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Respiratory effects of potent hypotensive derivatives of veratrum.
RESPIRATORY EFFECTS OF POTENT HYPOTENSIVE DERIVATIVES OF VERATRUM

by

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Table of Contents

Introduction ............................................. Page 1
Methods .................................................. 9
Results .................................................... 13
Discussion ................................................ 16
Summary (Abstract) ...................................... 21
Illustrations .............................................. 23

Table 1. Effect on Ventilation Volumes in Anesthetized Dogs subsequent to 10 minute I.V. Infusion of Pure Alkaloids and Mixtures from Veratrum at Equi-hypotensive Doses ........................... 13.

Figure 1. Dose-response Relationship of three Veratrum preparations ........................................... 13.

Table 2. Time Relationships in Decreased Minute Expiratory Volume ......................................... 14.

Figure 2. Respiratory and Blood Pressure Tracings following administration of Acetic acid-propylene glycol extract of crude dried root of Veratrum viride .................................................. 14.

Table 3. Results of studies to determine the site of action of Veratrum Derivatives in the production of respiratory depression ................................................. 15.

Bibliography .............................................. i
Introduction

Veratrum has been known for centuries as a potent biologic substance. Hippocrates (12) in an early reference to the drug, which was first used as an emetic, speaks of toxic phenomena of "spasm" following administration of Hellebore. He further warns against giving Hellebore as a purgative to persons suffering from respiratory diseases since "dangers may arise from this practice which may be blamed upon the drug".

Early animal experimental work on Veratrum album (Hellebore) was done largely in Germany. Among the early workers was von Meissner, who first isolated Veratrine from the crude root extract. (22) Veratrine was at that time regarded as a pure alkaloid and the active principle of the root. Its discovery stimulated research into its pharmacologic properties by workers of the early nineteenth century. Not until the work of von Bezold in 1865, 1866, and 1867, however, was there definitive investigation of these properties. Von Bezold, in his publication in 1867 (1) mentions many other workers, among them Magendie in 1821 and von Bardisley, Turnbull, Ebers and Vogel in 1836, who had reported observations regarding the activity of the extract of the crude root of Veratrum album. According to von Bezold, their reports were in general agreement, describing
toxic phenomena of convulsions, respiratory arrest as the chief cause of death in animals given lethal doses of the drug, and emesis in those animals capable of vomiting. A few workers described a slow weak pulse in addition to the other manifestations of the drug. Original interest in the drug lay chiefly in its effect upon nerve and muscle, which consisted of prolongation of the twitch response into spasm, with rapid loss of ability of the nerve-muscle preparation to respond at all.

Von Bezold, (1) in 1865, began the most ambitious investigation of the properties of this drug which had been yet attempted. He tried to locate and define physiologic mechanisms responsible for the action of the agent in both frogs and several species of mammals. The preparation he used was an acetic acid extract of the dried ground root of Veratrum, which he administered orally, sub-cutaneously, and intravenously. He reported respiratory depression resulted from the action of the drug, and ascribed this effect to the action of the agent upon nerve and muscle, particularly the diaphragm. Circulatory effects of the drug were described, and he concluded that the profound hypotension he observed was the result of bradycardia and of vasodilatation. In this connection he described the reflex that bears his name, ascribing hypotension and bradycardia to a reflex arc
whose afferent limb was the vagus, with receptors located somewhere within the heart. Other effects of the drug which were noted were emesis, gastrointestinal irritation, and effects upon both nerve and muscle.

In 1887, Lissauer, (22) stimulated by the separation of Cevadine from Veratrine, reinvestigated the pharmacologic properties of Veratrine. He studied the effects of a substance he described as crystalline Veratrine (which may have been Cevadine) upon isolated muscle-nerve preparations of frog; studied and described in great detail the lethal manifestations of the agent; and investigated circulatory and respiratory effects in mammals, chiefly rabbits, but also cats and dogs. The lethal dose in frogs was found to vary between 0.25 and 1.0 mg; in rabbits it varied between 2.5 and 8.0 mg. He found that atropine prevented death in about 1/2 of the animals in which it was administered prior to dosage with Veratrine. Circulatory changes he described were bradycardia and hypotension. The hypotension he concluded was due to a central action of the agent since blood pressure could be promptly raised by asphyxias. Of most interest to the present study, however, were his findings in regard to respiration. Respiratory arrest in the expiratory phase was the immediate cause of death in all animals given a lethal dose of Veratrine. This observation was particularly
noticeable in dog experiments where arrest was preceded by marked dyspnea. In those animals which survived, the rate of respiration decreased by about 1/2 and the dyspnea was followed rather shortly by more regular respiration. He felt that the respiratory effects were due to depression of central respiratory innervation.

