and another ketone possibly beta-thujone. Acetic and formic acids are present and the presence of terpenes and sesquiterpenes is indicated.

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## THE HYPOGLYCEMIC PROPERTIES OF WHITE SNAKEROOT. (EUPATORIUM URTICAEFOLIUM.)

BY G. F. CARTLAND, F. W. HEYL AND E. F. NEUPERT.

It is now well established that the ingestion of white snakeroot by cattle leads to a condition known as trembles and that the use of milk from such cattle may cause milk sickness in man. A historical review of this subject is given in an article by Couch (1) who, as a result of animal experiments, has concluded that the toxic constituent of white snakeroot responsible for trembles is an unsaturated alcohol, tremetol.

That white snakeroot may contain one or more principles which affect the blood sugar is indicated by the fact that both a hyperglycemia and hypoglycemia have been reported in cases of poisoning with this plant. Couch (1) observed that in sheep poisoned with tremetol the blood sugar was markedly increased. On the other hand, Bulger, Smith and Steinmeyer (2) observed the development of hypoglycemia, even to the extent of convulsions and death in rabbits poisoned with white snakeroot. The experiments of these authors indicate a close relationship between the hypoglycemia and the toxic factor inasmuch as a lowered blood sugar was observed only in those animals which were obviously sick. The oral administration of dextrose produced marked improvement in most cases. Bulger, Smith and Steinmeyer regard hypoglycemia as an important symptom of white snakeroot poisoning and suggest the use of a high carbohydrate diet for treatment. These authors interpret the results of their preliminary experiments as indicating that such studies present a new field for the investigation of ketosis and of carbohydrate and fat metabolism. A tea prepared from white snakeroot has been used in some parts of the country for the treatment of diabetes under the belief that this plant contains an "insulin-like" substance.

The experiments here reported were conducted to investigate the "insulin-like" properties of white snakeroot with the object of finding out whether the changes in blood sugar following the oral administration of this plant are due to

a specific action upon sugar metabolism, or whether these changes may be merely secondary as a result of injury to the liver or kidney of the poisoned animal. Very early in this work it became apparent that the dried plant lacks the toxic constituents which are responsible for milk sickness and for the severe poisoning associated with the ingestion of the green plant. Similarly, the dried plant apparently lacks the principle responsible for the severe hypoglycemia noted by Bulger, Smith and Steinmeyer. Consequently our experiments agree with those of these authors in indicating a close relationship between the toxic and hypoglycemia factors since both are apparently destroyed by drying.

However, aside from the severe hypoglycemia sometimes associated with poisoning by the green plant, our experiments with extracts of the dried plant indicate the presence of a hypoglycemic substance which, although slight and irregular in its action upon the blood sugar of fasting dogs, was, nevertheless, deemed of sufficient importance to warrant further study. Further experiments concerned with the effects of oral administration of white snakeroot upon alimentary hyperglycemia indicated that the mildly hypoglycemic action of the extracts is exerted without toxic effects upon the liver or kidney. Attempts at isolation of an active fraction were unsuccessful. We obtained no fraction of greater activity than the entire 70 per cent alcoholic extract of the dried plant.

## EXPERIMENTAL.

The white snakeroot used in these experiments was collected from a "milk-sick" area during September 1929. The entire part of the plant growing above the ground was air dried and ground for extraction.

(*A*) *Effect of Alcoholic Extract upon the Fasting Blood Sugar.*—The dried plant was exhausted by percolation with 70 per cent alcohol. Immediately before use the alcohol was removed by distillation *in vacuo* below 60° and the residual emulsion made up to a volume such that 1 Gm. of dried drug is represented in 5 cc. of extract. This emulsion, called "Extract A" was administered by stomach tube to dogs in doses representing 5 to 20 Gm. of white snakeroot. The dogs were starved for 16 hours previous to the experiment and were allowed no food or water during the period in which the blood sugar was followed. Control experiments were performed using equivalent amounts of water substituted for the snakeroot extract. The results, summarized in Table I, are irregular but in two of the three cases in which the largest dose was administered a mild hypoglycemia was observed after 3 to 5 hours.

