measured by $t_{10\%}$) in unbuffered aqueous pharmaceutical systems, the exact expressions (*e.g.*, Eqs. 13–15) must be employed. The examples of computed $t_{10\%}$-values shown in Table IV indicate that significant errors can be made in estimates of stability if pseudo-first order degradation is assumed.

The models discussed fail to take into account general acid and base catalysis or reversible reactions such as those which might be observed in simple ester hydrolysis. However, these effects certainly can be neglected for estimation of $t_{10\%}$.

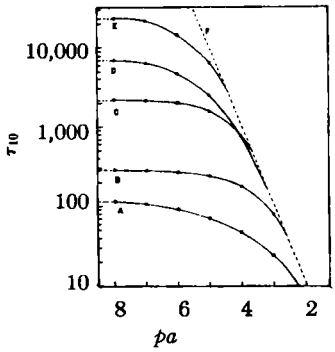

Fig. 8.—Computed τ_{10}-values shown as a function of $a = k_0/k_H$: curves A ($[N_0] = 0.1\ M$) and D ($[N_0] = 0.001\ M$), degradation product is a strong acid, defined by Eq. 5; curve B ($[N_0] = 0.1\ M$), degradation product is a weak acid ($pK_{a_2} = 4.0$), defined by Eq. 14; curves C ($[N_0] = 0.1\ M$) and E ($[N_0] = 0.001\ M$), degradation product is a weak acid ($pK_{a_2} = 6.0$), defined by Eq. 14. Curve F represents τ_{10}-values computed assuming pseudo-first order degradation at constant $pH = pH_0 = 7.0$.

Other cases can be considered, such as degradation of neutral molecules or weak bases which form weak bases as products and are subject primarily to specific base catalysis in unbuffered solution. Treatment of these cases leads to solutions of the same form as those described for *Cases II* and *III*.

The power of the analog computer as a tool in analyzing cases of this sort can readily be ascertained by noting the complexity of the solutions obtained to the differential equations. The computer will prove especially useful in analyzing systems where analytical solutions cannot readily be arrived at—for example, cases complicated by reversible reactions.

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Effect of Antiemetics and Other Compounds on Protoveratrine Induced Emesis in Dogs

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Several known antiemetics and other compounds were evaluated for their ability to prevent protoveratrine induced emesis in dogs and found to be ineffective.

Protoveratrine produces a potent reflex vasodepressor effect in dogs and humans (1). However, the range between the therapeutic and the toxic dose is quite narrow (2), and it is often difficult to obtain good clinical results without some side effects; the major side effects are nausea and vomiting. Alterations in the protoveratrine molecule to increase the margin between effectiveness and nausea have been attempted (3). A second approach has been directed at the prevention of nausea and vomiting by the administration of antiemetic agents.

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Generous supplies of haloperidol (Searle), perphenazine (Trilifon, Schering), triflupromazine (Vesprin, Squibb), prochlorperazine (Compazine, Smith Kline and French), pipamazine (Mornidine, Searle), meclizine (Bonine, Pfizer), and trimethobenzamide (Tigan, Roche) are greatly appreciated.
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TABLE I.—RELATIVE EMETIC POTENCIES OF VERATRUM ALKALOIDS IN DOGS FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION

	Emetic Dose 50					
	Intravenous			Oral		
Drug	No. Dogs	ED_{50}, mcg./Kg.	Slope	No. Dogs	ED_{50}, mcg./Kg.	Slope
Protoveratrine A	29[a]	3.24 (2.63 to 3.99)[b]	1.6 (0.9 to 3.0)	24	3.4 (2.9 to 4.0)	1.5 (1.3 to 1.7)
Protoveratrine B	40	3.63 (3.03 to 4.36)	1.4 (1.1 to 1.8)	20	20.0 (15.9 to 25.2)	1.3 (0.9 to 2.1)
PAB	37[a]	3.82 (3.38 to 4.32)	1.3 (1.1 to 1.5)	...	...	...

[a] From Abreu, Richards, Alexander, and Weaver (1954). [b] 95% confidence limits.

This report explores the feasibility of the latter procedure.

METHODS

Adult mongrel dogs, unselected as to sex, were used in all experiments. They were maintained on an adequate diet consisting of canned horse meat and dry pellets with water given *ad libitum*, except during the period of testing.