Isolation of pure alkaloids of veratrum stimulated further investigation into the pharmacologic effects of each. In 1892 Watts-Eden (36) published observations upon the effect of Protoveratrine, contrasting it with the actions of Veratrine. He showed that Protoveratrine was much more potent than Veratrine with a relative potency of 25:1 on a basis of lethality. He described the same general effects of convulsions, respiratory depression, emesis and hypotension with bradycardia, which differed only quantitatively from those produced by Veratrine. The only qualitative difference found was the "vagus paralyzing" action of Protoveratrine upon the sensory component of the nerve.

The effect of Cevadine was similarly studied in 1902 by Heinz. (11) No different actions were described for Cevadine than had been described for other potent veratrum derivatives. He observed respiratory arrest in the expiratory phase, and remarked upon the dyspnea that preceded it.
The next pure alkaloid to be described was Veratridine in 1915. More modern workers have investigated the effects of pure alkaloids of veratrum as they became available. The effects of Germerine were described by Haas in 1938. The action of Protoveratrine was reinvestigated by Krayar and his associates in 1944. Cevine and Veratridine were studied by Mendez and Montes in 1943 and again by Moe and associates in 1944. Krayar stated that the alkamine, Veratramine, produced no respiratory depression, in contrast to all the potent ester alkaloids. To date, there has been no study of respiratory effects of the Veratrum alkaloids which has measured the depression in quantitative terms or which investigated the sites of action of these agents at all conclusively. The availability of newly isolated potent hypotensive alkaloids of veratrum has made possible the study of additional compounds in this investigation.

Present day interest in veratrum derivatives stems from their ability to depress blood pressure. Since hypotensive potency is the desired therapeutic effect of these compounds, it seemed desirable and of interest to measure respiratory depression produced by therapeutic hypotensive doses or by multiples of those doses. A method of measuring hypotensive potency by bio-assay depending upon the fall in mean
arterial blood pressure in anesthetized dogs has been developed by Maison, Gotz, and Stutzman. (24) Therapeutic trial indicated that the dosage which produces a fall in mean arterial blood pressure of 32% as measured by this assay, is the effective therapeutic dose in man. (6,33) The present study concerning respiratory effects of veratrum preparations uses as a basis of comparison of the separate alkaloids and mixtures of alkaloids their effect at equi-hypnotic dosage.

It has been suggested in the literature that more than one mechanism plays a part in the production of respiratory depression. (3,21) The existence of both reduction in central drive and of broncho-constriction seemed probable in view of the frequency of apneic periods, the production of respiratory arrest, particularly in the expiratory phase. Methods of measuring broncho-constriction available in the past have included contraction of isolated tracheal rings; perfusion of isolated rabbit lungs with water, with measurement of resistance to flow; (35) Jackson plate methods where the anterior chest wall is replaced surgically with a plate so that intrathoracic pressure relations may be measured; and a similar method which utilized a Jackson spear to measure pressure changes. (14,15) Serious objections may be raised to all of these methods. The use
of isolated tissue may be condemned since its response cannot be assumed to be representative of the actions of tissue in the intact animal. The reaction of tracheal rings cannot be assumed to be identical with that of the bronchial musculature. Perfusion with fluid in no way approximates the normal air flow throughout the bronchial tree. Methods which depend upon the use of the Jackson breast plate, or the spear inflict considerable trauma upon the test animal and are technically difficult to prepare; with air leaks about the operative site a frequent complication. They depend upon positive pressure respiration which has been shown by Sarnoff (32) to alter circulatory dynamics within the pulmonary circuit.

