Bulletin No. 94 - The Chemical Examination of Death Camas

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UNIVERSITY OF WYOMING
Agricultural College Department.

Wyoming Experiment Station
LARAMIE, WYOMING.

BULLETIN NO. 94
JULY, 1912

The Chemical Examination of Death Camas
(Technical Bulletin)

By F. W. HEYL, S. K. LOY, HENRY G. KNIGHT and O. L. PRIEN

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Wyoming Agricultural Experiment Station

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The Chemical Examination of Death Camas.

BY F. W. HEYL, S. K. LOY, HENRY G. KNIGHT, AND O. L. PRIEN.*

INTRODUCTION.

The poisonous character of the several species of plants designated as Death Camas has long been known to the stockmen of certain western states. These plants belong to the genus Zygadenus and are found in Montana, Wyoming, Colorado, and other states of the northwest, where they have from time to time caused heavy stock losses, particularly among sheep.

Chestnut and Wilcox† in an extensive survey of stock poisoning plants state that during the season of 1900, 3,070 cases of sheep poisoning by Zygadenus came under their immediate observation, and that probably only about one-fourth of the actual cases were brought to their attention. Of this total, 651 cases proved fatal. According to their observations, only one other stock poisoning plant is more destructive to sheep than Zygadenus. This is the Lupine.

Although deaths due to Lupine poisoning are more numerous, yet the mode of poisoning is entirely distinct. So far as Chestnut and Wilcox were able to observe, lupines are not very extensively eaten by sheep during the spring or summer, except where they are being trailed through a strange country or when they have just been unloaded from cars, and are in a hungry condition, when they eat lupines in any stage of growth. The leaves of the lupines remain green and the

*Dr. Charles L. Ralford co-operated on this problem for a year in the Wyoming Experiment Station Laboratory.
plants offer slightly succulent forage after the other plants have become dry. Again, lupine poisoning has frequently resulted from the feeding of lupine hay, when it has been cut in full pod, with the ripe seeds. From these considerations of *Lupine*, it follows that Death Camas is the most prominent poisonous plant of the early spring, and death is caused by it most extensively during the latter part of May and early in June.*

In Wyoming the most common species is the *intermedius*, and the greatest stock losses noted have usually occurred in the early spring when this plant is in bloom, and before the early forage plants are plentiful. In addition to this, there prevails the idea that poisoning is more frequent immediately after rainstorms, and it has been suggested that this might be accounted for on the general knowledge that in some plants, the active constituent is found chiefly in the roots, and that when the ground is moist and soft the bulbous portion could be more easily uprooted by cattle. Such a view, however, is scarcely supported by our experiments, for analyses show that the bulb and the leaf differ but little in the amount of alkaloid contained. It will be shown that the flower contains a higher percentage of alkaloids than any other part of the plant. In the light of these facts the more probable explanation of the greater frequency of poisoning immediately after rainstorms, would seem to be that at such a period either the flowers are more numerous, or the animal then eats a greater quantity of leaves than usual. A possible explanation of the tendency to eat such a plant at all when anything else is available, at which some surprise has been expressed, may be found in the fact that the plant as a whole has a relatively high food value, while the bulb, which is doubtless eaten to some extent, contains a high percentage of sugar.

*From research carried out at the Wyoming Experiment Station, Woody Aster (*Xylorhiza Parryi* Gray) is apparently a greater factor in the spring losses in this state than Death Camas. Bul. 88, Wyo. Exp. Sta.

†Blankinship, *Montana Exp. Sta.*, Bull. 45, 91 (1906), states that the bulb of Death Camas is the most poisonous part of the plant, but this statement is not supported by experimental evidence.
A knowledge of the constituents of the several species of the genus Zygadenus, or of the details of the behavior of animals subjected experimentally to their influence is confined to the report of a study of the stock poisoning plants of Montana, by Chestnut and Wilcox∗, who conducted feeding and other experimental tests with extracts of Zygadenus venenosus, and to a preliminary chemical study of the constituents of the same species by Slade†. The latter obtained by means of color tests described by Merck‡, and Wright and Luff§, evidence which led him to conclude that the poisonous character of the plant is due to the presence of the alkaloids sabadine, sabadinine and veratralbine. Another report of work on this plant by Reid Hunt** gives some reason for suspecting that the effect of the plant is due to an alkaloidal mixture very closely resembling veratrine.

Thus the subject of the toxicity of this plant is in a rather confused state and offers an interesting field for further investigation. In the present bulletin the discussion of the plant will be considered under the following topics:

1. Description, Habitat, and Distribution.
2. Proximate Analysis of the Plant.
4. The Percentage of Alkaloid.
5. The Physiological Effects of the Alkaloid and Antidote.

I. DESCRIPTION, HABITAT AND DISTRIBUTION.

The work here described was carried out on the species available in Wyoming, which was identified as Zygadenus intermedius Rydb. by Professor Nelson. This species is a near relative of the non-western and better known Zygadenus

∗Loc cit.
†Am. J. Pharm., 77, 262, (1905).
‡Arch. Pharm., 229, 164 (1891).
**Am. J. Physiology, Vol. 6, 19 (1901).
venenosus Wats. It is also known as poison camas, lobelia, squirrel food, wild onion, poison sego, poison sego-lily, mystery grass, etc.

The following has been drawn in part from the original description:

Bulb elongated-ovoid: stem rather stout, 2-5 dm. high: leaves light green, scabrous on margin and midrib, 1-3 dm. long, 5-9 mm. wide, keeled and often more or less folded, with scarious sheathing base: flowers greenish or yellowish-white: perianth free from ovary; segments ovate or oblong, obtuse or acute, 5-8 mm. long, short-clawed; the outer sub-cordate at base; the inter acute or rounded; gland semi-orbicular, the upper margin thin and not well defined: capsule ovoid-cylindrical.

