The effects of veratrum derivatives upon the isolated intestine of the rabbit and upon the intact intestine of the trained unanesthetized dog.

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BY

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This thesis deals with the study of the effects of Veriloid and several pure alkaloids of veratrum on isolated small intestine of the rabbit and intact intestine of the trained unanesthetized dog.

Part I contains a discussion of the nature of the problem, a description of the pharmacologic agents employed, some pertinent words regarding the physiology of isolated and intact intestine, and the methods utilized in the investigation. Part II discussed the specific effects of these agents upon the intestine.
PART I * INTRODUCTION

A. The Nature of the Problem:

Renewed interest in veratrum preparations has occurred within the past several years. The promising results of clinical (25) and experimental (12, 13, 21) efforts to reduce blood pressure by means of these agents have prompted the search for purified fractions of the crude drug.

Considerable effort has been expended in studying the effects of veratrum on cardiovascular dynamics and skeletal muscle (7). Until recently, Veratrine, a mixture of several alkaloids, was the predominant preparation investigated. Pure alkaloids and reproducible extracts of veratrum have now, to a great degree, supplanted Veratrine. Extensive pharmacologic investigation of Veriloid and development of an assay method using unit blood pressure fall in the anesthetized dog, have shown it to be a reproducible mixture of active veratrum alkaloids (13). This same method of assay has been applied to the pure veratrum alkaloids and their hypotensive potencies are available (12). The aims of this study were (a) to obtain information concerning the relative spasmogenic effects of these agents and the locus of this action and (b) to determine the relationship between the hypotensive and spasmogenic potencies. Such information might offer potentialities as a more accurate means of assay of clinical preparations or as a method of following the concentration of veratrum in the blood or other body fluids.

B. The Veratrum Alkaloids and Extracts:

(1) Chemistry
Veratrum alkaloids and extracts are derived from several sources. The most common of these are the roots of Veratrum viride - Aiton (green hellebore) found in North America, the roots of Veratrum album - Linne (white hellebore) of European origin, and Asagroea officinale - Lindley (sabaddilla), native to South America. These plants contain a variety of alkaloids, alkamines, organic acids, acrid resins, gums, fat, and starch (7). The potent depressor alkaloids are esters whereas their hydrolysis products (7), alkamines and organic acids, are relatively impotent. (12) (Table 1). The chemical configuration of the alkamines has not yet been completely defined although present evidence suggests the presence of the basic sterol structure (7).

Veriloid is a mixture of amorphous alkaloids extracted from Veratrum viride (21). Present evidence suggests that its depressor activity is destroyed by saponification, that a highly potent neogermitrine-like alkaloid which may account for as much as 50 per cent of its total activity, and protoveratrine, which accounts for at most 5 per cent of total activity, are present. The relatively impotent alkamines rubijervine and isorubijervine which were present in the original Veriloid standard reference powder to the extent of 25 per cent. (21), are not present in the existing clinical material.

(2) Pharmacology

The outstanding pharmacologic effects of veratrum compounds are the production of hypotension (13, 21, 25), and bradycardia (7, 12). Cross-circulation studies on dogs have shown the hypotensive action to be of central origin (24). Positive inotropic
effect on the isolated and in situ mammalian heart by Veriloid (20) and pure alkaloids (7), has been demonstrated.

Other effects include emesis and mild bronchoconstriction. The former has been reported to be of central origin (23), while the latter was attributed to a combination of non-cholinergic local constriction and depression of central respiratory drive (15).

Acute (22) and chronic (5) toxicity studies on these compounds are also available.

C. The Movements of Intestine:

The smooth muscle of the isolated rabbit intestine displays rhythmical segmental activity (pendular movements) and tonus changes. The intact intestine of the trained unanesthetized dog presents the phenomenon of peristalsis in addition to the movements above. The activity of both intact and isolated intestine is affected in a qualitatively similar fashion through autonomic innervation or by the administration of pharmacologic agents.

(1) The Pendular Movements

These are the most prominent type of movement seen in the empty intestine, both intact and excised. They consist of rhythmical contractions chiefly of the longitudinal muscle. In the rabbit ileum, their incidence per unit time may be as low as 10 per minute (2). This activity usually serves to sway the intestine to and fro and side to side, churning the contents without propulsion. It is an index of the excitability of the intestine. The rhythm and amplitude may be altered by temperature change and by autonomic drugs in vivo and/or in vitro. The rate of these movements decreases
as one proceeds caudally, reflecting the "gradient of irritability" described by Alvarez (2).

(2) Tonus

Increases and/or decreases in tone reflecting a change in the resting length of the intestine, likewise are an index of irritability of the intestine in vivo and in vitro. These changes occur in both isolated and intact intestine when either the ganglia, parasympathetic or sympathetic terminations, or muscle cells are stimulated by appropriate pharmacologic agents. Tonus increase culminates in spastic rings, bands and chords of both longitudinal and circular musculature. Relaxation in tone causes flaccidity and suppression first of peristalsis and then of pendular movements.