The method used in the present study was that of electrophrenic respiration as modified from Sarnoff. (29,30) (The technique will be described in the section on methods). This technique provided an artificial central drive which was constant and could be controlled by the experimenter. The operative procedure was not extensive or excessively traumatic, and the observation of peripheral changes in ventilatory function could be made with normal physiologic mechanisms intact in the lung and bronchiolar tissue. Circulatory dynamics were observed to remain relatively unchanged from those of the animal breathing spontaneously. (32)
In summary, the objectives of this work were (a) to explore the respiratory depressant action of equi-hypotensive doses of a number of alkaloids, some well known and some never before tested for their respiratory action; (b) to establish the nature of the dose response relation of characteristic pure and mixed agents and; (c) to investigate the contribution of central and peripheral components of the respiratory action of a veratrum mixture now in clinical use and which contains many of these potent ester alkaloids.
Methods

Drugs tested included the hypotensive ester alkaloids, (1) Protoveratrine (Lilly, lot P44366, m.p. 246°C, and Riker, lots 23-2cc-22-24P and 23-3cc-3, from Veratrum viride, m.p. 262°C); (2) Germitrine (Squibb, lot JF94ABC3, m.p. 199-210°C); (4) Germidine (Squibb, lot JF66G8, m.p. 230-232°C); (5) Veratridine (Riker, lot 5-18-50, m.p. 171°C); (6) Cevadine (Ringel, m.p. 209°C); and (7) Neogermitrine (Squibb, lot JF115G5, m.p. 231-232°C). Veratramine (Penick, lot RHP 79/A51, m.p. 204-209°C) the only potent alkamine, was tested as were the mixtures Veriloid* (Riker); Veratrine (Penick, lot 1801 IGB); an acetic acid-propylene glycol extract of dried ground root of Veratrum viride#; and PNPlll (Squibb, a mixture of Germitrine and Neogermitrine). All drugs were administered by 10 minute administration.

*Veriloid (trade mark of the Riker Laboratories, Los Angeles, is a potent hypotensive reproducible derivative of Veratrum viride.

#The extract was made by stewing 100 mg. of dried root in 5% acetic acid for 1 hour, filtering and washing with 0.1% acetic acid. The residue was stewed in propylene glycol for 20 minutes, filtered and washed with 0.1% acetic acid in saline as before. The two extracts were then combined. Volume was controlled so that final concentration represented the extract from that amount of crude root.
intravenous infusion to dogs anesthetized with pentobarbital sodium. Dose-response curves were obtained for Protoveratrine, Veriloid, and Veratridine at 1, 1.75, and 3 times the standard hypotensive dose as determined by dog assay. (24) (The standard hypotensive dose is defined as that dose which produced in anesthetized dogs an average fall of pressure equal to 32% of the pre-existing mean arterial blood pressure.) Other hypotensive agents were administered at 1 and 3 times this standard hypotensive dose. All respiratory effects, therefore, were measured at equivalent hypotensive dosage or multiples of that dosage for each of the substances tested.

Criteria of respiratory depression were established as (a) the reduction to the lowest minute observation of expiratory ventilation volume expressed as a percentage of the normal volume; and (b) the occurrence of apnea and ventilatory arrest. Minute volume, measured by wet test gasometer, was recorded for 10 minutes preceding, during, and for 20 minutes following infusion of the test material. Animals were intubated with cuffed endotracheal tubes and were connected with the gasometer through a polarizing flutter value. One criterion of significance for reduction of minute volume was that it exceeded twice the standard deviation of the mean control minute volume. The effect of
injection and time upon the minute expiratory volume was
determined by control infusions of saline.

Respiratory movements were recorded kymographically
with a heart lever connected by cord sutured to the anterior
chest wall at the tip of the xyphoid process. Blood pres-
sure from the femoral artery was recorded simultaneously
in certain of the animals.

In order to delineate bronchoconstriction, the method
of electrophrenic respiration (EPR) modified from Sarnoff
(29, 30, 31) was used. The contributory segmental roots of
the phrenic nerve derived from C₅, C₆, and C₇ were isolated
in the root of the neck and dissected free. Shielded hook
silver electrodes, connected in series, were placed so that
both nerves were stimulated. Nerves were fired with volleys
from a Grass Stimulator at a voltage of 0.6V and a frequency
of 60 cycles. Rate of volleys was controlled with a Photo-
switch timer with an added Cornell-Dubilier decade capacitor,
and so regulated that electrophrenic stimulation produced a
minute expiratory volume exceeding by about 50% that observed
while the animal was breathing spontaneously. Duration of
the stimulating volley was set on the Photoswitch so that
the inspiratory phase occupied about 1/4 of the total respir-
atory cycle. Expiratory volume was measured as described
above.
In certain experiments vagi were isolated from the carotid sheath in the neck and sectioned bilaterally in intact animals and EPR animals, with observations on the effect on expiratory volume. The effect of atropine sulphate, 1.0 mg/kg, (checked for complete cholinergic blockade by the U.S.P. epinephrine assay method) upon the respiratory response to veratrum was also observed in EPR dogs.

