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THE HEMOLYTIC ACTIVITY OF ATRIPLEX CANESCENS.*

BY M. R. MILLER.[1]

DESCRIPTION OF THE PLANT.

Atriplex canescens, James, is a shrub found growing in the desert flats or washes of the Mohave and Colorado Deserts, west to San Bernardino and San Diego; east to Nevada and Dakota and south to Mexico. It appears as a roundish gray shrub, 1 to 5 feet high with linear, entire leaves, narrowed at the base, $^3/_4$ to 1 $^1/_4$ lines long, finely scurfy and canescent. The flowers are mostly dicœcious and very dense in fruit. The fruiting bracts form a thick, hard body, 3 to 4 lines long.[2] The plant has several common names as "saltbush," "chamiso brush," and "shad scale," the latter name being used in reference to the plant in this article.

* Published by permission of the Director of the Nevada Agricultural Experiment Station.

[1] Chemist, Nevada Agricultural Experiment Station.

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Shad scale is generally held by stockmen to be a valuable browse and forage plant of the vast areas of a large portion of the West. It affords a succulent browse at a time of the year when there are but few forage plants available and its abundance in the wide valleys is such that quantities of feed are provided by it.

Attention has been called, from time to time in Nevada, to losses of sheep in areas in which shad scale is abundant and in which no plants known to be poisonous could be found. Feeding experiments at the Nevada Experiment Station with both sheep and rabbits have indicated that shad scale does not possess the nutritive qualities which might be expected of a plant of such luxurious growth and that it is toxic under certain conditions. In the laboratory it has been demonstrated that shad scale contains a substance, or group of substances, which are capable of producing pathological conditions and which have the properties of the class of compounds known as saponins.

SAPONINS AND HEMOLYSIS.

The most outstanding property of the saponins, aside from their foam-forming properties, is that of hemolytic activity. A substance which is hemolytically active is, in brief, one which has such an action on red corpuscles of blood as to destroy or to break them up. This action is readily distinguished by the so-called "laking" of a suspension of the corpuscles. A suspension of red blood corpuscles in an isotonic salt solution presents an opaque appearance. If left to stand quietly, the corpuscles will soon settle to the bottom of the vessel leaving a clear and colorless supernatant liquid. If "laking" has taken place, *i. e.*, if the corpuscles have been broken up, the liquid will appear transparent and of a red color due to the liberation of the coloring matter of the corpuscles. On standing there will be no settling as in the suspension above described.

Laking may be produced in a number of ways. It may be produced by many chemical substances, both organic and inorganic. The hemolytic reaction serves as a differential biological test for certain organisms and may be applied as a test for the presence of saponins. The saponins, as a group, are hemolytically active, their relative activity varying with their constitution, when other conditions as time, temperature and concentration are equal.

The hemolytic test for saponins is conveniently carried out at ordinary temperatures by adding a solution of the active substance in physiological salt solution to a suspension of washed red blood corpuscles. The suspension will then become clear after a time interval depending on the concentration of the saponins and corpuscles and the hemolytic power of the saponin. By using uniform concentrations it is possible to judge the relative concentration of the saponin in question; or the relative hemolytic activity of a given saponin may be determined. The results here reported on the variation of the hemolytic activity of the plant throughout the year were obtained by the following technique:

Corpuscle Suspension.—Twenty cubic centimeters of fresh defibrinated rabbit blood are centrifuged and the corpuscles washed several times with physiological salt solution (0.9% NaCl). When the washings are colorless and apparently free from serum, the corpuscles are suspended in 1000 cc. of the saline solution.

Plant Extract.—Two grams of the air-dried and powdered plant are shaken

with 100 cc. of saline solution and allowed to stand at least an hour. The mixture is then filtered clear and the filtrate used for further dilution.

Making the Test.—Into each of a series of test-tubes is pipetted 10 cc. of the corpuscle suspension. Care must be taken that the suspension be shaken up each time before removing a portion. To each of the tubes is added 10 cc. of the solution to be tested. The time required for clearing is noted. The point at which the liquid may be considered clear is when coarse printing is legible through the tube and there are no corpuscles settled at the bottom of the tube. This time is taken as the time for hemolysis.

VARIATION OF TIME OF HEMOLYSIS WITH DILUTION.

To demonstrate the variation of time for hemolysis to take place with a change in concentration of a saponin solution the following experiment was performed.