(3) Peristalsis

Peristaltic waves are waves of contraction preceded by a wave of relaxation moving caudally. They are deep contraction rings or bands chiefly of the circular muscles, which form and relax rhythmically in a descending direction, generally traveling for some distance, propelling the contents before them. This constitutes a very important difference from the pendular movements. At the height of the peristaltic rush, however, pendular excursions tend to be diminished by the rigidity of the contracted intestine; after the wave passes, the pendular movements may be temporarily suspended. The contractions resume gradually with increasing intensity until another peristaltic rush is initiated. Such activity occurs in the excised intestine as well as in the living animal provided the proper stimulus is present. This stimulus is furnished most simply by distention of
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the intestinal lumen with moderate pressure.

In our studies, we were interested mainly in the segmental activity and tonus change of both the intact and isolated preparations. Peristalsis may be studied in the intact preparation by utilizing balloons set in tandem which progressively record the wave. The Magnus method minimizes, if not precludes, the possibility of any peristaltic activity occurring, since one is dealing with segments which are too short to permit initiation of peristaltic activity. Furthermore, the activity of the circular muscles is quite negligible (see under Method).

(4) Factors Influencing Intestinal Activity in Vivo and in Vitro

a. Automaticity

The observation of the various types of intestinal movements tends at first to create the impression of complexity, but from this nebulous condition, a certain orderliness becomes apparent in the regularity of rhythm and the graded polarity of the small intestine.

The occurrence of these co-ordinate phenomena, in excised as well as in the intact organ, suggests that they are autonomic and do not depend upon the central nervous system. The excised intestinal segment, although free of central connections, maintains a rhythmic integrity. The enteric plexuses of Meissner and Auerbach were at one time considered to be the regulators of rhythmicity. This apparently was confirmed by the initial experiments of Magnus (10), wherein the rhythmic movements were abolished when the enteric plexuses were stripped away. Subsequently, both Magnus (11) and Alvarez (5) showed that the loss of rhythmicity was merely the result of traumatic injury which depressed muscle tonus and irritability below the
threshold required for rhythmicity. With careful technique in extirpating these plexuses, the intestine retains the basic rhythmicity although the sensitivity to pharmacologic agents is decreased and its activity is atypical in comparison to intact preparations. Therefore, the myogenic element of intestinal rhythmicity must be taken into account. The current trend of thought on this argument appears to favor the theory that although the relation of rhythmicity to tonus and irritability does not require central nervous system linkage, the integrity of the innervation assists by maintaining the excitability and tonus of the muscle at a higher level (1).

b. Exogenous Influence on Rhythmicity

Pharmacologic agents which act upon the autonomic nervous system or which have a direct muscle effect, may act upon the excised as well as the intact organ. Similarly, the effects of agents upon the gut may be blocked by use of appropriate drugs; the quaternary ammonium compounds may block effects at the ganglia; the belladonna group exerts its action on the parasympathetic terminations; and the adrenergic-blocking drugs, on the sympathetic terminations. Therefore, by appropriate choice of blocking drugs, one may inhibit the effects of an agent acting either to depress or to stimulate intestinal activity and determine the mechanism of action of such a drug.

Variables which alter activity of intact and isolated intestine have to be carefully avoided. Electrolyte imbalance causes marked alterations in the activity of isolated strips (19); excess magnesium ion depresses activity, while deficiency in bicarbonate ion markedly impairs normal rhythm. Excess acidity of the solvent in which
veratrum compounds are dissolved likewise depresses in vitro intestinal activity (19). Deficient oxygenation and deviation from physiologic temperature (38° C.) can contribute to irregular rhythm and unexpected tonus change (1). Excitement and/or restlessness in the unanesthetized dog may cause depression of intestinal motility probably through release of epinephrine by the adrenal medulla, whereas undue abdominal tension produces an exogenous increase in intestinal tone.

D. Methods:

(1) In Vitro Studies on Rabbit Intestine

a. The Magnus Method

A biologic method widely used to evaluate pharmacologic agents is the employment of strips of intestine; Magnus in 1904 (9) first described this technique. The intestine is excised from the rabbit, placed in a bath containing warm aerated Locke's solution, and attached to a recording lever. Such preparations exhibited excellent rhythmical activity for considerable periods of time. This method afforded the investigator a simplified means of studying the dynamics of smooth muscle, the effects of exogenous and/or endogenous chemical substances, and the interrelationship of its autonomic innervation. Its simplicity was particularly attractive for this study, as only qualitative effects were to be studied pro tem. The small intestine of the rabbit is best suited for this work because it possesses excellent survival properties and is readily available. It exhibits a minimum of confusing tonus waves which may be present in the intestinal activity of larger animals. The main disadvantage of this preparation is that it only presents a partial picture of intestinal movements, i.e., the stimulus
of distention is lacking and the circular muscles contribute little to the record; however, the direction of response is the same (19).

