described fluid extract.

Feb. 14, rabbit was given 300 cc. of above-described fluid extract.

Feb. 15, at 9 A.M. rabbit was given 135 cc. of above-described fluid extract.

Animal showed signs of toxicity resembling the therapeutic action of this weed on cattle and sheep. Rabbit died at 12:15 P.M. Autopsy was performed immediately with the following results:

Heart—dilated, pale in color, muscles pale. *Lungs*—lower lobe showed areas of deep red color indicating hyperemia, otherwise entire lung normal. *Stomach*—strong odor of drug upon opening. Lining showed slight irritation. *Intestines*—The veins seem distended, especially those of the small intestine. *Adrenals*—normal. *Kidneys*—Capsule stripped easily leaving a mottled surface; pale; boundary between cortex and medulla very much congested. The kidneys appeared to be slightly larger than normal. *Brain*—normal. *Spleen*—dark in color, otherwise normal.

Two hundred grams of the marc from this percolation were fed to a hen with no apparent effect. This would indicate that the $^1/_{10}$ of 1% aqueous-hydrochloric-acid menstruum extracted all the toxic principle of the drug.

Another portion of this fluid extract was tested for the presence of alkaloids. None were present. A third portion was tested for glucosides. There was a *very* slight reduction of the Fehling solution, but not enough to indicate the presence of glucosides. These experiments were repeated with the same results. Therefore we believe that the toxic principle is neither an alkaloid nor a glucoside.

Third Experiment.—Another 500-gram portion of the ground drug was macerated and percolated with a $^1/_{10}$ of 1% aqueous solution of HCl. We were unable to give this fluid extract to a rabbit for a day or two after percolation. When we

were ready to administer it, we noticed a pronounced fermentation odor which would indicate that the fluid extract had undergone some process of fermentation. The thought occurred to us, "Does fermentation destroy the active principle of the drug?" To determine this point, sufficient fermented fluid extract to represent 263 grams of drug was given to a rabbit with no effect. In fact the animal gained weight. This experiment was repeated with the same result. Therefore we concluded that fermentation destroys the active principle of the drug.

Fourth Experiment.—The finely ground drug was again macerated and percolated with $^1/_{10}$ of 1% of aqueous HCl as in Experiments 2 and 3. This fluid extract was "shaken out" with petroleum ether, next with chloroform, and finally with anhydrous ether. Each of these solvents was evaporated in a current of air and little or no residue was obtained from either of them. It is interesting to note, however, that very disagreeble emulsions were formed with both the chloroform and anhydrous ether menstruums. There may be saponins in the plant and these may have caused the emulsions.

The above fluid extract was made alkaline and "shaken out" with ether, petroleum ether, chloroform, toluene, and amyl alcohol. All of these solvents were evaporated in a current of air, but no residue other than slight stains was obtained.

Fifth Experiment.—Volatile Oil Experiment. Several grams of the drug were moistened with water and distilled with steam. A small amount of a volatile oil lighter than water was obtained. This volatile oil had a distinct odor of pinene. Time did not permit us to determine whether this volatile oil was toxic or not.

Another portion of the ground drug was percolated with the same menstruum ($^1/_{10}$ of 1% HCl) used in Experiments 2, 3, and 4. This fluid extract was made distinctly acid with strong HCl for the purpose of decomposing any glucoside if present and testing for same. As soon as the strong acid was added, a pronounced apple-like odor was noted. This made us think that there might be some constituent freed or changed in some way by the acid, giving a new volatile constituent which produced this apple-like odor.

To determine whether the above was true or not, 200 cc. of this fluid extract were then treated with strong HCl and distilled in a partial vacuum and the distillate collected in a container cooled with ice. About 1 cc. of a volatile constituent (either a volatile oil or ester) was obtained. This volatile constituent was heavier than water, rather thick, and of yellowish brown color. It has a penetrating odor which to some was nauseous and seemed to cause a slight dizziness, while to others it was a pungent, pleasing odor. A suspension of this constituent in water gave the agreeable apple-like odor previously mentioned. This oily-like volatile constituent was shaken with sodium carbonate to neutralize any acid and then washed with water. It was then injected into the ear vein of a five-months old rabbit, and no toxic results were apparent.

An attempt was made to secure more of this volatile constituent in order to determine whether it was toxic if given by way of mouth. We were able to liberate a sufficient amount of it to give the pronounced odor, but, when we distilled it, we were unable to secure more than a suspension of it in water. This suspension was given to a rabbit by way of mouth, but no toxic symptoms were apparent.

We attribute our failure to secure a second portion of this volatile constituent to the fact that our fluid extract was not sufficiently concentrated as was perhaps our

first one. Time did not permit a repetition of this experiment to determine this point. We believe this part of the work worthy of further investigation.

Sixth Experiment.—Four hundred grams of the crude drug were shaken for an hour with acetone, the drug expressed and the acetone filtered. This acetone extract was evaporated in a current of air to avoid any possibility of decomposition by high temperatures. A heavy resinous residue was obtained. To obtain the pure resin, this residue was taken up in absolute alcohol and the alcoholic solution poured into water. This gave a very fine colloidal suspension of the resin, which, was unfilterable with our best laboratory filters. This colloidal suspension of the resin was acidified with HCl and a flocculent precipitate of the resin was obtained. This was filtered out and washed free from acid. The filtrate (A) and washing were reserved for future tests. The filtered acid-free resin was dissolved in the least amount of absolute alcohol and this solution poured into water, giving us a suspension of the *pure* resin in an hydroalcoholic menstruum. The alcohol was evaporated in a current of air, leaving a fine aqueous suspension of the resin. This resinous suspension was given to a rabbit by stomach tube, but no toxic symptoms were observed, indicating that the resin is not the toxic principle.

The filtrate (A) was tested for the presence of aluminum salts and a heavy gelatinous precipitate of $Al(OH)_3$ was obtained, thus confirming the reports of previous workers regarding the presence of aluminum salts in the drug. The toxic principle cannot be due to inorganic aluminum salts or it would not have been destroyed by heat and fermentation. It is possible that there are toxic organic aluminum compounds present which are decomposed by heat and fermentation. We believe that this is worthy of further investigation.

CONCLUSIONS.

We believe that the above experiments have demonstrated the following facts:

1. That the toxic principle of the plant is destroyed at the temperature of the water-bath.

2. That an aqueous menstruum containing $^1/_{10}$ of 1% of HCl extracts the toxic principle from the plant.

3. That there is neither alkaloid nor glucoside present in the plant.

4. That the fluid extract with the $^1/_{10}$ of 1% HCl menstruum readily ferments and this fermented fluid extract is non-toxic.

5. That the following menstruums: petroleum ether, ether, chloroform, amyl alcohol, and toluene do not shake out from either the acid or alkaline fluid extract of the plant more than the slightest of residues, in most cases mere stains.

6. That by steam distillation of the plant a volatile oil is obtained having the odor of pinene.

7. That the addition of strong hydrochloric acid to the fluid extract of the plant produces a volatile constituent with a pronounced apple-like odor. This constituent is heavier than water, yellowish brown in color. We believe that this is worthy of further investigation.

8. That the drug contains a great deal of resin, but the pure resin is not the toxic principle.

9. That the drug contains considerable aluminum salts, but the toxicity of the drug is not due to inorganic aluminum salts.