Although the doses of white snakeroot administered were as large as could be retained by the dogs without emesis, no toxic effects other than slight nausea were observed. This absence of toxicity together with the very mild lowering of the fasting blood sugar observed in our experiments indicate that our extracts do not contain appreciable quantities of the principle which causes the severe poisoning and marked hypoglycemia reported by Bulger (2).

Since these experiments established the absence in the dried snakeroot of a powerful hypoglycemic substance capable of reducing the fasting blood sugar when administered orally, we continued our experiments to see if there might be present a mildly hypoglycemic principle capable of reducing alimentary hyperglycemia. Since the slight hypoglycemia observed in the early experiments oc-

curred 3 to 5 hours after administration of the extract, a latent period of $4^1/_2$ hours was allowed between the administration of the white snakeroot and the glucose in the following experiments.

(*B*) *Effects of White Snakeroot upon Alimentary Hyperglycemia.*—As in the above experiments the dogs were starved for 16 hours. After taking the initial blood sample, the white snakeroot was administered in the form of Extract A by stomach tube. After a period of $4^1/_2$ hours a second blood sample was taken and immediately afterward a dose of glucose calculated according to body weight was administered orally, dissolved in 100 cc. of water. Blood samples were taken first at one-half hour intervals and later at hourly intervals and analyzed for sugars by the Shaffer-Hartmann (3) method, amino acid nitrogen by the method of Folin (4), and urea nitrogen by the method of Koch (5). Blood amino acid and urea nitrogens were followed as an index of liver and kidney damage. Red cell counts were made for the purpose of detecting changes in blood volume. No food or water was allowed during the course of the experiment. Control experiments were performed upon the same dogs using exactly the same procedure except that an equal volume of water was substituted for the extract of white snakeroot. In order to remove as far as possible variations in glucose tolerance due to variations in diet, the dogs were fed upon a diet of milk and whole-wheat bread for several days preceding and during the entire time in which the dogs were under active experimentation. In spite of these precautions, a considerable variation was observed in the hyperglycemia produced in different dogs and in the same dog at different times following the oral administration of constant amounts of glucose. The experiments are listed in Table II in series arranged in the order of their performance. Each series represents one or more experiments with white snakeroot extract paralleled by a control experiment upon the same dog. Thus, each series is considered as a unit in judging the power of the extract of white snakeroot to depress the hyperglycemia curve following the oral administration of glucose.

The results, summarized in Table II, show that white snakeroot administered $4^1/_2$ hours before the administration of glucose exerts a depressant action upon the alimentary hyperglycemia. In each of the four series of experiments the maximum rise in blood sugar following the oral administration of glucose is reduced by the previous administration of white snakeroot in the form of Extract A, as compared to the corresponding controls. The degree of this reduction shows considerable variation with equivalent doses of Extract A and apparently is not increased by increasing the dose of white snakeroot from 10 Gm. to 20 Gm. In those cases where the hypoglycemic action of white snakeroot is most pronounced, not only a lowering of the maximum blood sugar is observed but also a quicker return to normal.

The changes in amino acid and urea nitrogens of the blood are insignificant indicating the absence of any toxic action upon liver or kidney. The determination of urinary sugar during and immediately following the experiments failed to indicate any increase in sugar excretion, which also shows that the hypoglycemic action is not due to a lowered renal threshold. The red cell counts fail to indicate changes in blood volume of any significance. No toxic effects were observed as a result of the administration of the extracts except a temporary nausea with the larger doses. Doses equivalent to 20 Gm. of the dried plant were retained with-

out emesis by distracting the dog's attention at the earliest appearance of symptoms of nausea. In spite of repeated experiments upon the same animal, none of the animals showed the toxic symptoms of lipemia, acidosis and acetonuria characteristic of severe poisoning with white snakeroot. These results indicate that the dried white snakeroot contains one or more principles which administered by mouth exert a hypoglycemic action independent of any toxic effects upon the liver or kidney.