Stock solutions of protoveratrine A and protoveratrine B were prepared by dissolving a sufficient amount of each in its stochiometric equivalent of glacial acetic acid, then adding sufficient distilled water to make a 0.1% concentration of the base. These stock solutions were refrigerated and dilutions freshly prepared from them prior to each experiment. A commercially available solution of the proveratrines A and B (PAB)[1] was used.

The emetic doses for 50% of animals (ED_{50}) and their slopes were calculated by the method of Litchfield and Wilcoxon (4).

An emetic dose of protoveratrine A was injected at different rates and in different volumes of solvent to determine the importance of these factors on the occurrence of the side effects which usually accompany its administration.

In some experiments the intravenous (i.v.) protoveratrine A challenge (6 mcg./Kg.) was repeated at 15, 30, 40, 120, or 135-minute time intervals to determine whether there was a development of tolerance to certain responses, particularly the convulsive (collapse and leg extension) and emetic effects, upon repeated administration.

Several drugs with known and unknown antiemetic properties were tested for their ability to prevent protoveratrine A emesis in dogs. All animals were observed for 60 minutes following the injection of protoveratrine A (5.6 mcg./Kg., i.v.). The procedure for the preliminary evaluation of 4'-fluoro-4(1-[4-hydroxy-4-(4'-chloro)-phenylpiperidino])-butyrophenone (haloperidol) can best be followed by referring to Table IV. A subsequent experiment was performed in which three dogs each received orally 1, 2, 5, 10, and 20 mg./Kg./day for 4 days each and then were challenged 4 hours after final dose with protoveratrine A.

Dogs being used to establish dosage levels of piperacetazine[2] for chronic toxicity studies were tested to determine the effects of piperacetazine on protoveratrine A emesis. One dog each received orally 0.1, 0.25, 0.5, or 1.0 mg./Kg./day for 7 days, respectively, then 0.5, 1.25, 2.5, or 5.0 mg./Kg./day for 14 days, followed by 7 days without drug, and finally 2.5, 5.0, 2.5, or 5.0 mg./Kg./day for 7 days. They were challenged with protoveratrine A 2 hours after the last dose. Two additional dogs received 5 mg./Kg./day of piperacetazine for 21 or 44 days. The first dog was challenged after 6 and 21 days; the other dog was challenged after 44 days.

PAB (6.3 mcg./Kg.) was employed as the emetic agent in the final experiment of this series. The challenging dose was administered to six dogs after chlorphenoxamine[3] (10 mg./Kg., i.v.) and simultaneously with reserpine (32 mcg./Kg., i.v.) in another six dogs.

TABLE II.—EFFECT OF ALTERING CONCENTRATION AND/OR INJECTION RATE ON UNTOWARD RESPONSES TO PROTOVERATRINE ADMINISTRATION

	Proto. A, 6 mcg./Kg., i.v.[a]				
Dog	Vol., ml.	Rate, sec.	Collapse and/or Convulsions	Salivation Diarrhea and/or Urination	Onset Emesis, min.
♀	0.42	2	+	+	11
7.0	5.0	30	0	+	3
Kg.	5.0	30	0	+	4
♂	0.46	2	0	+	5
7.7	5.0	30	+	+	4
Kg.	5.0	30	+	+	3
♀	0.39	2	+	+	15
6.5	5.0	30	0	+	5
Kg.	5.0	30	+	+	4
♂	0.64	2	+	+	6
10.6	5.0	30	+	+	2
Kg.	5.0[b]	30	0	+	60

[a] Time between protoveratrine A injections in the same dog was 48 hours or more. [b] A portion of the drug went subcutaneously.

RESULTS

The emetic potencies of the veratrum alkaloids in dogs following intravenous and oral administration are shown in Table I. It is readily apparent that there was no significant difference among the emetic potencies of the three preparations following intravenous administration or between the two routes of administration following protoveratrine A. On the other hand, protoveratrine B was considerably less active following oral administration.

Neither the rate of injection nor volume of solvent administered consistently altered the side effects usually seen after the intravenous injection of an emetic dose of protoveratrine A. The side effects usually observed in the customary order of their appearance were: collapse, tonic extension of all legs,

[1] Veralba, Pitman-Moore Co. brand of protoveratrines A and B, containing 53% A and 47 B standardized chemically, both for total alkaloids and for A and B content.