In our procedure, a modified Magnus apparatus was adopted. Male albino rabbits, ranging in weight from 1.8 to 3.6 kg, were sacrificed by means of a sudden blow over the occipital region of the skull. Ileal and/or jejunal sections were then excised, thoroughly cleansed, divided into segments 2-3 cm. in length, and placed in refrigerated Tyrode solution until used.

The perfusion apparatus consisted of a 75 ml. muscle bath surrounded by a constant temperature water reservoir (38 ± 0.5° C.). The water in the reservoir was circulated continually by a rotary mixer and the temperature maintained by a bimetallic thermostat regulating a 125 watt heating element. A glass aeration tube (Figure 1), so bent as to permit the use of two strips of intestine from different rabbits at the same time in a muscle bath, was utilized. The physiologic solution employed for all experiments was Tyrode 2 (19). It was definitely advantageous to employ this solution for the muscle bath in lieu of the classic Locke's or the modified Sollman-Raedemaker because the addition of magnesium ion to the solution, although tending to depress the initial excitability of the muscle, prevents rapid

2- The Tyrode solution had the following per liter composition: Sodium chloride 8.0 g (0.8%); sodium bicarbonate 1.0 g (0.1%); potassium chloride 0.2 g (0.02%); calcium chloride 0.1 g (0.01%); magnesium chloride 0.1 g (0.1%); sodium bisphosphate 0.05 g (0.005%); glucose 1.0 g (0.1%); and distilled water q.s. ad 1000 ml. A stock concentrate of Tyrode's was prepared omitting the sodium bicarbonate and the glucose. This procedure eliminated possibility of fermentation of the glucose or precipitation of calcium carbonate occurring in the finished solution left at room temperature. Appropriate amounts of glucose, bicarbonate and concentrate, diluted to volume with distilled water were added as needed for each day's experiment.
fatigue (19). Reserve Tyrode solution was warmed by the same water reservoir. Medicinal oxygen was bubbled through the test bath constantly. Intestinal contractions were recorded on Teledeltos paper (Western Union Telegraph Company) by modified heart levers attached to the strips. Levers were so weighted as to maintain a tension of approximately 2.0 gm. on the strips. The kymograph speed was adjusted to permit adequate accuracy in the measurement of change in the rate of contraction. Time interval was recorded by a Telechron timer directly on the tracing.

b. Preparation of Veratrum Drug Solutions

Veratrum derivatives are quite insoluble in distilled water but readily soluble in dilute acid and organic solvents (7, 21). The exact concentration of acid required to effect solution varies with the individual derivative. Therefore, it was desirable to achieve an optimum concentration with a minimum of deviation from physiologic pH. The stock drug solutions were prepared using 2.0 ml. absolute alcohol, 2.0 ml. propylene glycol, and 0.5 - 1.0 ml. glacial acetic acid per 10 mg. drug. Physiologic salt solution was then added to form appropriate dilutions.

c. Procedure for Single Dose Experiments

Control tracings were obtained after allowing the strips to attain a normal level of tone and segmental activity. Preliminary solvent controls were performed to ascertain the pharmacologic impotence of the solvent. The drug as a stock solution was then instilled into the test bath equidistant between the two strips with a hypodermic syringe. This technique aided the mixing which resulted from
aeration, and avoided unequal initial response. Variability in the magnitude of response of strips from different rabbits (biologic variation), necessitated pilot experiments be conducted for each agent to determine the approximate concentration at which strips demonstrated visible drug effect. Subsequently, four to six graded concentrations of each compound, bracketing the minimal effective concentration were chosen. Intestinal strips from each of at least ten rabbits were exposed to each concentration. A single strip was exposed to only one concentration, but strips from each rabbit were exposed to each concentration.

1. Evaluation of Results

Responses of the strips to the test drugs were recorded as either positive or negative. A positive response was either an increase in the height of segmental activity, an increase in tone, or both. These discrete changes in the rhythmic activity of the strips were easily defined when compared to the control record of the same strip. Rate was counted directly from the tracing.

The resultant data for each agent, of incidence of spasm at each of four to six concentrations were analyzed by the method of Litchfield and Wilcoxon (8). The effective dose 50 (ED50) was calculated for each veratrum derivative tested. These ED50 doses were studied for possible interrelationship with the hypotensive potency of each test drug.

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3 The minimal effective concentration as herein used signifies that dose which just elicits a response in strips from each rabbit.
d. Determination of Locus of Action

Since the effect of veratrum derivatives on the isolated intestine of the rabbit is spasmogenesis, five possible sites of action are suggested:

1) Stimulation of the parasympathetic ganglia
2) Inhibition of cholinesterase at the myo-neural junction
3) Acetyl-choline (Ach-like) effect
4) Inhibition of sympathetic myo-neural transmission
5) Direct stimulation of the muscle

By initially blocking the parasympathetic system with atropine, the first four sites may be eliminated if the spasmogenic effects of the test drugs are not blocked by atropine. Therefore the following routine was devised:

1) Control tracing
2) 0.05 mg. per liter bath Ach
3) Wash; repeat control tracing
4) 1.0 mg. per liter bath atropine sulfate
5) 0.05 mg. per liter Ach to test adequacy of atropine blockade
6) 1 mg. per liter bath Veriloid or 0.065 mg. per liter germerine or 0.27 mg. per liter bath cevadine

These doses represent 175, 70, and 34 times the respective ED50 spasmogenic doses. Marked spasm of the strips was produced by these doses, although the spasm was not maximal. The 1 mg. per liter dose of atropine adequately blocked the effects of the test dose of Ach without causing severe atonia of the gut as was occasionally seen with higher doses of atropine.
e. Effects of Other Drugs

The effects of Ach and epinephrine on the response of the isolated gut to Veriloid and vice versa were determined. The drug concentrations used in this experiment were:

1) Veriloid - 1 mg per liter bath
2) Epinephrine - a maximum relaxing dose (0.27-0.5 mg. per liter bath)
3) Ach - 0.05 mg. per liter bath

The response to Veriloid while the strips were at a maximum relaxation due to epinephrine was noted. Likewise, Veriloid was added to the muscle bath while the strips were at a peak response to a test dose of Ach. Conversely, the effects of epinephrine and Ach were determined by adding these agents to the bath when the peak response to Veriloid was noted in the test strips.

(2) In Vivo Studies on Trained Unanesthetized Dogs.

a. Preparation, Surgery (14), and Training

Three female mongrel dogs were chosen at random from the animal stock. Male dogs proved useless for this type of preparation because fluid from the intestinal loops would cause irritation and excoriation of the external genitalia. The dogs weighed approximately 15, 18, and 21 kg. at the time of selection. The animals were exercised daily and fed once daily. The diet consisted of a balanced diet of dog pellets (Ralston Purina Co.) and one-half pound meat daily for two weeks. Prior to surgery, the dogs were bathed, and the abdomen shaved and scrubbed.

Anesthesia was induced with pentobarbital sodium (USP) 30 mg.
per kg. intravenously. Additional anesthesia was administered as needed during the operation in 5 per cent. glucose solution which was infused constantly during surgery. The peritoneal cavity was entered through a mid-line incision extending from below the xyphoid to the pubis. A section of ileum 50 cm. in length was selected, approximately 70 cm. above the ileo-cecal valve, and divorced by section from the intestinal continuity retaining its mesentery, nerve, and vascular supply intact. The ends of this segment were exteriorized through two stab wounds adjacent to the midline incision and sutured securely to the fascia of the exterior oblique muscles. This formed the classic Thiry-Yella fistula (14). Next, the meso-appendix was divided and the appendix brought through a third stab wound in the lower right quadrant and sutured to the superficial muscle fascia. The exteriorized tip of the appendix was cut off on the 4th - 6th post-operative day and the fistula thus produced formed a cecostomy. Finally, to reestablish the continuity of the ileum, a side-to-side anastomosis was performed at a point 20 cm. caudal to the cut distal ileal segment. This allowed the free end of the distal portion to be pulled through a fourth stab wound (left upper quadrant) attached to the abdominal wall, and formed an ileostomy (Figure 2).

The post-operative treatment consisted of daily injections of 100,000 U. penicillin intramuscularly and 5 per cent. glucose or of whole blood intravenously; milk and light food were presented to the animals on the second or third post-operative day, and normal feeding was usually reestablished by the tenth post-operative day.

The training period began about 3 weeks post-operatively and
consisted of allowing the animal to become accustomed to lying quietly on its right side on a foam rubber cushion in a quiet room. Latex balloons attached to soft rubber catheters (French 10), were then inserted gently into the loops and recordings made from a multiple bromoform manometer on Teledeltos paper (Figure 3).

1) Procedure for Intravenous Administration of Test Drugs

Control tracings were taken for a period of 30 minutes to an hour. Thereupon, one of a series of graded doses of Veriloid was infused intravenously over a period of ten minutes. The doses included 5, 10, 15, and 20 microgm. per kg. in a volume of 10 ml. of physiologic salt solution. A 20 to 30 minute record was then observed following drug administration.

2) Procedure for Intraloop Drug Administration.

Following an adequate control tracing, a control dose of 5 to 10 ml. of a warm solution of 0.01 per cent glacial acetic acid in physiologic saline was introduced via catheter into either the ileostomy or Thiry-Vella fistula posterior to the balloon. After 10 to 15 minutes, the test drug was instilled at the same site as the control at the appropriate concentration. An hour of record following drug instillation was taken to ascertain any pharmacologic effect.