This plant grows from a rather deep set bulb from which the few grass-like or onion-like leaves develop very early in the spring. These leaves are soon followed by the flower stalk which becomes six or ten inches high, terminating in a spike-like cluster of nearly white flowers. As the season advances the flower stalk lengthens out slightly into a nearly naked seed stalk, bearing rather large capsules. The plant grows on the sandy plains as well as in the drier and stonier foot-hills. It is, however, more abundant in sandy swales where the soil remains moist for a comparatively longer time. It is never abundant on the open plains except in such locations as just mentioned.

II. PROXIMATE ANALYSIS OF THE PLANT.

Preparation of Material.—Portions of Zygadenus intermedius which together weighed 5 kilograms in the green state were collected between May 26 and June 2, 1910. The first lot consisted of plants that had not reached the flowering stage, while the last contained many plants with flowers, though the latter were not mature. In preparing for analysis the leaves and flowering tops were separated from the bulbs, and the latter in all cases deprived of the outer layer and the roots. The average weight of the plant was 7.4 grams, and
moisture determinations at 95-100° showed losses of 75.50 per cent. and 68.93 per cent. on the leaves and flowering tops, and on the bulbs and roots, respectively. The various parts were allowed to dry in the air at a somewhat elevated temperature, under which condition the leaves dried readily, while the bulbs long remained sticky and had to be sliced. Six weeks were required to dry them to such a state that they could be ground easily. The grinding and sieving necessitated the shielding of one's face in order to avoid the extremely irritating dust, the character of which was probably due to one or more of the sternutatory veratrum alkaloids, possibly veratralbine.

The investigation was begun by extracting separate portions of 2 grams each in a Soxhlet apparatus with ligroin, ether and alcohol, respectively. The quantities removed are given in percentages in Table I.

TABLE I.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Leaf</th>
<th>Flower</th>
<th>Bulb</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligroin extract (dried at 93°)</td>
<td>2.04</td>
<td>2.80</td>
<td>0.62</td>
<td>0.89</td>
</tr>
<tr>
<td>Ether extract, total</td>
<td>5.47</td>
<td>5.21</td>
<td>3.18</td>
<td>3.05</td>
</tr>
<tr>
<td>Ether extract, volatil (110°)</td>
<td>1.33</td>
<td>1.07</td>
<td>1.30</td>
<td>0.80</td>
</tr>
<tr>
<td>Alcohol extract (dried at 110°)</td>
<td>35.12</td>
<td>46.27</td>
<td>39.60</td>
<td>lost</td>
</tr>
</tbody>
</table>

The proximate analyses were conducted in accordance with the usual methods*, and gave the results stated in Table II.

TABLE II.

<table>
<thead>
<tr>
<th></th>
<th>Leaf</th>
<th>Flower</th>
<th>Bulb</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>6.91</td>
<td>6.13</td>
<td>7.50</td>
<td>7.56</td>
</tr>
<tr>
<td>Starch by diastase</td>
<td>absent</td>
<td>absent</td>
<td>23.53</td>
<td>absent</td>
</tr>
<tr>
<td>Pentosans</td>
<td>10.81</td>
<td>7.95</td>
<td>4.41</td>
<td>12.04</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>16.17</td>
<td>10.58</td>
<td>5.08</td>
<td>21.53</td>
</tr>
<tr>
<td>Protein</td>
<td>13.25</td>
<td>19.73</td>
<td>6.19</td>
<td>6.78</td>
</tr>
<tr>
<td>Ash</td>
<td>8.12</td>
<td>8.91</td>
<td>4.29</td>
<td>18.41</td>
</tr>
</tbody>
</table>

The material removed by extraction with alcohol, the amounts of which were given in Table I, was examined for resin (insoluble in water), for sucrose and hexose sugars. The results are given in Table III, to which have been added the figures representing the dextrin determinations.

TABLE III.

<table>
<thead>
<tr>
<th></th>
<th>Leaf</th>
<th>Flower</th>
<th>Bulb</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin</td>
<td>5.03</td>
<td>undet.</td>
<td>2.58</td>
<td>5.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5.60</td>
<td>undet.</td>
<td>18.44</td>
<td>1.02</td>
</tr>
<tr>
<td>Reducing sugar*</td>
<td>5.89</td>
<td>undet.</td>
<td>6.69</td>
<td>2.45</td>
</tr>
<tr>
<td>Dextrin</td>
<td>3.25</td>
<td>trace</td>
<td>1.40</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*Calculated as invert sugar.

PREPARATION AND ANALYSIS OF ASH.—Portions of the ground and seived leaves and tops, and of the bulbs, respectively, were ignited below red heat in a muffle furnace. The charred material was extracted with hot water and filtered, the residue burned to a white ash and the filtrate mixed with this ash, and the whole evaporated to dryness on the water bath. The last portions of water were expelled by heating at 110°. The resulting solid was at once powdered and preserved in glass stoppered bottles until analyzed. Table IV gives the constituents of the ash of the leaf and of the bulb.

TABLE IV.