A two-gram sample of the dry powdered plant was macerated for a short time with 100 cc. of the physiological salt solution, filtered and the clear filtrate used. This dilution (2:100) when used with equal parts of suspension gives a dilution of 1:100 in the test. Three dilutions were made with this solution. The times necessary for hemolysis are given in Table I.

TABLE I.

EFFECT OF DILUTION ON TIME FOR HEMOLYSIS.

Dilution.	Time.
1:100	Immediate
1:500	15 minutes
1:1000	30 minutes
1:5000	No action in 15 hours

VARIATION OF HEMOLYTIC ACTIVITY THROUGHOUT THE YEAR.

To determine whether there is a variation in the hemolytic activity in shad scale through the year a series of small collections were made from the same plants in one locality. The samples were air dried, ground to 100 mesh and preserved in tightly stoppered bottles.

The corpuscles of rabbit's blood were used prepared as outlined above. The test-tubes used were selected for uniformity of bore in order to give more comparable results.

Two dilutions were selected, 1:1000 and 1:1500, as these were found to give laking in a convenient time. Duplicate tests (A and B) were run at each dilution.

The variation in the time for hemolysis with the season indicates (*a*) a change through the year of the quantity of the active substance, its constitution remaining

TABLE II.

HEMOLYTIC ACTIVITY ON SAMPLES TAKEN THROUGH THE YEAR.

Sample collected. Dilution.	Time for hemolysis, seconds. 1:1000.		1:1500.	
	A.	B.	A.	B.
May 3, 1925	60	58	80	76
June 3	105	92	135	142
July 2	90	66	105	99
Aug. 11	120	95	225	173
Sept. 20	150	157	420	375
Nov. 22	90	80	135	123
Jan. 8, 1926	60	65	85	81
Mar. 10	45	40	55	49

constant, or (*b*) a change in the chemical character of the substance, its quantity remaining constant, or, (*c*) more likely, a change in both the quantity and constitution.

From these data it may be seen that the plant has its least hemolytic activity, *i. e.*, may be assumed to be least toxic, in late summer and fall, increasing to a maximum in early spring. There is also a small drop in the summer about during the periods of June and July. What the significance of this drop is, is not known. It may refer to a fluctuation in the weather which influenced the growth of the plant, moisture conditions or other causes. The increase in the hemolytic activity of the plant, however, coincides with the observed periods of increased toxicity of the plant for sheep during the feeding experiments.

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p_H DETERMINATIONS IN ALCOHOLIC SOLUTIONS.

BY RALPH B. SMITH.

The effect of changes in the hydrogen-ion concentration of many types of reactions has been widely studied during the past few years and these investigations have covered almost every field of chemistry. In the field of bio-chemistry a vast amount of work has been done and many interesting and valuable results have been obtained.

These p_H studies in the bio-chemical field have been almost entirely limited to water solutions; there are, however, many cases in which alcoholic extractions of vegetable or animal products must be made. The hydrogen-ion concentration is known to be a factor of great importance in aqueous solutions and must also be an influential factor in alcoholic solutions. These considerations have led to a study in this laboratory of the hydrogen-ion concentration of alcoholic solutions and tinctures.

It is known from the work of Lapworth, *et al.*[1] that stable and reproducible e. m. f. values could be obtained from concentration cells in which absolute to 98% alcohol was used as a solvent. They also used calomel half-cells made up with alcohol instead of water.

Bishop, Kittredge and Hildebrand[2] made a series of electrometric titrations of various acids and bases in alcohol as a solvent. They used $N/10$ NaBr half-cells made up with 95% and absolute alcohol. Smooth titration curves similar to those obtained when water is used as a solvent were obtained with the difference that there was a greater e. m. f. interval between the acid and the basic parts of the curve. This is due to the fact that the dissociation constant of alcohol is smaller than that of water. This difference in the dissociation products gives rise to a new and different system each time the concentration of the alcohol is changed. All these systems must have contact potentials which many investigators have practically ignored. The data accumulated by Lapworth, *et al.*[1] shows that these contact potentials cannot be ignored even over small changes in the water content of the alcohol.

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[1] *Jour. Chem. Soc.*, 99 (1911), 1417; 99 (1911), 2242; 101 (1912), 2249; 105 (1914), 2553 and 107 (1915), 1520.

[2] *Jour. Amer. Chem. Soc.*, 44 (1922), 135.