3) Evaluation of Results

The difficulties in attempting to assess positive responses to a pharmacologic agent by the intact intestine are many. Small qualitative responses are concealed in the variability of intestinal motility. Increase in tone, however, could be noted and this was the main criterion of effect. Similarly, emesis was regarded as an end point in certain
experiments wherein increased dose caused emesis without appreciable local gut effect.
PART II - RESULTS

A. Effects of Veriloid and Pure Alkaloids on Isolated Rabbit Intestine:

(1) Single Dose Effects

The general effects of both Veriloid and the pure alkaloids may be discussed under a single heading because they are qualitatively similar. The predominant effect seen in initial experiments using concentrations up to 10 mg. per liter bath was marked and immediate increase in tone. (Figure 4b) This appeared to be related to the bath concentration in the dose range tested. As the bath concentration was lowered to the vicinity of the minimal effective dose, the response was mainly an increase in the height of segmental activity. (Figure 5a & 4c). The segmental rate remained constant throughout. No other response to these agents was evident even at subthreshold doses. Effective doses of these agents always caused stimulation of rabbit intestine. ED50 doses with 95 per cent. confidence limits for all compounds studied are presented in Table 2.

No difficulty was encountered with the solvents involved for the ester alkaloids. Faulty electrolyte balance of the test bath or poor oxygenation were rarely encountered in the course of the experiments. Such conditions, when occurring, were rather easily discernible, and proper steps were taken to remedy the condition. (Figure 4d, e, and g). The study of the pure alkamines jervine and vertramine had been abandoned because excessive solvent acidity (pH 5.5) caused marked depression of intestinal activity (Figure 4f). Buffering systems added to the solvent failed to eliminate this difficulty.
(2) The Locus of Spasmogenic Action

Atropine did not prevent spasmogenesis in the isolated rabbit intestine initiated by Veriloid, germerine, or cevadine tested in 24 rabbits, (Figure 5b). In all cases, the dose of atropine was sufficient to produce complete blockade of a test dose of Ach. Conversely, in 10 rabbits, Veriloid given prior to atropine did not interfere with atropine blockade of Ach (Figure 6a).

(3) Tachyphylaxis

Repeated applications of 0.006 to 1.0 mg. per liter bath Veriloid, despite thorough washing between doses, produced progressively diminished spasm (Tachyphylaxis). This tachyphylactic phenomenon appeared to be relieved by time. (Table 3) If up to an hour was allowed to elapse between doses of Veriloid, recovery occurred to the extent that the response to a second dose was approximately 23 per cent. of the initial response. (Figure 7a) This time factor was shorter for the pure alkaloids. 75 per cent of all strips tested displayed a response approximately equal to the control after 30 minutes had elapsed between doses. (Figure 8) The doses of the test drugs employed for this particular study ranged from 1 to 175 times the calculated ED50 dose. (Table 2)

(4) Effects of Other Drugs on the Response of the Isolated Rabbit Intestine to Veriloid

The effects of Ach. and epinephrine when given at the peak spasmogenic response to Veriloid, and of Veriloid when given at the peak response to Ach. and epinephrine, were studied on strips from 10 rabbits. The maximal Veriloid spasm could be augmented in all cases by
administration of Ach. at the summit of this response. (Figure 6b) The same was true for Veriloid when given during the response to Ach (Figure 6c) Epinephrine always markedly relaxed the intestine when it was at the peak of a spasm due to Veriloid. (Figure 6c) Similarly, Veriloid caused marked spasm in a strip relaxed by epinephrine. (Figure 6d) B. The Effects of Veriloid on the Intact Intestine of the Trained, Unanesthetized Dog:

(1) Intravenous Injection

No local intestinal stimulation attributable to Veriloid was seen prior to emesis. Emesis in all 3 dogs occurred at a dose of 2.0 microgm. per kg. per minute for 10 minutes, which was within the 95 per cent confidence limits of the intravenous ED50 emetic dose (23).

(2) Intraloop Administration of Veriloid

Graded doses of Veriloid up to 40 microgm. per kg. instilled in either the ileostomy or the Thiry-Vella fistula produced no visible alteration of the normal control record in the 3 dogs tested. (Figure 9)

DISCUSSION

The results of this study corroborate the rather meager literature (4, 6, and 16) - qualitatively at least - concerning the effects of veratrum on isolated intestine. Effective doses of such compounds always produce either an increase in the height of segmental activity in the intestinal strip, an increase in tone, or both.

The calculated ED50 doses (Table 2) for the pure alkaloids bring out quantitative differences in their spasmogenic potency. This difference roughly parallels the hypotensive potency (Table 4); however, the two exhibit no apparent mathematical relationship. The spasmogenic
potency of germerine, veratridine, and cevadine appeared to be greater than their hypotensive potency; the reverse was true for protoveratrine and germidine.

Backman (4) was unable to prevent spasmogenic effects of Veratrine chloride on isolated rabbit intestine with "normal" doses of atropine, whereas "extensive" atropinization (several mg. in an 80 ml. bath) did depress the response. The criteria of "normal" and "extensive" atropinization are important here. Extensive atropinization will not only block at the parasympathetic nerve endings, but will also depress the intestinal musculature directly, causing a marked diminution of normal activity. The dose of atropine finally chosen for this study produced adequate parasympathetic blockade without excess muscle depression.

Since the spasmogenic effects of veratrum could not be blocked by atropine, it is apparent that the locus of action is directly on the muscle.