<table>
<thead>
<tr>
<th></th>
<th>Leaf</th>
<th>Bulb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>3.79</td>
<td>2.04</td>
</tr>
<tr>
<td>Chlorine</td>
<td>.30</td>
<td>.19</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>18.05</td>
<td>16.61</td>
</tr>
<tr>
<td>Sand</td>
<td>8.31</td>
<td>7.01</td>
</tr>
<tr>
<td>Carbon</td>
<td>.71</td>
<td>.48</td>
</tr>
<tr>
<td>Soluble Silica</td>
<td>4.39</td>
<td>3.55</td>
</tr>
<tr>
<td>Sulphur Trioxide</td>
<td>2.89</td>
<td>3.33</td>
</tr>
<tr>
<td>Phosphorous Pentoxide</td>
<td>5.03</td>
<td>8.73</td>
</tr>
<tr>
<td>Ferric Oxide</td>
<td>1.03</td>
<td>1.08</td>
</tr>
<tr>
<td>Aluminum Oxide</td>
<td>2.55</td>
<td>1.08</td>
</tr>
<tr>
<td>Manganese</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Calcium Oxide</td>
<td>25.37</td>
<td>26.48</td>
</tr>
<tr>
<td>Magnesium Oxide</td>
<td>5.34</td>
<td>5.02</td>
</tr>
<tr>
<td>Sodium Oxide</td>
<td>5.58</td>
<td>4.68</td>
</tr>
<tr>
<td>Potassium Oxide</td>
<td>20.64</td>
<td>20.35</td>
</tr>
<tr>
<td>Summation</td>
<td>102.98</td>
<td>100.63</td>
</tr>
</tbody>
</table>

*These figures represent the total sulphur determined by the peroxide method and calculated as sulphate in terms of the ash.
III. METHOD FOR THE PREPARATION FOR THE ALKALOID.

In the first method, which was used for the preparation of the alkaloid in quantity, we proceeded as follows: One hundred and fifty grams of the ground and seived material was boiled for several hours with 1200 grams of 95% alcohol in which 6 grams of glacial acetic acid had been dissolved. The tincture was filtered and the partially exhausted drug after pressing upon the Büchner funnel was again boiled with 1000 gm. of 95% alcohol and 5 gm. acetic acid. The extraction was repeated once more with 750 gm. alcohol and 4 gm. acetic acid.

The united liquids were next evaporated under reduced pressure to a volume of 500 c.c. and then poured into a mixture of 120 c.c. of normal $\text{H}_2\text{SO}_4$ and 1380 c.c. of water, and allowed to stand over night. The mixture was next filtered and the resinous precipitate washed by decantation with dilute sulphuric acid of the above concentration.

The acid filtrate was extracted with ether. This ether extract undoubtedly contains a crystalline body, on which we will report later. The aqueous layer which had been freed from acidic material soluble in ether was now rendered ammoniacal and repeatedly extracted with a mixture of chloroform and ether (3-1). This was a long, tedious process and not to be recommended. The resulting extract was next shaken with normal $\text{H}_2\text{SO}_4$ to remove the alkaloid. The acid liquid was then rendered alkaline with ammonia and extracted again with the chloroform-ether mixture. Thirteen extractions were found necessary. Upon evaporating and drying the residue amounted to 2.0715 gram.

This was dissolved in 0.1 N $\text{H}_2\text{SO}_4$ and precipitated with Mayer's reagent. The mercury double salt separated as a flocculent white precipitate. This was filtered off on suction, washed with water, and then decomposed with hydrogen sulphide while suspended in dilute $\text{H}_2\text{SO}_4$. 
The mercury sulphide was filtered off. The filtrate containing the alkaloidal sulphate was very gently warmed to expel the excess of H₂S and the liquid was rendered ammoniacal and shaken repeatedly with ether. The alkaloid thus recovered weighed 0.8835 gram.Repeated efforts to crystallize the material failed and this method was abandoned as a means of preparation.

The most satisfactory method for obtaining material which may be crystallized is to avoid the presence of acids and the use of prolonged boiling as a method of extraction. The following method led to an alkaloidal product which is now being crystallized repeatedly in order to isolate a distinct alkaloid.

Three kilograms of the ground leaves and tops were divided into six lots of 500 gm. each and 2500 c.c. of 95% alcohol was added to each. This mixture was allowed to macerate for seven days with occasional application of very gentle heat. The partially exhausted drug was pressed dry and the extraction repeated exactly as before, using alcohol which was recovered by partial concentration of the first extract.

The combined alcoholic extracts were concentrated to 700 c.c., and the residue was allowed to concentrate further by standing in a desiccator over H₂SO₄. This concentrated extract was poured into 4 liters of water which had been acidified with 10 gm. of tartaric acid. This was allowed to stand until the soft resin had settled compactly on the bottom. The resin was now removed by filtration, and the brownish-red aqueous solution was repeatedly extracted with ether.

The aqueous layer was now rendered distinctly alkaline with Na₂CO₃ and extracted with ether twice, whereupon the third extraction removed nothing further. (In another experiment it was found necessary to shake many more times with ether.)

After shaking with ether, the remainder of the alkaloid was removed by extraction with chloroform until the original aqueous liquid gave no test with Mayer’s reagent.
The etherial extract was now shaken with a 5% solution of tartaric acid several times, which readily extracted the alkaloid. This tartaric acid solution of the alkaloid was rendered strongly alkaline with Na$_2$CO$_3$ and thereupon a gummy, sticky mass separated. This was allowed to stand for several days, during which time it hardened. The material was now filtered off on suction and washed with water. It weighed 2.3 gm. The crude material contained a small amount of coloring matter which was removed by dissolving in 5 c.c. of alcohol, and adding 50 c.c. of ether which precipitated a small amount of silmy, foreign material.

The behavior of this material indicates, as has been suggested by Reid Hunt, a close analogy to commercial Veratrine. Upon powdering it caused intense irritation and sneezing. It was but slightly soluble in water, soluble in alcohol, ether, and chloroform. It was insoluble in petroleum benzin. The melting point was indefinite but closely resembled that stated for Veratrine. When titrated with sulphuric acid an orange red color is at first observed, which goes over to a brilliant cherry red in a short time.

A titration of this material (freed from coloring matter) gave the following result:

0.1788 gm. dissolved in 3.5 c.c. 0.1 N H$_2$SO$_4$ required 2.3 c.c. 0.02 N alkali for neutralization.