In all studies determining the site of action of veratrum, Veriloid was used as the test substance because of its availability in unlimited quantity and uniform potency; because it is being used therapeutically; and because the confidence limits of its potency were the smallest of any derivative studied.** Dosage was established at 3 mcg/kg/minute for 10 minutes in the mechanism studies in order to produce a response more readily measurable. A dose-response relationship was determined at 1, 3, and 5 times the standard hypotensive dose.

Repeated infusions of Veriloid were given to animals breathing spontaneously and on electrophrenic stimulation to test for possible tachyphylaxis. No infusion was repeated until blood pressure returned to control levels.

**Actually the studies of potent hypotensive derivatives of veratrum indicated no qualitative differences among these substances.
Results

The effects of the various veratrum derivatives upon expiratory minute volume are shown in Table 1. It will be noted that there are qualitative differences in the response manifested at the 3X dose. Veratramine, Cevadine, and Veratrine produced both ventilatory depression and stimulation as indicated by the two separate results reported for each substance. The effect of the first two substances was predominantly stimulant and the latter substance shows essentially a diphasic relationship which is especially interesting in view of its composition. All of the other potent hypotensive derivatives of veratrum tested produced decreases in minute volume which fell in the range of 40-60% and which by the "r" test (17,18) were shown not to differ statistically from each other.

The results of saline control infusions showed that no influence could be attributed to the effect of injection or to the passage of time, with the limits of these experiments. Tachyphylaxis was not demonstrated.

Dose-response curves were obtained using Veratridine, Veriloid, and Protoveratrine (Lilly, and Riker, from Veratrum viride). Figure 1 is the composite graph of these curves; in addition, the dose-response relationship obtained on EPR animals using Veriloid as the test substance
may be seen in this graph. Regression lines were obtained for each test substance, and parallelism between the lines determined. (23, 4, 19) It was found that the lines were linear, and that no significant difference existed between their slopes.

In table 2 are summarized the data on time relationships observed in the production of decreased minute volume by those agents that depressed respiration. It includes similar observations made upon EPR animals. It will be noted that the duration of action varies among the different agents, though the onset and time of greatest decrease are relatively less variable.

Respiratory tracings as obtained from the excursion of the anterior chest wall frequently showed a change in pattern indicating increased expiratory effort, as well as a decrease in frequency of respiratory movement during the period of action of the depressant drugs. Figure 2 is a kymographic record produced by administration of acetic acid - propylene glycol extract of dried root of Veratrum viride, which shows a period of apnea, and a pattern of tracing which, when combined with decreased ventilation volume, suggested bronchoconstriction. Direct observation of the animals during the infusion of the drugs showed increased expiratory effort in most cases.
The results of procedures done to determine the site of action of respiratory depression are summarized in table 3. It was found that no procedure completely abolished a decrease in expiratory volume, though comparison of the results obtained in animals breathing spontaneously with those on EPR shows significant modification in the degree of decreased ventilatory response. Bilateral vagectomy did not abolish the decrease in minute volume in either the EPR or the spontaneously respiring animals. The tremendous variation encountered in the vagectomized, spontaneously breathing dogs precludes comparison with other animals, though these experiments demonstrate that the presence of the vagus is not essential for depression of ventilation.
Discussion

The variation in response to veratrum derivatives seen in these experiments was so great that interpretations must be made most conservatively. It would appear that with the exception of Cevadine, Veratramine, and Veratrine, potent hypotensive derivatives of veratrum depress ventilatory function to a degree that was about equal when dosage was determined by equi-hypotensive potency. Variation in response and the error inherent in the method prevent further conclusion as to relative respiratory depressant potency. The production of respiratory arrest by these agents was only statistically significant in the difference between no arrests and 5 arrests in 15 dogs. The occurrence of periods of apnea combined with the production of respiratory arrest suggested a decrease of central drive as a possible mechanism of production of reduced ventilation. Respiratory tracings combined with observed expiratory effort in the presence of decreased minute volume suggested that bronchial constriction might also be a contributing factor.