Tachyphylaxis to repeated doses of veratrum in isolated rabbit intestine has been reported by Okazaki (16), and was ascribed to a "poisoning" of the muscle. Krayer (7) reported tachyphylaxis in circulatory effects when using high doses of pure alkaloids in heart-lung preparations. However, Maison et al. (13) observed no significant change in the hypotensive response to repeated doses near the clinical level in the anesthetized dog.

In this study, marked tachyphylaxis to all veratrum derivatives was observed in doses ranging from 1 to 175 times the calculated ED50 spasmogenic dose. That it was a true tachyphylactic phenomenon and not a poisoning was proved by challenging the strips with a known dose of
Ach before and after the veratrum was given. No diminution of the response to Ach was ever seen, although the strip was tachyphylactic to veratrum.

Inspection of the size of the ED50 dose reveals the marked sensitivity of rabbit intestine to veratrum. The ED50 intestinal spasmogenic dose for Veriloid was 5.7 microgm. per liter bath, whereas demonstrable effects (positive inotropism) in the isolated heart preparation were seen at dosage levels of 26 microgm. per liter of perfusate. The comparative sensitivity of other smooth muscle organs such as ureter, seminal vesicle or guinea pig ileum to veratrum remains to be investigated. Such marked sensitivity may perhaps prove useful clinically in the detection of minute amounts of veratrum circulating in the body fluid of patients.

Whether spasmogenic effects are within the scope of clinical dosage is difficult to determine, since the distribution of veratrum in the animal body is unknown. Assuming an ideal equal distribution, a theoretical blood titer for Veriloid may be calculated. If 9 percent of the total body weight were blood (17), a clinical dosage of 1 microgm. per kilogram per minute for ten minutes would produce a circulating titer of 10 microgm. per liter blood; this is far greater than the calculated ED50 spasmogenic dose for isolated rabbit intestine. However, the probability of such a blood level producing discrete stimulation of the intact intestine is remote. Intravenous administration of a 2X clinical dose to trained, unanesthetized dogs has elicited emesis without prior local intestinal stimulation.

The failure of Veriloid in high dose to cause local stimulatory
effects on the gut when administered intravenously or intralopp has several possible explanations. In the former case, the threshold of the emetic center was reached; whether the intact intestine would demonstrate visible local effects at higher dosage, remains unanswered. In the latter instance, poor absorption or loss of drug from the Thiry-Vella loop and passage of the drug into the cecum when instilled into the ileostomy, may explain the lack of gut response as well as the inordinately high emetic threshold. In normal animals, the emetic dose is the same by oral and intravenous routes (23). Thus, if adequate absorption had occurred, emesis would have resulted at the higher dose range. The answer may lie in administering the liquid drug orally in capsules and allowing absorption to occur in the duodenum while rhythmic changes are recorded from the ileostomy. Such investigation is now in progress.

SUMMARY

(1) Veriloid and pure veratrum alkaloids cause stimulation of isolated rabbit intestine. Doses in the range of the ED50 produced mainly an increase in the height of segmental activity. Increase in tone, although sometimes seen at the minimal effective dose range, was primarily a phenomenon of higher dosage.

(2) The ED50 for gut spasm of the following compounds has been measured: Veriloid, germerine, protoveratrine, germidine, veratridine, and cevadin.

(3)

a. Graded doses of Veriloid administered intravenously to the trained unanaesthetized dog produced no local intestinal stimulation
prior to emesis.

b. Intraloop administration of Veriloid in doses up to 40 microgm. per kg. caused no alteration of normal control tracings.

(4) The locus of spasmodic action of these agents on the isolated rabbit intestine appears to be directly upon the muscle.

(5) Tachyphylaxis to veratrum compounds has been demonstrated; this has been shown to be relieved by washing and passage of time.

(6) The spasmodic potency of these agents bears no apparent relationship to the hypotensive potency.
## TABLE 1*

### Hydrolysis Reactions of Ester Alkaloids

<table>
<thead>
<tr>
<th>Alkaloid **</th>
<th>Alkamine</th>
<th>Organic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Germerine $+ \text{H}_2\text{O}$</td>
<td>Protoveratridine</td>
<td>Methyl ethyl glycolic $+ \text{H}_2\text{O}$ Germine $+ \text{Methyl acetic}$</td>
</tr>
<tr>
<td>2) Proroveratrine $+ 2\text{H}_2\text{O}$</td>
<td>Protoverine</td>
<td>Methyl ethyl acetic Acetic Methyl ethyl glycolic</td>
</tr>
<tr>
<td>3) Germidine $+ \text{H}_2\text{O}$</td>
<td>Germine</td>
<td>Acetic Methyl butyric</td>
</tr>
<tr>
<td>4) Veratridine $+ \text{H}_2\text{O}$</td>
<td>Cevine</td>
<td>Veratric</td>
</tr>
<tr>
<td>5) Cevadine $+ \text{H}_2\text{O}$</td>
<td>Cevine</td>
<td>Tiglic $+$ isomeric Angelic $+$ mixture</td>
</tr>
</tbody>
</table>