One cubic centimeter of 0.1 N H$_2$SO$_4$ is therefore equivalent to 0.5881 gm. of this alkaloidal mixture. The calculated value of cevadine is 0.590 gm. per cubic centimeter. This figure will be used as a basis for the assay by titration.

The filtrate from the material precipitated with Na$_2$CO$_3$ was twice extracted with ether. The ether was washed with water, dried over anhydrous Na$_2$SO$_4$ and evaporated to dryness. An alkaloidal residue remained which weighed approximately 1.5 gm. It was dissolved in chloroform and precipitated with ether. When heated in a capillary tube it shriveled at 174° and had entirely decomposed at 186° C.
The aqueous alkaline liquid which had been extracted with ether still contained alkaloid which was removed with chloroform. The weight was not taken but approximated one gram.

The main alkaline liquid from which about 4.8 gm. of alkaloid had been removed by the ether extraction was now extracted several times with chloroform, and the chloroform was extracted with 5% tartaric acid. When this acid solution of the alkaloidal tartrates was rendered alkaline with Na₂CO₃ a sticky precipitate resulted. After standing several days it was removed by filtration and allowed to dry in a desiccator. It was very sternutatory and had an indefinite melting point. It began to frit at 152° and no definite melting point could be assigned. The weight was 0.69 gm. The liquid which was filtered from this precipitate was shaken with ether and with chloroform; the former extracted about 0.6 gm. and the latter 0.8 gm. further. The original alkaline liquid now contained no further alkaloid.

The first method was used to prepare the alkaloid used in the toxicological experiments, whilst it is hoped that a pure alkaloid may be derived from the material obtained according to the second method.

The alkaloidal preparation, which upon titration showed a molecular weight approaching very closely to cevadine, was subjected to the action of concentrated sulphuric acid. It gave the color changes which are those of cevadine.* In the concentrated acid, the alkaloid dissolved with a yellowish-orange coloration, which upon warming gave a brilliant cherry red. The same red coloration was obtained upon allowing the material to stand with cold concentrated sulphuric acid.

IV. THE PERCENTAGE OF ALKALOID.

Determination of Alkaloid in the Leaf.—The presence of an alkaloid in the leaf having been detected by a preliminary assay, it was decided to make determinations in more than one way. Accordingly, portions of leaf were next assayed by three different methods, as follows:

I. The first method* tried is that official in the United States Pharmacopeia for the assay of belladonna leaves, which we modified to the extent of using ether instead of chloroform in the final extraction. Duplicate determinations in this way gave crude alkaloidal residues weighing 0.0883 and 0.0910 gram, which required for neutralization 1.13 and 1.07 c.c. respectively, of 0.1 N sulfuric acid.

The residues so obtained, which were contaminated with conspicuous amounts of resin that was insoluble in dilute acid, were united, the mixture acidified and filtered, and the clear filtrate precipitated with Mayer’s reagent. The insoluble mercury compound that resulted was collected on a filter, washed with water, suspended in dilute sulfuric acid, and decomposed by hydrogen sulfide. After standing over night the mixture was filtered, the mercuric sulfide washed with water, the filtrate made alkaline with ammonia and extracted with ether. Evaporation of the ether left a crystalline alkaloidal residue that weighed 0.0815 gram, and which corresponded to 0.41 per cent. of the drug employed. The combined, partially exhausted drug residues obtained in the first part of the operation were next exhausted by percolation with 5 per cent. sulfuric acid. The percolate was made alkaline with ammonia, and worked up for alkaloid in the usual way. A further quantity of alkaloidal residue weighing 0.0230 gram and requiring 0.11 c.c. 0.1 N acid for neutralization, was obtained.

II. A second portion of leaf weighing ten grams was assayed by the method specified above and modified to the

extent of using Prolius' solution instead of the ether-chloroform mixture as a menstruum. The crude alkaloidal residue obtained by this method weighed 0.0807 gram, and required 1.04 c.c. 0.1 N acid, and 0.78 Mayer's reagent. Decomposition of the mercury salt in the manner already described gave 0.0280 gram of crystallin residue, representing 0.28 per cent. of the original material. Further percolation of the partially exhausted drug with 5 per cent. sulfuric acid yielded an alkaloidal residue that weighed 0.0107 gram and sufficient to neutralize 0.10 c.c. 0.1 N acid.

III. A third portion of leaf weighing 40 grams was exhausted as far as possible by repeated extractions with boiling 95 per cent. alcohol. The extract was then concentrated under reduced pressure to a volume of 200 c.c., the dissolved solids determined in an aliquot of 5 c.c., and the remainder poured into a mixture of 40 c.c. N sulfuric acid, and 460 c.c. water. A brown, resinous mass amounting to 4.9 per cent. of the weight of the drug was precipitated. This was collected on a filter and washed with dilute acid. The acid filtrate was now made alkaline with ammonia, and worked up for alkaloid in the usual way. The crude residue obtained weighed 0.2382 gram, and required for neutralization 3.70 c.c. 0.1 N acid, and for precipitation 7.48 c.c. Mayer's reagent. The residue was known to be contaminated with resin, and in order to correct for this the mercury compound was decomposed as described above, and a crystallin alkaloid obtained. The latter weighed 0.1033 gram, and represented 0.26 per cent. of the drug.

The residue of leaf left after extraction with alcohol was percolated with 5 per cent. sulfuric acid until the percolate no longer reacted with Mayer's reagent. A further yield of alkaloid amounting to 0.0139 gram and neutralizing 0.18 c.c. 0.1 N acid was secured.

*Alcohol 8 c.c., ether 88 c.c., ammonia (10 per cent.) 4 c.c.
The Chemical Examination of Death Camas.