(*C*) *Attempts at Fractionation with the Purpose of Concentrating the Hypoglycemic Activity.*—The dried plant was treated by the method of Bourquelot (6) for the detection of glucosides. The plant tissue was exhausted with boiling 95 per cent alcohol in the presence of calcium carbonate. The combined alcoholic extracts were concentrated to dryness *in vacuo*. The glucoside fraction is the water-soluble part of the residue obtained from the alcoholic extracts. The water-insoluble material is the resin fraction. Although considerable inulin and levulose were found in the glucoside fraction, no glucoside capable of being split by emulsin was present. Couch (1) reports the presence of a non-toxic glucoside in the plant. Jordan, Whelan and Gidley (8) on the other hand report that the dried plant contains no glucoside. It may be that in their material as well as in ours the glucoside found by Couch to be present in the green plant was destroyed during the drying process. Although our 70 per cent alcoholic extract (Extract A) was concentrated *in vacuo* and that of Jordan *et al.* was evaporated on the water-bath, our extract apparently is no more toxic to dogs than Jordan's extract was to rabbits. Both the glucoside fraction and the resin fraction were tested for hypoglycemic activity using the same type of experiment described above. The results, reported in Table III, indicate that both of these fractions are inactive.

Attempts to fractionate Extract A, which was used in the experiments listed in Table II, resulted only in the loss of the hypoglycemic activity. Extract A was separated by centrifugation into water-soluble and water-insoluble fractions, both of which were tested on the dogs for their ability to reduce alimentary hyperglycemia. The results, summarized in Table III, are negative. The entire Extract A, however, shows its original activity.

It was thought that possibly the hypoglycemic activity may be due to myrtillin or some similar substance in white snakeroot. The "myrtillin fraction" was pre-

TABLE I.—EFFECT OF WHITE SNAKEROOT UPON THE BLOOD SUGAR OF FASTING DOGS.

| Dose of dried* plant administered as Extract A. *Gm.* | Sugar per 100 cc. Blood. Initial *Mg.* | 1 hr. *Mg.* | 3 hrs. *Mg.* | 5 hrs. *Mg.* | 7 hrs. *Mg.* |
|---|---|---|---|---|---|
| 0 (control) | 101 | | | 95 | |
| 5 | 89 | | 89 | 92 | |
| 10 | 98 | | 92 | 91 | 89 |
| 0 (control) | 92 | 95 | 86 | 87 | 89 |
| 20 | 90 | 93 | 92 | 87 | |
| 20 | 90 | | 71 | 63 | 70 |
| 20 | 92 | | | 77 | 84 |
| 0 (control) | 91 | 92 | 91 | 84 | 87 |

*Administered orally immediately following the initial blood sample.

pared from the plant using the procedure of Allen (7), which consists of extracting the plant with hot, acidulated, 50 per cent alcohol, precipitation of the protein with the addition of more alcohol, and precipitation of the filtrate with ammonium sulphate. The ammonium sulphate precipitate from 100 Gm. of dried white snakeroot weighed 2.7 Gm. This is called the myrtillin fraction since it corresponds

TABLE II.—EFFECT OF THE ORAL ADMINISTRATION OF WHITE SNAKEROOT UPON ALIMENTARY HYPERGLYCEMIA IN THE DOG.

