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STUDIES IN EXPERIMENTAL TREMBLES OR MILK-SICKNESS[1]

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In the fall of 1931, six members of the Moss family residing near Baxter, Tennessee, were afflicted with milk-sickness. Four of these six died from the disease and after the death of the fourth, the remainder of the family became thoroughly frightened and came to the Vanderbilt hospital for examination and treatment. It was thus that our interest in this disease was aroused and we decided to do some experimental work in the hope of a better understanding of the syndrome and the possibility of suggesting more effective treatment.

Milk-sickness, though at the present time a fairly rare disease, was one of the chief scourges of the early settlers. It was common in Maryland, North Carolina, Kentucky, Tennessee, Alabama, Missouri, Illinois, Indiana, and Ohio. Epidemics frequently occurred which wiped out as much as one-fourth of the population of the area and in a few cases almost the whole population of certain villages succumbed to this dread disease. It was regarded with horror by the pioneers, because the attacks were so fatal, the cause so mysterious, and all preventive measures so unavailing. Frequently large areas were deserted because the "milk-sick," as it was commonly called, had been found to abound there. A typical epidemic in which the mother of Abraham Lincoln died is described by Nicolay and Hay, biographers of Lincoln (8).

The early settlers were also well acquainted with a disease of cattle known as the "trembles." It was noticed even by the earliest chroniclers that trembles existed only in the same areas, and during the same periods as human milk-sickness was prevalent. It was also observed that suckling calves and other animals, fed on the milk of cows which were permitted to range the woods, often developed trembles and died. The similarity of the attacks of these animals

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to human milk-sickness and the observation of the fact that both types of disease prevailed in the same area at the same time soon led to the conclusion that milk-sickness was caused by some deleterious substance present in the milk of the cows at certain seasons of the year. This conclusion also received support from the fact that all persons who refrained from eating milk or milk products in the late summer or the fall escaped the attacks of milk-sickness.

The nature of this substance present in the milk caused much speculation. In early days there was much superstition connected with this mysterious malady. In fact, even now, among the hill farmers in regions where trembles is still not infrequently seen, there is disagreement as to the cause and some rather absurd beliefs are held. As an illustration, some of the theories held by the farmers who reside near "Milk-sick Mountain" just south of Sparta may be repeated. This mountain has been fenced off for many years and it is well known by all farmers who live in the vicinity that animals which wander onto the mountain very frequently develop trembles. Human milk-sickness cases were reported in the region three years ago and the older settlers can remember when the disease was fairly common. One farmer told us that he was convinced that trembles was caused by "arsenic on lead" which "comes out the ground," presumably in a volatile form and "gets on the plants." Another said that cattle got trembles only on a foggy morning and that there was "something poisonous in the fog." Others believed that there was a poisonous dew which collected upon the plants. Another was fully convinced that the disease was caused by the animals eating "buckeye sprouts."

It is likewise interesting that several hypotheses have been advanced by scientific investigators of the subject. In 1909, Jordan and Harris (3) isolated from the excreta of animals sick with trembles, a bacillus which they termed the "bacillus lacti morbi" and which they believed was responsible for the disease. However, they later admitted that the facts did not surely point to this organism as the cause of the disease as it was found in the excreta of healthy animals and on plants which could in no way be connected with milk-sickness. The investigators were never able to produce typical trembles by inoculation with the bacillus.

It was noticed that, as the land was cleared and the cattle were confined in pastures, the incidence of the disease diminished. This led to the suspicion that feeding on a poisonous plant was the responsible factor. A large number of plants received the blame at one time or another but the one which has received the most widespread interest is *Eupatorium urticaefolium* (white snakeroot or richweed). In the past twenty-five years, the view that it is this plant which is responsible for trembles and milk-sickness has received a great deal of experimental support and this is the only hypothesis as to the cause of the disease which at present has any standing. Mosely (5, 6, 7), in a long series of experiments (1906-1917) was able to produce trembles by feeding the leaves of this plant, and a similar disease in animals which were fed on the milk of animals receiving the plant.

He also found that the meat of animals which died of trembles would produce trembles when fed to other animals. Similar results have been obtained by many observers.

Several efforts have been made to determine just what substance in richweed is responsible for its toxic properties. The most complete piece of work of this kind was published by Couch in 1927 (2). He fractionated the material very carefuly and tested the toxicity of the various fractions. He was able to obtain from richweed a viscous, oily liquid, having the characteristics of an alcohol, which he named "tremetol," which, when fed to sheep, produced typical trembles.