DETERMINATION OF ALKALOID IN THE BULB.—When three separate portions of ten grams each of the bulb were subjected to the process designated as I under the leaf, there were obtained crude alkaloidal residues that weighed 0.0560, 0.0530, and 0.0450 gram, and which neutralized 0.32, 0.34, and 0.36 c.c., respectively, of 0.1 N acid. As with the leaf, the partially exhausted drug residues from twenty grams were percolated to exhaustion with 5 per cent. sulfuric acid, and the percolate yielded 0.0168 gram crude alkaloidal residue that neutralized 0.13 c.c. 0.1 N acid, and precipitated 0.37 c.c. Mayer's reagent.

II. Two ten-gram portions of bulb were assayed in accordance with method II outlined under the leaf and gave impure alkaloidal residues weighing 0.0876 and 0.0770 gram, equivalent to 0.97 and 0.96 c.c. 0.1 N acid, and 1.90 and 1.51 c.c., respectively, of Mayer's reagent. Decomposition of the mercury salt of the first of these in the usual way gave 0.0390 gram of crystallin residue, equivalent to 0.39 per cent. of the drug.

III. Fifty grams of bulb were exhausted as nearly as possible by repeated extraction with boiling 95 per cent. alcohol. When the first portion of the extract, measuring 500 c.c., was concentrated, it deposited crystals of sucrose. The combined extracts were then concentrated under reduced pressure to a small bulk and finally allowed to dry out over sulfuric acid in a desiccator. The residue was next boiled with 150 c.c. 95 per cent. alcohol and the undissolved portion (which had a sticky consistency) removed and boiled with a second portion of alcohol. The final residue, which was granular in character, weighed 3.771 grams and represented 7.54 per cent. of the bulb. When heated upon platinum foil the substance produced caramel; tested with Fehling's solution, it showed slight reduction. Examined with the polariscope it showed the constants for cane sugar mixed with a trace of
reducing sugar. The specific rotation found was $[a]_D = +62.70$ at $20^\circ$, while that for the pure sucrose is $+66.48$.

The alcoholic extract obtained above, which had a strong reducing action on Fehling’s solution, was now concentrated to the consistency of a fluid extract, and then diluted with a mixture of 10 c.c. N sulfuric acid and 240 c.c. water. A brown, flocculent resin precipitated. This was collected on a filter, washed with water and dried. It weighed 1.2875 grams and was equivalent to 2.58 per cent. of the bulb.

The acid filtrate obtained above was now shaken repeatedly with ether, which removed a brown tar, weighing 0.2922 gram, equivalent to 0.58 per cent. The liquid was then made alkaline with ammonia, and the alkaloid removed by extraction with ether as already described. From the ether the alkaloid was recovered by shaking out with sulfuric acid in the usual way. The liquid obtained was made alkaline with ammonia a second time, extracted with ether and the latter evaporated off. The residue weighed 0.0905 gram, was completely soluble in 0.1 N acid, of which 1.10 c.c. were required for neutralization, and 2.90 c.c. of Mayer’s reagent for precipitation.

It was found in the determination just specified that ether failed to remove all the alkaloid from the second ammoniacal liquid. A further extraction with chloroform yielded 0.0307 gram of alkaloid that required 0.23 c.c. 0.1 N acid, in which it was completely soluble, and 1.30 c.c. Mayer’s reagent for precipitation. The combined alkaloidal residues constitute 0.24 per cent of the bulb.

The flower and root were next assayed by the method designated above as I. Duplicate determinations on ten-gram portions of the flower gave crude alkaloidal residues weighing 0.1358 and 0.1842 gram, which required 1.82 and 2.32 c.c. 0.1 N acid for neutralization, and 3.79 and 3.60 c.c., respectively, of Mayer’s reagent for precipitation.
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Assays of duplicate portions of the root in the same way gave crude alkaloidal residues weighing 0.0504, and 0.0422 gram. These required for neutralization 0.52 and 0.56 c.c. 0.1 N acid, and 1.15, and 1.15 c.c., respectively, of Mayer's reagent for precipitation.

In view of the fact that we have quite definitely determined the value of standard acid against a fair sample of the alkaloid, the results will unquestionably be more accurately stated by calculating the weight from the titration values. The following work is based upon the titration value stated above, i.e., 1 c.c. 0.1 N acid = 0.5881 gm. alkaloid.

<table>
<thead>
<tr>
<th>Method</th>
<th>I</th>
<th>II</th>
<th>Method</th>
<th>I</th>
<th>II</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>0.665</td>
<td>0.629</td>
<td>0.612</td>
<td>0.585</td>
<td>0.570</td>
<td></td>
</tr>
<tr>
<td>Bulb</td>
<td>0.188</td>
<td>0.211</td>
<td>0.565</td>
<td>0.570</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flower</td>
<td>1.07</td>
<td>1.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>.306</td>
<td>.329</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Taking these results by titration, it will be observed that the alkaloidal determination in the bulb is apparently very low by the method designated as I.

By the second method it is found that the bulb, and the leaves and tops contain approximately 0.60% of alkaloid, while the root contains but half that amount. The alkaloid is most abundant in the flowers, which contain approximately 1.25%.

*Obtained by making correction for the amount further removed by the sulphuric acid percolation.
V. THE PHYSIOLOGICAL EFFECTS OF THE ALKA-LOID AND THE ANTIDOTES.

Chestnut and Wilcox* have carried out experiments on the injection of watery and alcoholic extracts of Zygadenus venenosus. The sheep and rabbits used in their experiments showed symptoms of poisoning such as dizzy movements, slow and labored or convulsive respiration, and finally a deep coma, which if sufficient poison had been administered, terminated fatally. The effects were apparently the same after injection of extracts or feeding of plants and were identical with those observed in animals poisoned on the open range. These authors showed that all parts of the plant contain toxic substances, so that the belief formerly held that animals must eat the bulb or root to be poisoned is erroneous. They found that of the substances which they tried as antidotes, potassium permanganate was most effective. For sheep and cattle they advised giving it in the form of a drench consisting of potassium permanganate dissolved in water.