| Series no. | Dose of dried plant administered as Extract A.* Gm. | Dose of glucose per Kg.** Gm. | | Blood Analyses. Initial Mg. | 4½ hrs. Mg. | 5 hrs. Mg. | 5½ hrs. Mg. | 6½ hrs. Mg. | 8½ hrs. Mg. | 24 hrs. Mg. | Maximum rise, blood sugar. Mg. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 3 | Sugar | 90 | 90 | 111 | 100 | 100 | 90 | | 21 |
| | 10 | 3 | Sugar | 100 | 89 | 106 | 104 | 99 | 83 | | 17 |
| | 0 | 3 | Sugar | 100 | 95 | 129 | 120 | 121 | 95 | | 34 |
| 2 | 10 | 3 | Sugar | 88 | 77 | 140 | 116 | 114 | 109 | 81 | 63 |
| | | | Urea N | 12.1 | 10.5 | 10.2 | 10.3 | 10.0 | 10.4 | 12.1 | |
| | | | Amino N | 7.4 | 7.1 | 6.7 | 6.1 | 5.5 | 5.5 | 7.5 | |
| | | | R B C | 5.3 | 6.3 | 5.9 | 5.6 | 5.5 | 5.4 | 6.4 | |
| | 0 | 3 | Sugar | 86 | 80 | 154 | 102 | 129 | 86 | 81 | 74 |
| | | | Urea N | | 10.6 | 11.1 | 11.0 | 10.9 | 10.7 | 9.0 | |
| | | | Amino N | 7.1 | 5.0 | 5.6 | 5.4 | 4.8 | 4.7 | 6.7 | |
| | | | R B C | 6.7 | 5.5 | 6.0 | 5.8 | 6.3 | 6.0 | 6.5 | |
| 3 | 10 | 2 | Sugar | 83 | 77 | 96 | 98 | 80 | 87 | 83 | 21 |
| | | | Urea N | | 8.3 | 8.3 | 7.4 | 7.5 | 9.8 | 9.2 | |
| | | | Amino N | 8.0 | 8.3 | 7.7 | 7.5 | 7.3 | 7.3 | 7.2 | |
| | | | R B C | 6.3 | 6.3 | 6.2 | 6.2 | 5.9 | 6.0 | 6.4 | |
| | 0 | 2 | Sugar | 81 | 82 | 111 | 114 | 78 | 76 | 74 | 32 |
| | | | Urea N | 7.0 | 7.8 | 7.1 | 6.6 | 6.6 | 7.2 | 6.9 | |
| | | | Amino N | 8.9 | 7.2 | 7.0 | 6.3 | 6.5 | 7.0 | 8.0 | |
| | | | R B C | 6.6 | 6.0 | 5.7 | 5.8 | 6.0 | 0.1 | 6.6 | |
| 4 | 10 | 2 | Sugar | 87 | 82 | 138 | 127 | 105 | 69 | 76 | 56 |
| | | | Urea N | 8.0 | 7.2 | 8.2 | 7.1 | 7.5 | 8.1 | 10.9 | |
| | | | Amino N | 6.5 | 6.9 | 6.5 | 5.5 | 5.5 | 5.5 | 6.7 | |
| | | | R B C | 6.4 | 5.7 | 5.7 | 5.6 | 6.0 | 5.4 | 6.5 | |
| | 0 | 2 | Sugar | 77 | 79 | 150 | 118 | 140 | 65 | 74 | 71 |
| | | | Urea N | 9.3 | 10.1 | 10.2 | 11.3 | 11.6 | 10.9 | 13.3 | |
| | | | Amino N | 7.2 | 6.6 | 6.7 | 5.8 | 5.6 | 5.5 | 6.5 | |
| | | | R B C | 6.6 | 5.6 | 6.0 | 5.0 | 5.0 | 5.5 | 6.0 | |
| | 10 | 2 | Sugar | 90 | 81 | 104 | 102 | 102 | 70 | 75 | 23 |
| | | | Urea N | 7.8 | 7.5 | 7.7 | 9.0 | 8.8 | 8.4 | 11.7 | |
| | | | Amino N | 6.9 | 7.0 | 6.5 | 5.9 | 5.5 | 5.6 | 6.6 | |
| | | | R B C | 6.4 | 5.2 | 5.9 | 6.5 | 5.6 | 5.7 | 6.2 | |

* Administered immediately after taking the initial blood sample.

** Administered immediately after taking the 4½-hour blood sample.

to a similar fraction of that name obtained by Allen from the myrtle and other plants. The entire precipitate from 100 Gm. of dried plant was administered in a single dose to a dog. Although this dose represents 5 to 10 times the amount of white snakeroot which was found active in the form of Extract A, the results, summarized in Table III, are negative. The hypoglycemic principle in white

snakeroot is probably not myrtillin. However, the hypoglycemic action of dried white snakeroot resembles that of myrtillin in that it apparently is demonstrated on the alimentary hyperglycemia curve (Table II) but to a much less extent upon the fasting blood sugar (Table I).