The author's chief interest in the problem lay in the metabolic changes which occur in the animal or person poisoned by richweed and in the resulting clinical symptoms. He has produced richweed poisoning in rabbits and goats. The symptoms seen in the goat are like those described as typical trembles in cattle and sheep. The first symptom noted is inactivity. The animal prefers to lie down and can only be made to get up and walk about by much prodding. After a day or two, the characteristic trembles gait is noticed. The hind legs become stiff, and the animal drags them in a peculiar manner when it is forced to walk. At about this stage, or a little later if he is made to exert himself, he begins to tremble violently all over and often sinks to the ground in a fit of trembling. Such an attack usually lasts only a few minutes. Between these attacks of visible trembling, fine tremors can be felt in the muscles, especially in the hind legs, though frequently the skeletal muscles of the whole body are involved. If the disease progresses. the animal becomes weaker and weaker and refuses to respond to any stimulation and finally dies in a state of profound depression. There is a rapid loss of weight.

The picture in rabbits is a little different. They rarely exhibit the fits of trembling although the fine muscle tremors can easily be felt and the limbs can often be made to tremble by holding them suspended. The most notable symptom in rabbits is profound weakness. They lie flat upon the abdomen with all four legs sprawled out to the sides. If placed in a sitting position, they will immediately slip back to the prone position.

Many observers have reported severe gastro-intestinal disturbances as accompaniments to the above symptoms. We have noticed only, in a few cases, a mild diarrhea.

The symptoms of human milk-sickness are in many ways similar. Mrs. Moss, the mother of the family which suffered from the disease last fall, had two typical and severe attacks. She described her symptoms as follows: For some time she had been feeling tired and weak. The acute attack, however, came on suddenly. She felt an extreme weakness and could hardly stand. She then began to vomit, and suffered from severe pains in her stomach and a feeling as though she were smothering. She became prostrated, semi-conscious and delirious and remembers none of the events of the next five days. The vomiting and smothering spells were frequent throughout this

period. As soon as the acute symptoms abated, she had a voracious appetite. She continued to have periods of extreme weakness. Whenever she got out of bed her feet and ankles became swollen.

The chemical changes which occur in the body after poisoning with richweed have not been very widely studied. The fact that animals or persons dying with the disease have a very noticeable sweetish odor to the breath, led to the suspicion that there is an acetone acidosis. When this substance has been tested for in the blood and urine, it has usually been found, especially late in the course of the disease. Bulger (1), in a series of experiments on rabbits found it practically always present in severely intoxicated animals. He also found that rabbits die from the disease with a profound hypo-glycemia. Walsch of Illinois (9) confirmed these findings on a number of patients, finding acetone in both the blood and urine and a low blood sugar. On this basis he prescribed as treatment, the use of soda, either by mouth or by vein, to combat the acidosis and the use of sugar as glucose by vein and as karo syrup by mouth to combat the low blood sugar. He found this treatment very effective.

The author was especially interested in the disease because the severe gastro-intestinal symptoms, the trembling and the extreme weakness, associated with a low blood sugar are similar to other conditions which have been studied in the laboratory during the past few years. Certain of the conditions showing these symptoms have been found to be associated with and probably in a large measure resulting from an increase of guanidine in the blood (4).

Guanidine is a nitrogenous compound with the formula $C{<}{=}{\begin{matrix}NH\\NH_2\end{matrix}}^{NH_2}$. Little is known about its source in the animal organism, but it seems to be a product of tissue breakdown and is extremely toxic. When it is administered artificially to animals, it produces vomiting and diarrhea, severe neuromuscular disturbances as tremors, convulsions, and loss of coordination. Death from guanidine poisoning usually follows a period of depression and coma which is accompanied by a profound hypoglycemia.

The same course of symptoms has been found to occur where guanidine is produced by the organism. Examples of such conditions are the acute liver injury which follows administration of carbon tetrachloride, chloroform, etc., and certain toxic conditions of infants, as acute diarrhea with extreme dehydration. If guanidine were found to occur in milk-sickness, a means of treatment would be at hand in calcium medication, since calcium and guanidine are antagonistic to each other and calcium has been found to be of great benefit in treating the other conditions mentioned where an accumulation of guanidine has been found (4).

It was planned to divide the experimental work into three parts: (1) the feeding of richweed to animals and a study of their blood chemistry; (2) the feeding of animals upon the milk of animals receiving richweed; and (3) extraction of "tremetol," the product in

richweed which Dr. Couch believes is responsible for trembles and its effect upon animals.

Considerable data upon the effect of feeding richweed have been obtained. The results of feeding the milk of poisoned animals, with one exception, have been negative, perhaps because it was difficult to force the animals used for producing the milk (cow and goat) to eat sufficient quantities of the weed. The author was not able to procure an extract of richweed which would produce characteristic symptoms. Hence, the conclusions here must be confined to results of the feeding of richweed.

The first specimens of weed which the author used were gathered from the Radnor Lake region about eight miles from Nashville. These weeds were fed to rabbits over a period of four weeks with the development of no symptoms. It was concluded that the weeds from this region must be either non-toxic or of a very low toxicity and it was arranged to have plants supplied from "Milk-sick Mountain" near Sparta where trembles is known to exist.