The following toxicological study of the alkaloidal preparation by the first method is a report of Dr. Philip Mitchell and Mr. George Smith.†

I. THE FATAL DOSE FOR GUINEA-PIGS.—To obtain some idea of the relative toxicity of the preparation, intraperitoneal administration to guinea-pigs was employed. Injections of the alkaloidal solution diluted with sterilized physiological saline were made aseptically.

In Table I the results are shown arranged in the order of the dose per 100 gm. of guinea-pig. The fatal dose is seen to be between 4.6 and 5.1 mgm. per 100 gm. of animal. It is probably nearer the lower than the upper of these limits, because the animal which received 4.6 mgm. per 100 gm. recovered only with great difficulty after a long period, eigh-

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† Amer. J. Physiology, 318 (Sept., 1911).
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It seemed important also to test the effect of administration per os. The undiluted alkoidal solution as furnished us was put in small gelatin capsules and fed to guinea-pigs by forcing the capsules into the back of the mouth through a glass tube. The results are arranged in Table II, and apparently indicate that a comparatively large quantity (0.2 gm.) is required for the fatal effect.

The actual fatal dose absorbed from the digestive organs is not, however, as large as these figures would seem to show. A considerable quantity of the material fed was vomited up before it was absorbed, because the substance, as will be explained below, is a very powerful emetic. Another factor tending to diminish the toxicity when fed is the power of the organism to effect an oxidative destruction of the alkaloid before a fatal amount has been absorbed. This effect is illustrated by the rather quick recovery of guinea-pigs from the effects of injection of small doses. For example, in the last experiment recorded in Table I, 3.5 mgm. produced very
profound symptoms of poisoning, but these had entirely passed away within three hours. In another experiment, not recorded above, the subcutaneous injection of 3.7 mgm. produced undoubtedly effects which had all disappeared in an hour and a half. It does not seem necessary then to conclude from these experiments that gastro-intestinal digestion destroys the alkaloid or diminishes its toxicity, but it seems reasonable to conclude that the tendency to vomiting, the slowness of absorption and the rate of destruction of the alkaloid account for the relatively large quantity required to kill when fed.

II. The Effects on Guinea-Pigs.—After subcutaneous or intraperitoneal injection or after feeding, the alkaloid produces the same symptoms of poisoning. There are marked insalivation and frequently repeated vomiting, which begin very soon after the substance is administered and persist nearly as long as any symptoms can be observed. During the first few minutes of the onset of the effects the animal jumps and runs about the cage excitedly in the intervals between vomiting. It soon begins, however, to lose control of its muscles. The hind legs are usually affected first, but finally all the limbs become useless and the guinea-pig lies on its side completely prostrated. The respiration, at first rapid, has by this time become slower than normal and very labored. It is frequently interrupted by short periods of very convulsive breathing. Although quite unable to make any co-ordinate movements

### TABLE II.
Per Os Administration to Guinea-Pigs.

<table>
<thead>
<tr>
<th>Weight of guinea-pig gm.</th>
<th>No. of capsules used</th>
<th>Amount of sulphate gm.</th>
<th>Dose per 100 gm. of animal</th>
<th>Result</th>
<th>Time between feeding and death</th>
<th>Time between feeding &amp; apparent recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>334</td>
<td>1</td>
<td>0.0175</td>
<td>0.0053</td>
<td>Recovery</td>
<td>30</td>
<td>About 12 hrs.</td>
</tr>
<tr>
<td>332</td>
<td>4*</td>
<td>0.140</td>
<td>0.040</td>
<td>Recovery</td>
<td>About 12 hrs.</td>
<td>About 12 hrs.</td>
</tr>
<tr>
<td>278</td>
<td>6</td>
<td>0.21</td>
<td>0.0755</td>
<td>Death</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Last two capsules given one hour after the first two.  
†Occurred during the night and was not exactly known.
the animal is exceedingly irritable and responsive to reflex stimuli. The lightest touch will start a struggle, and as the symptom complex advances there comes a stage when the struggles become spasmodic and later typical tetanic spasms occur. The guinea-pig now behaves quite like an animal poisoned with strychnine, and at the slightest irritation, such as a jar of the case or a breath of wind, exhibits profound tetanus. All of its muscles are rigid and the body stiffened out in an extended position sometimes for ten seconds at a time. The heart rate, as detected by allowing the guinea-pig to lie in the observer's hand so that the cardiac impulse may be felt, is found to be much slower than normal, and this condition persists until the recovery is quite far advanced or death occurs. Defecation takes place at frequent intervals throughout the development of effects. Micturation is also generally observed. If the fatal dose has been given by injection, death occurs after an interval of about twenty to thirty minutes. No relationship between the dosage administered intraperitoneally and the time required to kill is to be seen in our results. Two typical protocols follow:

Experiment No. 1. Jan. 30, 1911.—Female guinea-pig; weight, 845 gm. Dose: 0.05 gm.; volume, 2 c.c.

3:25 p. m. Intraperitoneal injection.
3:27 p. m. Vomiting; claws mouth.
3:29 p. m. Spasmodic twitching; retching; jumping; gagging; squealing.
3:30 p. m. Defecation; vomiting; lies on side. Cannot right self.
3:32 p. m. Respiration very irregular.
3:33 p. m. Short, recurring tetanic spasms.
3:35 p. m. Respiration shallow and infrequent; spasms stop. Trembling.
3:36 p. m. Perfectly limp.
3:38 p. m. Infrequent, spasmodic respiration.
3:40 p. m. Muscular spasms.
Experiment No. 2. Jan. 30, 1911.—Male guinea-pig; weight, 352 gm. Dose: 0.0037 gm.; volume, 1.5 c.c.