---

* - From Kraye, O. and Acheson, G.H.; Physiol. Reviews, 26:383, 1946

** - The substances utilized were kindly provided by the following sources:

- Eli Lilly and Company, Indiana: Protoveratrine
- Riker Laboratories, Los Angeles California: Veriloid and Veratridine and Cevadine
- Squibb Institute for Medical Research, New Brunswick New Jersey: Germerine and Germidine.
Figure 1

The muscle bath, showing the bent aeration tube with two intestinal strips in place.
Figure 2

Abdomen of a Loop dog

a. Proximal end, Thiry-Vella fistula
b. Distal end, Thiry-Vella fistula
c. Ileostomy
d. Cecostomy
Figure 3

The Multiple Bromoform

Manometer
Figure 4

A. Normal tracing

B. At V, 5.0 mg. per liter bath Veriloid

C. At V, 6.2 microgm. per liter bath Veriloid

D. At M, 250 mg. per liter bath MgCl₂

E. Tracing showing the effect of poor oxygenation

F. At S, 0.5 ml. solution of glacial acetic acid, propylene glycol, absolute alcohol and physiologic saline (pH 5.5) used as solvent for jervine.

G. Left- Effects of bicarbonate ion deficiency
    Right- bicarbonate ion balance restored
FIGURE 4

TRACINGS - ISOLATED RABBIT INTESTINE
Figure 5

A. At C, 8.0 microgm. per liter bath cevadine

B. At A, 1.0 mg. per liter bath atropine sulfate
   At Ach, 0.05 mg. per liter bath Ach
   At C, 0.27 mg. per liter bath cevadine
CEVADINE - RABBIT GUT

A.

8.0 MCG/LITER

B.

FIGURE 5
## Calculated ED50 – Veriloid and Ester Alkaloids

<table>
<thead>
<tr>
<th>AGENT</th>
<th>DOSE Mgc/Liter Bath</th>
<th>No. Individual Rabbit Strips Tested</th>
<th>No. Strips Giving Positive Response</th>
<th>Calculated ED50 with 95% Confidence Limits Mgc/Liter Bath</th>
</tr>
</thead>
<tbody>
<tr>
<td>VERILOID</td>
<td>4.0</td>
<td>11</td>
<td>1</td>
<td>(4.62 - 7.1)</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>17</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>14</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>GERGEMINE</td>
<td>0.5</td>
<td>12</td>
<td>3</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>14</td>
<td>6</td>
<td>(0.65 - 1.4)</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>12</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>PROTOVERATINE</td>
<td>2.4</td>
<td>12</td>
<td>4</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>14</td>
<td>10</td>
<td>(2.2 - 3.3)</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>GERMIKINE</td>
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<td>12</td>
<td>4</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>14</td>
<td>11</td>
<td>(2.3 - 3.4)</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>14</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>VERAATIDINE</td>
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<td>10</td>
<td>3</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>11</td>
<td>6</td>
<td>(4.1 - 5.4)</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
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<td>7.5</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>CIVADINE</td>
<td>6.3</td>
<td>10</td>
<td>2</td>
<td>7.8</td>
</tr>
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<td></td>
<td>8.0</td>
<td>10</td>
<td>6</td>
<td>(6.7 - 9.0)</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6

A. At V, 1.0 mg. per liter bath Veriloid
   At A, 1.0 mg. per liter bath Atropine sulfate
   At Ach, 0.05 mg. per liter bath Ach

B. At V, 1.0 mg. per liter bath Veriloid
   At Ach, 0.05 mg. per liter bath Ach

C. At Ach, 0.05 mg. per liter bath Ach
   At V, 1.0 mg. per liter bath Veriloid

D. At E, 0.5 mg. per liter bath epinephrine
   At V, 1.0 mg. per liter bath Veriloid

E. Same as Part D.
FIGURE 6

TRACINGS - ISOLATED RABBIT INTESTINE
**Comparative Tachyphylaxis - % Recovery**

*Isolated Rabbit Intestine***

<table>
<thead>
<tr>
<th>Agent</th>
<th>Time - minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Veriloid</td>
<td>0</td>
</tr>
<tr>
<td>Germerine</td>
<td>12</td>
</tr>
<tr>
<td>Protoveratrine</td>
<td>22</td>
</tr>
<tr>
<td>Germidine</td>
<td>15</td>
</tr>
<tr>
<td>Veratridine</td>
<td>18</td>
</tr>
<tr>
<td>Cevadine</td>
<td>15</td>
</tr>
</tbody>
</table>

* Magnitude of initial response = 100%.