[2] Available from the Pitman-Moore Co. for experimental use under the trademark, Quide.

[3] Available from Pitman-Moore Co. under the trademark, Phenoxene.

TABLE III.—EFFECT OF DRUGS ON PROTOVERATRINE A INDUCED EMESIS IN DOGS

Drug	Dose, mg./Kg.	Route	Interval,[a] min.	No. Emesis/ No. Dogs
		Antiemetics		
Perphenazine	0.1	i.v.	15	1/1
	0.1	i.v.	35	2/2
	0.1	i.v.	60	1/1
Piperacetazine	1.5[b]	i.g.	60	4/4
Pipamazine	0.002	i.v.	30	3/3
Prochlorperazine	0.1	i.v.	15	1/2
	0.25	i.v.	20	2/2
Triflupromazine	5.0	s.c.	5	2/3
Trimethobenzamide hydrochloride	25.0	i.g.	60	1/2
	50.0	i.g.	60	2/2
	100.0	i.g.	40	2/2
Meclizine	5.0	i.v.	10	3/3
		Miscellaneous		
Atropine sulfate	1.0	i.v.	45	3/3
	1.0	i.v.	60	3/3
	2.0	i.v.	15	3/3
Nicotinic Acid	10.0	i.v.	10	2/4
	10.0	i.v.	20	2/3
P-275[c]	250.0[b]	i.g.	60	4/4

[a] Between drug administration and protoveratrine A (5.6 mcg./Kg., i.v.) challenge. [b] Dose repeated three times—challenge 60 minutes after last dose. [c] *N,N*-Diisopropyl-*N'*-*n*-butyl-*N'*-diethylaminoethylurea.

central nervous system depressant side effects from haloperidol appeared during the 5 mg./Kg./day dosage and continued throughout the remainder of the experiment.

Complete protection against protoveratrine A induced emesis was obtained in the four dogs receiving increasing dosage of piperacetazine but not by the other two dogs. Subsequent attempts to control protoveratrine emesis have not been successful; the conclusion is that chronic administration is also ineffective.

Chlorphenoxamine and reserpine were each successful 50% of the time in preventing PAB-induced emesis.

DISCUSSION

Protoveratrines A and B are very potent hypotensive and emetic agents in the dog. Protoveratrine A is equally potent as an emetic by the intravenous and oral routes. On the other hand, protoveratrine B is only one-fifth as potent as an emetic by the oral as by the intravenous route. Similar findings have been reported for protoveratrine B in the human (5).

It is interesting that neither the rate of injection nor the volume of solution containing the emetic dose of protoveratrine A were critical within the limits of this test. Furthermore, successive doses at various intervals produced emesis but not convulsive type activity in six of seven cases. These results do not conflict with earlier work of a similar nature (6).

TABLE IV.—EFFECT OF HALOPERIDOL ON PROTOVERATRINE A INDUCED EMESIS IN DOGS

Day	Dog 1			Dog 2			Dog 3			Dog 4			Dog 5		
	H[a]	P[b]	E[c]	H	P	E	H	P	E	H	P	E	H	P	E
1	0	+	+	100	+	0	100	+	+	200	+	+	200	+	0
2	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0
3	0	0	0	0	0	0	100	0	0	200	0	0	0	0	0
4	200	+	+	0	+	+	100	+	0	200	+	+	0	+	+
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	200	+	+	100	+	+	180	+	0	200	+	+	200	+	+

[a] Haloperidol dose in mcg./Kg., i.v.—challenge 60 minutes after drug. [b] Protoveratrine A: +, 5.6 mcg./Kg., i.v.; 0, no drug. [c] Emesis: +, present; 0, absent.

some convulsive-like activity, urination, diarrhea, salivation, and emesis. Often these effects occurred in such a rapid sequence that it was difficult to determine which came first. The results are presented in Table II.

When the protoveratrine A challenge (6 mcg./Kg., i.v.) was repeated after an interval of 15, 30, 40, 120, or 135 minutes, all dogs vomited regardless of the time interval. Of the seven dogs used in this experiment, six vomited after the first injection of protoveratrine A. Only the dog which failed to vomit after the first dose showed the convulsive movements after the second protoveratrine A dose.