These results are essentially in agreement with those appearing in the literature, in the conclusion that most veratrum derivatives depress respiration. In the case of Veratrine and Cevadine, earlier workers have observed
decrease in respiratory function following administration of these drugs. (22,11) An examination of the dosage utilized by these workers provides one explanation of the disparity with present results since reported doses exceeded those utilized in this study by factors of 2 or 3 times, and were not dictated by hypotensive action. Krayer (21) reported no depression following the administration of Veratramine. Other derivatives tested did not demonstrate a quantitative difference in the degree of respiratory depression when dosage was determined in terms of the relative hypotensive potency of these substances. Respiratory effects of veratrum derivatives appear to be nearly as intimately related to hypotensive potency as emetic properties. (34)

Utilization of the method of electrophrenic stimulation combined with measurement of minute expiratory volume provided a measure of the degree of broncho-constriction produced by veratrum.*** The parallelism of the dose-response curves obtained using Veriloid in animals breathing spontaneously and in EPR animals indicates that the proportion of ventilatory depression caused by bronchoconstriction remained constant at all doses tested. By direct subtraction

***While this study was in progress, the use of electrophrenic respiration as a measure of bronchoconstriction was reported by Alexander, W.M. and Abreu, B.E., Fed. Proc., 10: 276, March 1951.
of the values obtained for EPR animals from those obtained during spontaneous breathing, the contribution of the depression of central drive to the total ventilatory decrease was found to be about 40\%, since bronchoconstriction, as measured by the method of electrophrenic stimulation, accounts for 60\%. The value obtained for expiratory minute volume following bilateral vagectomy in the spontaneously breathing animal suggested that one possible mechanism for central depression may be mediated by vagal afferents, which have been described in the literature, \(^{(3)}\) although the tremendous variation observed made impossible the drawing of conclusions from present data.

The observed bronchoconstriction was not considered to be dependent upon reflex vagal mechanisms since no statistical difference was found between the decrease in minute volume observed in EPR animals and that seen in EPR-Vagectomized dogs. Similarly no difference (in EPR dogs) was found between the atropinized as compared with the non-atropinized condition. This indicated that the bronchoconstrictor action was not cholinergic in nature. It was concluded therefore, that veratrum derivatives depress ventilation: (a) centrally by either direct depression of the respiratory center or through a vagal afferent reflex; and (b) peripherally to produce bronchoconstriction either by
direct action on the bronchial smooth muscle or by extra-
vagal, non-cholinergic reflexes. Veratrum derivatives have
been shown to have a direct musculotropic effect in the
isolated gut (9) which bears some resemblance to their bron-
choconstrictor action in that it is unmodified by atropine
blockade. It seems possible that a similar mechanism may
operate upon the bronchiolar smooth muscle.

Action of these agents upon the phrenic nerves or
upon the striated muscle of the diaphragm as postulated by
von Bezold (1) cannot be completely eliminated by these
experiments, but seems improbable since the electrophrenic
preparation which is essentially a muscle-nerve system
appeared to function normally through the entire period of
each experiment, and did not demonstrate the characteristic
action of veratrinized nerve.

Dose-response relationships indicated that the reports
in the literature which related respiratory depression to
increasing dose were essentially correct. No tachyphylaxis
was found in the respiratory response of spontaneously
breathing or EPR animals, if the interval between infusions
was sufficiently great to permit recovery of blood pressure.