** Each value calculated from data on strips from ten rabbits.

**TABLE 3**
Figure 7

A. At $V_1$, 1.0 mg. per liter bath Veriloid

At $W$, wash

At $V_2$, 1.0 mg. per liter bath Veriloid
VERATRUM TACHYPHYLAXIS

'VERILOID'

53 MIN. INTERVAL BETWEEN $V_1$ AND $V_2$

FIGURE 7
At $V_1$, 6.27 mg. per liter bath cevadine
At $V_2$, 0.27 mg. per liter bath cevadine
At Ach, 0.05 mg. per liter bath Ach

N.B. The waning of tachyphylaxis to $V_2$ as time progresses.
VERATRUM TACHYPHYLAXIS-CEVADINE-
ISOLATED RABBIT GUT

5 MIN. 30 MIN.

FIGURE 8
Figure 9

Normal control record – trained, unanesthetized Loop dog
CONTROL - LOOP DOG

ILEOSTOMY

THIRY-VELLA LOOP

CECOSTOMY

FIGURE 9
Comparative Hypotensive and Spasmogenic Potencies

<table>
<thead>
<tr>
<th>Agent</th>
<th>Hypotensive Potency*</th>
<th>Spasmogenic Potency**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% B.F. Fall in the Anesthetized Dog</td>
<td>Isolated Rabbit Intestine</td>
</tr>
<tr>
<td>Veriloid</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Germerine</td>
<td>540</td>
<td>600</td>
</tr>
<tr>
<td>Protoveratrine</td>
<td>435</td>
<td>210</td>
</tr>
<tr>
<td>Germinidine</td>
<td>210</td>
<td>200</td>
</tr>
<tr>
<td>Veratridine</td>
<td>120</td>
<td>73</td>
</tr>
<tr>
<td>Cevadine</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

* - Hypotensive potency of alkaloids subject to error of ± 25%.

** - Calculated from comparison of the ED50 spasmogenic doses of the alkaloids to that of Veriloid (100%).
BIBLIOGRAPHY


11. Ibid.; IV. Mitteil: Rhythmizitat und Refractare Periode. 103: 525, 1906


18. Riker Laboratories; Personal communication, 1951.


ABSTRACT

The aims of this study were: (1) to determine the qualitative effects of Veriloid and pure veratrum alkaloids upon the isolated intestine of the rabbit and the intact intestine of the trained unanesthetized dog, and, (2) to determine the locus of such effect.

A modified Magus apparatus utilizing a constant temperature bath and Tyrode solution were employed for the in vitro studies. Strips from two different rabbits were exposed at the same time in a 75 ml. muscle bath to appropriate concentrations of the test drugs.

After preliminary experiments had proved the common spasmogenic property of these agents, it was decided to find the ED50 spasmogenic dose for each compound tested. Intestinal strips were exposed to minimum dilutions of the veratrum drugs, and the response noted as either positive or negative. The criteria for a positive response were either an increase in the height of segmental activity, an increase in tone, or both. At minimal effective dosage, the former was the more common response, while the latter was mainly a phenomenon of higher dosage. ED50 spasmogenic doses of the pure alkaloids were compared to that of Veriloid (100 per cent) and the resulting ratio formed the spasmogenic potency for the particular agent. The spasmogenic potency for each agent was compared to its hypotensive potency for possible relationship and application to a method of assay; none was demonstrable.

The locus of spasmogenic action was determined to be directly upon the muscle since the effects of veratrum were not altered by atropine. Similarly, Veriloid did not alter atropine blockade of acetylcholine (Ach), when given prior to atropine.
The maximum Veriloid spasm could be augmented in all cases by administration of Ach. at the summit of this response; the same was true for Veriloid when given in response to Ach. Epinephrine always markedly relaxed the intestine when it was at the peak of a spasm due to Veriloid; similarly, Veriloid caused marked spasm in a strip relaxed by epinephrine.

The tachyphylactic effects of these agents were studied. Tachyphylaxis of the strips to second doses of Veriloid and pure alkaloids gradually waned as time passed, although the per cent response to a second dose of Veriloid was still only 25 per cent of the initial response after 1 hour with thorough washing; however, tachyphylaxis to pure alkaloids was nearly ended after 30 minutes. The size of the dose used bore no relationship to this phenomenon.

The marked sensitivity of isolated rabbit intestine to minute amounts of veratrum, may offer an avenue for the measurement of any amount of this drug circulating in the patient's body fluids.

The study on the intact intestine was made using 3 trained, unanesthetized female mongrel dogs, each operatively prepared with a Thiry-Vella fistula, an ileostomy, and a cecostomy. Kymograph tracings were taken directly via a balloon inserted into the intestinal loops, and attached to a multiple bromoform manometer.

Intravenous Veriloid caused emesis in the dogs at a dose of 20 microgm. per kg. Emesis occurred without prior intestinal stimulation.

Intraloop administration of Veriloid in doses up to 40 microgm. per kg. (2X oral ED50 emetic dose), produced neither local intestinal stimulation nor emesis. This was attributed to poor absorption from
either the Thiry-Vella loop, or ileostomy, and no absorption from the cecostomy.

The experiments on the intact animal appeared to indicate that intestinal spasmodogenesis is not within the range of clinical intravenous dosage. Whether oral dosage causes local intestinal stimulation may be answered by recording intestinal motility in the dog following oral administration of veratrum.