3:41 p. m. Respiration, 17 per minute.
3:44 p. m. Lid reflex absent.
3:45 p. m. Tetanus.
3:46 p. m. Dead. Heart in complete diastole. Peristalsis of the intestines very marked.

Experiment No. 2. Jan. 30, 1911.—Male guinea-pig; weight, 352 gm. Dose: 0.0037 gm.; volume, 1.5 c.c.

4:06 p. m. Subcutaneous injection.
4:09 p. m. Insalivated.
4:11 p. m. Vomits a little.
4:13 p. m. Vomits repeatedly.
4:39 p. m. Labored respiration interrupted by rapid respiration. Spasm.
4:40 p. m. Control of limbs is lost. Labored and slow breathing.
4:41 p. m. Respiration, 49-50 per minute.
4:42 p. m. Slight spasm.
4:44 p. m. Respiration very irregular; heart fast. Spasm.
4:46 p. m. Respiration, 24 per minute; sometimes nine seconds elapse between breaths. Tetanic spasms; heart rate, 81 per minute.
4:49 p. m. Respiration, 23 per minute; still tries to right itself, although unable to control limbs.
4:52 p. m. Spasms.
4:53 p. m. Tetanus in back muscles when touched; very bad spasms.
4:58 p. m. Heart rate, 52 per minute.
5:00 p. m. Respiration, 29 per minute; lid reflex present.
5:02 p. m. Very acute spasms; tetanus continues for three minutes without stopping.
5:11 p. m. Acute spasms: lid reflex present.
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5:14 p. m. Spasms; great respiratory distress.
5:17 p. m. Hyperpnoea alternating with apnoea.
5:21 p. m. Spasms; tetanus; defecation.
5:29 p. m. Heart rate, 48 per minute.
5:32 p. m. Spasms; defecation; much urine passed.
5:33 p. m. Heart very slow.
5:35 p. m. Spasms; eyes closed. Reflex present.
5:39 p. m. Respiration, 32 per minute.
5:42 p. m. Spasm; respiration, 46 per minute.
5:54 p. m. Respiration, 23 per minute.
5:55 p. m. Acute spasms; defecation.
5:58 p. m. Spasms; tries to gain feet.
6:01 p. m. Copious flow of urine.
6:12 p. m. Acute spasms; spasms now follow slightest stimulus.
6:30 p. m. Regained control of fore limbs. Spasms no longer follow stimuli.
6:57 p. m. Labor and spasmodic respiration.
6:59 p. m. Respiration easier.
7:01 p. m. Heart beat, 100 per minute.
7:10 p. m. Vomits again. Appears nearly recovered.

Jan. 31, 1911.—9:00 a. m. Entirely recovered.

III. The Effects on Frogs.—The effects of administration to frogs were not very striking. It was thought that because of the tetanic spasms in guinea-pigs this poison might affect the spinal cord somewhat as strychnine does. Experiments with frogs did not fulfill that expectation. Two injections into the dorsal lymph sac of frogs with the brain pithed in which 0.175 gm. of material was administered in one case and twice that amount in the other caused some slight irritability, but the animal soon became entirely limp and unresponsive to stimuli. To another frog about 0.1 gm. was injected without pithing any part of the nervous system. Some twitchings and spasmodic movements occurred, but in a few minutes the animal seemed paralyzed, gave no further response
to stimuli, and soon died. In all three frogs there was a noticeable reddening of the skin, and the blood vessels in the web of the foot seen under the microscope appeared to be dilated.

IV. THE EFFECTS ON DOGS.—To more accurately trace the action of the alkaloidal preparation, intravenous injection into dogs was employed. The animals, seven in all, were anaesthetized in each case with ether followed by A-C-E-mixture. The alkaloidal material diluted with physiological salt solution was injected into the right femoral vein, while a tracing of blood pressure in the left carotid artery was recorded by the aid of a mercury manometer. A record of respiratory movements made by using a simple cord and pulley device to connect a recording lever with a skin suture in the thoracic or abdominal wall of the dog was simultaneously obtained.

The first injection invariably caused a fall in the blood pressure within a few seconds. Its amount was roughly proportional to the amount of material injected. 0.0035 gm. of the alkaloidal sulphate given in 1 c.c. of a fifty-fold dilution of the original preparation to a dog of 13 kilos caused a fall of blood pressure equal to 41.6 mm. of mercury, while the injection of 0.0105 gm. in 5 c.c. of the same dilution in a dog of 20 kilos produced a fall of 82 mm. The proportionality, however, was not always maintained. The quickness of the recovering rise also depended on the amount injected, and if more than 10 mgm. of the alkaloidal sulphate was given the recovery was very slow. In no case did the blood pressure ever return to quite as high a level as that recorded before the first injection. The cause of this depressor effect is not a simple one. The most potent factor in the initial drop is the marked slowing of the heart rate. That this is due for the most part to an effect on the cardio-inhibitory centre appears from the fact that after section of both vagi an injection of the quantity usually employed did not cause the usual striking depression of the heart rate but only a very slight decrease.
A second factor involved in the fall of blood pressure is vaso-dilation. Even when the pulse had quite recovered to its initial rate, as seen in several tracings, the blood pressure was found to be still low, and only recovering so slowly that after some half-hour's observation it did not reach its original level. Also, the arterial pressure fell too much when injections were given after double vagotomy to be accounted for apparently by the slight slowing of the heart.

The effect on respiration is, in general, to slow it by a prolongation of the expiratory phase. This effect is slightly modified after the vagi have been severed.

A tracing to show the effect of injection without previous vagotomy is given (Fig. 1). The tracing seems to show that the alkaloid acts on the cardio-inhibitory and respiratory centres, or at least produces effects which involve them.