TABLE III.—FRACTIONS OF WHITE SNAKEROOT TESTED FOR THEIR ACTION UPON ALIMENTARY HYPERGLYCEMIA† IN THE DOG.

| Fraction. | Dried plant represented in dose administered.* | Sugar per 100 cc. Blood. Initial | 4½ hrs. | 5 hrs. | 5½ hrs. | 6½ hrs. | 7½ hrs. | Maximum rise in blood sugar. |
|---|---|---|---|---|---|---|---|---|
| | *Gm.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* |
| *Series 1.* | | | | | | | | |
| Glucoside fraction | 30 | 82 | 79 | 140 | 130 | 116 | 68 | 61 |
| Extract A | 20 | 85 | 80 | 115 | 122 | 104 | 71 | 42 |
| Water insol. fraction of Extract A | 20 | 85 | 88 | 164 | 135 | 95 | 73 | 76 |
| Extract A | 20 | 85 | 82 | 126 | 110 | 90 | 79 | 44 |
| Control | 0 | 81 | 75 | 149 | 152 | 132 | | 77 |
| *Series 2.* | | | | | | | | |
| Resin fraction | 30 | 76 | 78 | 141 | 103 | 82 | 67 | 63 |
| Water-sol. fract. of Extract A | 20 | 85 | 78 | 133 | 90 | 71 | | 55 |
| Water-sol. fract. of Extract A | 20 | 83 | 84 | 144 | 108 | 94 | 68 | 60 |
| Control | 0 | 81 | 87 | 150 | 118 | 64 | 87 | 63 |
| *Series 3.* | | | | | | | | |
| Myrtillin fraction | 100 | 85 | 84 | 127 | 128 | 109 | | 43 |
| Control | 0 | 85 | 88 | 137 | 135 | 101 | | 49 |

* White snakeroot extracts administered immediately after taking the initial blood sample.

† Two grams of glucose per Kg. administered immediately after taking the 4½-hour blood sample.

## SUMMARY AND CONCLUSIONS.

White snakeroot contains a principle which administered by mouth exerts a mildly hypoglycemic effect upon alimentary hyperglycemia in dogs. This principle resists drying, is without toxic action upon the liver or kidney, and is apparently not related to the factor or factors concerned in the production of trembles and milk sickness. Attempts at fractionation yielded no fraction of greater hypoglycemic activity than the entire 70 per cent alcoholic extract (Extract A). The hypoglycemic activity of dried white snakeroot is not of sufficient magnitude or regularity to warrant its use in the treatment of diabetes.

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## DETERMINATION OF OIL AND ALKALOIDS IN DELPHINIUM SEED.

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Previous researches on Delphinium seed, conducted by the writer,[1,2] made it desirable to determine the alkaloid content of the seed. The usual (Keller) process[3] when applied to this type of seed was found objectionable as, on account of the high oil content, intractable emulsions were found. The procedure here described was developed especially for seed of high oil content and makes possible a clean separation of the oil and alkaloids.

A mixture of several alkaloids is present in Delphinium seed. These alkaloids are soluble in most of the usual organic solvents, especially chloroform, but not in petroleum ether. Owing to their solubility in the oil of the seed, however, a petroleum ether extract of the seed contains some alkaloids. Ammonia liberates only part of the alkaloids from acid solution, but sodium hydroxide effects complete liberation. Alkaline solutions yield the alkaloids readily to chloroform, but acid solutions are not so rapid in their extraction of the alkaloids from chloroform. Sulphuric acid is the best medium for extracting the alkaloids from an organic solvent, and in this work 5% acid (by weight) was adopted as this strength accomplishes quicker extraction than weaker strengths without harmfully affecting the alkaloids. The alkaloids are partially extracted from chloroform by pure water

### PROCEDURE.

Extract 10 Gm. of the well-ground seed (ground as fine as the oily nature will permit) by percolation with low boiling petroleum ether until the oil is apparently all removed. Dry the marc. Extract the petroleum ether solution, concentrated if necessary to about 100 cc., with 5% sulphuric acid until the alkaloids are completely removed. Five extractions of 15 cc. each usually suffice. The last acid extract should give not more than a faint turbidity with Wagner's reagent (iodine in potassium iodide solution), the test being made on approximately 5 cc. of solution. If a precipitate forms, dissolve it by dilute sodium thiosulphate, return to the main solution and continue the extraction.

Filter the petroleum ether extract through paper into a weighed beaker, wash paper and funnel well with the solvent, and evaporate on the steam-bath. If the oil residue is not clear, dissolve it in petroleum ether, filter, wash as before and evaporate again. To insure complete removal of the solvent, which is tenaciously retained by the oil, add a little ethyl alcohol and thoroughly heat on the

* 1001—15th St., Washington, D. C. This work was performed in the U. S. Department of Agriculture.

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