The richweed from that region was fed to nine rabbits and two goats and the expressed juice of a considerable quantity of it was fed to two dogs. Seven of the nine rabbits became severely ill with the symptoms described above after eating the weed for periods varying from three to ten days. One of the goats developed trembles but recovered when the supply of the weed gave out. Neither of the dogs showed any ill effects from the juice of the weeds, although fairly large quantities were given.

A study of the blood chemistry of the poisoned rabbits revealed the following abnormalities:

I. The observation by other workers of a low blood sugar was confirmed. The blood sugar fell from the normal level of about 100 mgm. per 100 cc. to from 30-40 mgm. per 100 cc. This fall occurs late in the course of the disease and seems to be a result of some other factor. It does not occur unless the intoxication is severe nor in the early stages of the illness. This fact may account for the failure of certain observers to find it.

II. There was a progressive retention of nitrogen, the N.P.N. frequently reaching a figure as high as 100-120 mgm. per 100 cc. before the death of the animal. Such figures are indicative of a severe nephritis.

III. There was an accumulation of guanidine in the blood which was progressive throughout the course of the intoxication. The normal level of guanidine in rabbits' blood is from 0.4 to 0.8 mgm. per 100 cc. In every case of intoxication, this figure was found to be increased and grew progressively higher until the death of the animal. At death the level ranged from 6 to 9 mgm. per 100 cc. If such a level of blood guanidine is maintained in rabbits by subcutaneous injection of guanidine salts, the animals will suffer from tremors, convulsions and coma. Guanidine interferes with carbohydrate metabolism so that a few hours after its administration the blood sugar falls rapidly. It seems certain therefore, that the amount of guani-

dine found in the blood of animals poisoned with richweed must be responsible for many of the symptoms of the illness, and is probably the primary factor in the production of the low blood sugars.

Because the antagonism of calcium and guanidine is well known and because of the author's success in the past in relieving symptoms due to guanidine by calcium medication, in a few cases the effect of the intravenous calcium gluconate was tried. In each case where this was tried there was a marked, though temporary, improvement noted in the condition of the animal—a prostrated animal becoming seemingly almost normal for a time after the injection. Injection of calcium resulted in raising the level of the blood sugar since one effect of calcium upon a guanidine-poisoned animal is to restore normal carbohydrate metabolism. Of course, clinically, the use of calcium medication would be supplemented by the use of glucose.

The one positive result from the use of milk from an animal receiving richweed was on a nursing kid whose mother was fed the weed. The kid was never allowed to eat the weed and subsisted entirely upon its mother's milk. After the mother had eaten richweed for five days, the kid developed the typical symptoms of trembles. A study of its blood chemistry showed a moderately increased guanidine, a slight fall in blood sugar and a small rise in the N.P.N. The richweed feeding to the mother was stopped and the kid recovered after about three days. While no conclusions can be drawn from this single experiment, it is felt that it indicates that the chemical changes which occur in an acute attack of milk-sickness are probably the same as those which occur in the intoxication produced by eating richweed itself.

From the present status of the author's results it may be concluded: (1) that an accumulation of guanidine is a complicating factor in acute richweed poisoning or trembles; (2) that this accumulation must contribute materially to the production of the severe symptoms of the intoxication and be responsible, in a large measure, for the low blood sugars found in this condition; and (3) that calcium medication should prove a valuable supplement to the treatment of trembles (and probably also milk-sickness) by alleviating the symptoms which are caused or aggravated by guanidine.

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MORRILL HALL BURNS

Morrill Hall at the University of Tennessee, Knoxville, Tennessee, burned to the ground on January 18. The building itself was old so that the loss of the building was not great, the building being estimated as worth only about $75,000. It housed the laboratories of the various departments of biology, classrooms for these subjects, and the offices of the various instructors in these fields. Dr. Jennison of the Department of Botany writes: "The personal losses sustained by individual workers is almost appalling. The material losses averaging, I should say, $5,000 apiece for the professors, is quite sufficient; but, after all, perhaps the most significant loss is represented in the destruction of manuscripts and collected data, of which every one of us had a considerable amount."

The State has suffered an irreparable loss in the destruction of the various herbaria deposited at the University in this building. The eight thousand specimens in the Gattinger Collection represented the life work of this pioneer Tennessee botanist. It constituted a fine memorial to Dr. Augustin Gattinger. Now it is gone and the only thing that remains is his library which is at George Peabody College for Teachers. The collection of the grasses of Tennessee by Lamson-Scribner, the collections of T. H. Kearney, S. M. Bain, Albert Ruth, and George Ainslie, were likewise destroyed. In addition the collections made by the professors now at the University and their students have all been swept away. Altogether more than fifty thousand specimens, including many types and several undescribed species, were destroyed. The immensity of this loss to Tennessee botanists is hard to conceive of. Here was the only adequate collection of Tennessee plants in the State. No other college or university in Tennessee has any herbaria to speak of. In no other place in the State could one go to compare his collections and check their identification.