The ventilatory decrease produced by the 1 X dose is
of interest since this is adequate clinical dosage. Res-
piratory depression has occasionally been noted in some 200
administrations of Veriloid, but was very transient and never gross enough to be deleterious. (33)
Summary

Respiratory effects of potent hypotensive pure alkaloids and mixtures of veratrum were determined in terms of their effect upon ventilation volume and by the occurrence of apnea and respiratory arrest. Pure alkaloids included Veratridine, Germidine, Germitrine, Neogermitrine, Germerine, Protoveratrine, Veratramine, and Cevedine. Mixtures tested were an acetic acid - propylene glycol extract of dried ground root of Veratrum viride, Veriloid, Veratrine, and PNP 111. Dosage was determined by hypotensive potency of these agents as determined by the dog assay. At equipotent hypotensive dosage, Cevedine, Veratramine, and Veratrine produced respiratory stimulation with an increase in minute expiratory volume. The other agents tested depressed ventilation 40-60% at the 3 X dose, with no statistical differentiation possible. The occurrence of periods of apnea and of respiratory arrest suggested a depression of central drive as one mechanism by which decreased minute volume had been produced. Altered respiratory tracings suggested the presence of bronchial constriction.

By the method of electrophrenic stimulation, differentiation of the central and peripheral effects of Veriloid was made possible. It was found that bronchoconstriction
accounted for approximately 60% of the observed decrease in minute volume, and that this decrease was independent of vagal factors and of cholinergic effects. It was concluded that the respiratory depression produced by veratrum derivatives was dependent upon (1) reduction in central drive, and (2) broncho-constriction due to either direct action of the agents upon the bronchial smooth muscle, or to non-vagal, non-cholinergic reflexes.
Table 1

Effect on Ventilation Volumes in Anesthetized Dogs Subsequent to 10 Minute I.V. Infusion of Pure Alkaloids and Mixtures from Veratrum at Equi-hypotensive Doses.

<table>
<thead>
<tr>
<th>Drug</th>
<th>B.P. Fall in % of Control</th>
<th>Ventilation Change in % of Control</th>
<th>Incidence of Respiratory Arrest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average 5 dogs</td>
<td>Mean and S.D.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1X 3X</td>
<td>1X 3X (Number of dogs)</td>
<td></td>
</tr>
<tr>
<td>Veratridine (21.3 mcg/kg)</td>
<td>36 53</td>
<td>-21 -60 ± 35</td>
<td>1/20 4/15</td>
</tr>
<tr>
<td>Germidine (4.16 mcg/kg)</td>
<td>33 49</td>
<td>-18 -61 ± 31</td>
<td>0/5 5/16</td>
</tr>
<tr>
<td>Protoveratrine (2.2 mcg/kg)</td>
<td>30 46</td>
<td>-17 -55 ± 21</td>
<td>0/5 2/15</td>
</tr>
<tr>
<td>Neogermitrine (1.1 mcg/kg)</td>
<td>57</td>
<td>-47 ± 19</td>
<td>0/5</td>
</tr>
<tr>
<td>Germitrine (0.75 mcg/kg)</td>
<td>46</td>
<td>-44 ± 14</td>
<td>0/5</td>
</tr>
<tr>
<td>Germerine (1.9 mcg/kg)</td>
<td>34 55</td>
<td>-33 -45 ± 22</td>
<td>1/5 1/15</td>
</tr>
<tr>
<td>Protoveratrine (Riker) (2.1 mcg/kg)</td>
<td>28 40</td>
<td>-28 -44 ± 14</td>
<td>0/12 0/12</td>
</tr>
<tr>
<td>PNP 111 (0.94 mcg/kg)</td>
<td>28</td>
<td>-27 -49 ± 27</td>
<td>0/5 2/15</td>
</tr>
<tr>
<td>Veriloid (10 mcg/kg)</td>
<td>29 53</td>
<td>-23 -47 ± 15</td>
<td>0/15 0/15</td>
</tr>
<tr>
<td>Acetic acid-PGA Extract V.viride (4.16 mg/kg)</td>
<td>26 59</td>
<td>-16 -39 ± 20</td>
<td>0/5 1/15</td>
</tr>
<tr>
<td>Veratramine (380 mcg/kg)</td>
<td>21</td>
<td>72 ± 42 (7 dogs)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-38 ± 22 (8 &quot;</td>
<td></td>
</tr>
</tbody>
</table>
Table 1 (cont.)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Duration</th>
<th>Lowest Minute Ventilation</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cevadine</td>
<td>36</td>
<td>-35±16 (4 dogs)</td>
<td></td>
</tr>
<tr>
<td>(23 mcg/kg)</td>
<td></td>
<td>58±34 (11 &quot;&quot;)</td>
<td></td>
</tr>
<tr>
<td>Veratrine</td>
<td>48</td>
<td>-73±27</td>
<td>2/15</td>
</tr>
<tr>
<td>(37 mcg/kg)</td>
<td></td>
<td>65±32</td>
<td></td>
</tr>
</tbody>
</table>

*Figures represent the lowest single minute ventilation in the period during and immediately after infusion of the agent.*
Figure 1.