The results so far discussed are those obtained by an initial injection of small quantities of the alkaloid. Subsequent injections or the administration of larger quantities produced more complex effects. Second, third, and fourth injections of quantities comparable to it ordinarily used for our first, produced a successively smaller effect in decreasing the already lowered blood pressure. After a considerable quantity, for example, 0.0665 gm. has been given, a compensatory hastening of the heart rate produced a slight rise in blood pressure, and this effect was obtained after section of the vagi with comparatively small quantities of the alkaloid. Fig. 2 shows the effect of injection of 0.0105 gm. into a dog of 8.2 kilos after a previous injection, about twenty minutes earlier, of the same quantity. The rise in blood pressure amounts to 36 mm. of mercury, and the change in the heart rate is from 117 per minute before the injection to 157 per minute shortly after. In this case the vagus nerves were severed at the beginning of the experiment and the first injection gave a slight fall in pressure.
Figure 1. Effect of first injection of small quantity with vagi intact. Upper tracing shows carotid pressure, lower one respiration. Time = 0.6 sec. At A 0.0035 gm. of alkaloid in 1 c.c. of saline was injected. Dog weighed 22 kilos.
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Figure 2. Effect of a second injection, after section of the vagi. Tracing of carotid pressure partly superimposed on respiration curve because the pressure was lowered from the effects of the first injection. Time = 0.5 sec. At A 0.0175 gm. of alkaloid in 5 c.c. of saline was injected.
When sufficiently large quantities of the alkaloid had been administered to a dog, its heart showed an effect distinctly different from that produced by the previous injection of small quantities. Instead of the decrease of the heart rate with increase in the force of the beat seen in the initial injections or the increase in rate with slight decrease in force observed after further injections, there appeared in the more advanced stages of poisoning a marked fluttering of the heart. A tracing of such an effect is shown in Fig. 3. Sometimes the heart action as shown by the blood pressure curve would produce a series of very irregular beats interrupted at quite regular intervals by groups of three or four regular but very quick, shallow beats.

**Figure 3.** The effect on the heart of cumulative action of the alkaloid. Upper tracing shows carotid pressure, lower one respiration. Time = 0.6 sec. At A 0.0175 gm. of the alkaloid in 5 c.c. of saline was injected. The vagi were intact. By means of three previous injections, 0.0315 gm. of the substance had been given.

The effects of larger quantities of the poison on respiration were also noteworthy. Instead of the mere slowing of
respiration, there appeared a considerable irregularity characterized by movements more or less convulsive. This effect in a mild degree can be seen in Fig. 2.

One of the most interesting effects of this substance was that on intestinal peristalsis. Even the smallest dose administered, 0.0035 gm., given intravenously to a dog of 20 kilos, caused in a few minutes unmistakable intestinal rumblings. In all the dogs used defecation occurred several times, even though stools had previously been passed at the time of etherizing. In the course of an hour, after three or more injections of small quantities, fluid or semi-fluid feces were observed in each case. Micturition also took place in many of the dogs during the experiment. This involuntary evacuation of intestines and bladder in anaesthetized dogs was plainly the result of the action of the alkaloid, because effects followed quickly after injection and in the case of defecation were proportional to the amount given. Such results confirm our observations with guinea-pigs. The influence of the substance on the vomiting mechanism of dogs was not tested, as in guinea-pigs, because no injections were made without previous anaesthesia.

Several of the dogs while still anaesthetized were killed by intravenous injection of a fatal dose. In each case the heart beat became very irregular and soon stopped. Invariably the heart failed before respiration. At the moment respiration ceased, however, the dog passed into a death struggle exhibiting tetanic spasms of the entire body. The convulsion was always brief, and though in two cases followed by a few spasmodic respiratory movements was never, so far as we observed, succeeded by any revival of the heart beat. The heart was found in the open thorax in complete diastole, as was seen also in post mortem examination of the guinea-pigs.

No attempt to determine the fatal dose for dogs was made. The smallest quantity which gave a fatal result was
Wyoming Experiment Station.

0.105 gm. in 3 c.c. of saline injected intravenously into a dog of 10 kilos without previous injections.

Our experiments have led to the following conclusions:

1. The alkaloidal preparation from *Zygadenus intermedius* slows the heart rate by action apparently on the cardio-inhibitory centre.
2. It slows respiration by an effect involving the respiratory centre.
3. It causes vaso-dilation.
4. In quantities approaching the fatal dose it hastens the heart rate and produces both irregularity of the heart beat and convulsive respiration.
5. The fatal dose given intravenously to dogs stops the heart before respiration ceases.
6. The fatal dose for guinea-pigs is between 4.6 and 5.1 mgm. per 100 gm. of animal.
7. It has a very powerful action, whether injected or fed, both as a purgative and an emetic.

Toxicity of Resin.—In order to ascertain the pharmacological properties of the resin a series of experiments were conducted, the results of which demonstrated it to be physiologically inert.

A dog weighing 7.2 kilograms was given 0.2 gm. of the resin in the form of pills, three days later 0.4 gm. further was administered, and lastly 0.5 gm. was fed. All of these trials were without untoward effects, as regards obvious symptoms. These results do not agree with those obtained by Vejux-Tyrode,* who found the resin of *Zygadenus* to be toxic. The original article is not at hand, but it is possible that another species was examined in this work.

We have deferred the study of the antidote until we have isolated in a pure condition a definite alkaloid. The properties of the alkaloidal material prepared by the second method de-
scribed above, gives good promise of such success as we have found it to be crystallizable. It should be stated in closing that Hunt† states that he succeeded in removing the alkaloid from the circulation, rapidly and with very happy results, by the use of diuretics such as diuretin or caffeine.

†Loc. cit.