None of the compounds tested proved to alter significantly the emetic effects of protoveratrine A (Table III) under the conditions of the experiment. For the most part, none of the other side effects were altered by these compounds. As expected, atropine prevented the salivation usually observed.

Haloperidol was inconsistent in preventing protoveratrine A induced emesis (Table IV) in the exploratory investigation and uniformly unsuccessful in preventing emesis and convulsive activity in the three dogs used in the subsequent study. Toxic

The investigator should not be misled by the constancy of emesis observed in data reported in Table II. Although most dogs showed emesis following a dose of about 6 mcg./Kg., we have found dogs who failed to respond or did so inconsistently. Thus, *protection* as first obtained with chronic administration of piperacetazine must be viewed with suspicion until several dogs are used.

That many agents have been reported to reduce the susceptibility of the dog to the emetic effect of apomorphine is well documented. On the other hand, no compound has been found successful in preventing emesis induced by protoveratrine A in the dog. A decade ago chlorpromazine was shown to be effective against apomorphine (7–9) but not veratrum (10). More recently, chlorpromazine, triflupromazine, perphenazine, and fluphenazine were also found effective against apomorphine induced but not veratrum induced emesis (11). We confirmed this work for triflupromazine and perphenazine. Furthermore, three additional phenothiazines (pipamazine, piperacetazine, and prochlorperazine) were shown to be ineffective against protoveratrine induced emesis.

Compounds other than phenothiazines were also investigated for their ability to block protoveratrine induced emesis. Three potent antiemetic agents, haloperidol, meclizine, and trimethobenzamide (12, 13) were found to be without effect. Similarly, atropine, nicotinic acid, and P-275, which have not been reported to be antiemetic, were ineffective. Furthermore, chlorphenoxamine and reserpine did not consistently block PAB emesis. Other workers (6, 14) have reported that parasympatholytics (atropine, scopolamine, and methantheline), a ganglionic blocker (tetraethylammonium), a sympathomimetic (ephedrine), an antihistaminic (dimenhydrinate) and reserpine failed to prevent veratrum emesis in dogs.

Until more data are forthcoming on the mechanism of the emetic action of protoveratrine A, the inability of potent antiemetics to prevent the effect will remain obscure.

SUMMARY

Protoveratrine A is equally potent as an emetic agent by the intravenous and oral routes. On the other hand, protoveratrine B is only one-fifth as potent orally as by the i.v. route.

Neither the rate of injection nor the volume of solution containing the emetic dose of protoveratrine A is important with respect to modifying the side effects which usually accompany the intravenous injection of the drug.

Acute tolerance does not develop to the emetic action of protoveratrine A. Six out of seven dogs which responded to a challenging dose also vomited when the dose was repeated after an interval of 15, 30, 40, 120, or 135 minutes. Tolerance developed to the convulsive-like effects.

None of the drugs tested in this study were effective in preventing protoveratrine induced emesis in dogs.

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Gas Chromatography of Esters of Plant Acids and Their Identification in Plant Materials

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The gas chromatographic retention data of methyl and ethyl esters of a number of common plant acids are reported. Special emphasis is placed on the acids of the Krebs cycle. Of four liquid phases used, Apiezon L appears to be most generally applicable and the only one which permits satisfactory separation of fumarate and succinate. The esters of citric acid and isocitric acid (lactone) are readily separated on all four columns. The method has been used for identification of the acids in various plant materials. It is sensitive and selective and should be valuable for biochemical studies concerning the production and function of organic acids in plants.

PLANTS CONTAIN a large number of organic acids, some of which may be characteristic of certain plant families. Others, such as a group of di and tricarboxylic acids, are found more widely distributed throughout the vegetable kingdom and are usually referred to simply as the plant acids.

In 1937, Krebs and Johnson (1) showed that these acids play a central role in cellular respiration, and the series of reactions which are involved is known as the Krebs cycle. Some of the acids of the Krebs cycle, such as succinic, fumaric, malic, and citric acids, are found in appreciable concentrations in practically every type of plant material. Most of the others are present only in small amounts and require sensitive and specific methods for their detection. The keto acids, particularly oxaloacetic acid, are unstable and may easily be lost during the isolation procedures commonly used for plant acids and may thus escape detection. The enzymes

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