- Protovatrine — Regression Equation is $Y = 44.96 + 56.9X$ and Correlation Coefficient is 0.559.
- Veriloid — Regression Equation is $Y = -25.84 + 48.19X$ and Correlation Coefficient is 0.427.
- Veriloid EPR — Regression Equation is $Y = -19.64 + 34.66X$ and Correlation Coefficient is 0.529.
- Veratridine — Regression Equation is $Y = -86.37 + 79.57X$ and Correlation Coefficient is 0.513.

(Statistical Analysis was done by Mr. Cliff Mountain of the Office of Statistical and Research Services, Boston University).
Table 2

Time Relationships in Decreased Minute Expiratory Volume Produced by the 10 Minute Infusions of Pure Alkaloids and Mixtures from Veratrum at Equihypotensive Doses in Anesthetized Dogs - 3 X Dose*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reduction of Ventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of Onset from beginning Infusion</td>
</tr>
<tr>
<td>Veratridine</td>
<td>2</td>
</tr>
<tr>
<td>Germidine</td>
<td>4</td>
</tr>
<tr>
<td>Germitrine</td>
<td>4</td>
</tr>
<tr>
<td>Neogermitrine</td>
<td>2</td>
</tr>
<tr>
<td>Germerine</td>
<td>4</td>
</tr>
<tr>
<td>Protoveratrine</td>
<td>4</td>
</tr>
<tr>
<td>Veriloid*</td>
<td>3</td>
</tr>
<tr>
<td>Veriloid*EPR (10 animals)</td>
<td>4</td>
</tr>
<tr>
<td>PNP 111</td>
<td>4</td>
</tr>
<tr>
<td>Acetic acid - PGA</td>
<td>4</td>
</tr>
<tr>
<td>Extract of ground root 6 of V. Viride</td>
<td>4</td>
</tr>
</tbody>
</table>

All times in minutes and representing average of 15 dogs unless otherwise noted.
Respiratory and blood Pressure tracings following administration of Acetic acid - propylene glycol extract of crude dried root of Veratrum viride.

A = the beginning of the infusion of 1.248 mg/kg/minute; B = the end of the infusion and the beginning of the wash of normal saline; C = the end of the saline wash; D = the period of apnea and beginning expiratory effort.
Table 3

Effect of Veriloid* Upon the Ventilation Volume of Anesthetized Dogs in Studies To Determine the Site of Action of Veratrum Derivatives in Respiratory Depression***

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>No. of Dogs</th>
<th>Ventilation Fall of Control</th>
<th>B.P. Fall of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous Respiration (SR)</td>
<td>15</td>
<td>47 ± 15</td>
<td>53</td>
</tr>
<tr>
<td>(30 mcg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrophrenic Respiration (EPR)</td>
<td>13</td>
<td>12 ± 9</td>
<td>29</td>
</tr>
<tr>
<td>(10 mcg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>28 ± 12</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>45 ± 21</td>
<td>63</td>
</tr>
<tr>
<td>EPR plus Bilateral Vagectomy</td>
<td>5</td>
<td>26 ± 7</td>
<td>42</td>
</tr>
<tr>
<td>(30 mcg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPR plus Atropinized (30 mcg/kg)</td>
<td>5</td>
<td>32 ± 8</td>
<td>39</td>
</tr>
<tr>
<td>Spontaneous Respiration</td>
<td>5</td>
<td>32 ± 24</td>
<td>56</td>
</tr>
<tr>
<td>plus vagectomy (30 mcg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figures represent the lowest single minute ventilation in the period during and immediately after infusion of Veriloid.

***Statistical treatment of data obtained by the 10 minute infusion of 30 mcg/kg showed significant difference between SR and EPR with a "p" value less than 0.01; between SR and EPR-Vagectomy with a "p" value less than 0.01; and between SR and EPR-Atropinized with a "p" value of less than 0.05. No statistical significance was found between the EPR, EPR-vagectomized, EPR-atropinized, SR-vagectomized.
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