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ASSAY IN MAN OF THE CHEMICAL FRACTIONS OF VERATRUM VIRIDE, AND IDENTIFICATION OF THE PURE ALKALOIDS GERMITRINE AND GERMIDINE AS POTENT HYPOTENSIVE PRINCIPLES DERIVED FROM THE DRUG[1]

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Recent clinical (1) and experimental (2) evidence indicated that the crude powdered roots and rhizomes of *Veratrum viride* may produce marked reductions of arterial pressure in patients with essential hypertension. However, therapeutic application of the available preparations has been limited by the frequency of their disturbing side effects and the inconsistency of their action (1). The present investigation was undertaken (1) to identify the hypotensive principle or principles of *Veratrum viride*, (2) to determine whether the hypotensive and toxic factors were separable, and (3) to obtain pure crystalline compounds for clinical use which can be standardized by weight rather than by biological assay.

METHODS. Chemical fractionation of the crude roots of *Veratrum viride* was carried out by Drs. Josef Fried, Howard L. White, and O. Wintersteiner of the Squibb Institute for Medical Research (3). The fractions and pure alkaloids made available to us by these investigators were tested by oral administration in known hypertensive patients admitted to the wards of the Massachusetts Memorial Hospitals and the Veterans Administration Hospital, Washington, D. C. All patients were hospitalized for at least 48 hours prior to study. With the patient in the supine position, arterial pressure was recorded with an arm cuff and mercury manometer, while pulse rate was counted at the wrist. Prior to administering an extract, readings were taken at minute intervals until the arterial pressure and heart rate became stabilized. Further determinations of arterial pressure and heart rate were recorded at intervals of one-half hour for three hours following the oral ingestion of an extract. The various fractions were dissolved either in small amounts of 5 per cent acetic acid or 95 per cent ethanol depending on their solubility, diluted with water and flavored with orange syrup. Over 400 separate oral assays were carried out in testing 102 fractions. The intravenous route was used only with the pure active alkaloids which were dissolved in 2 per cent acetic acid (1 mgm. per cc.) and diluted further to a concentration of 0.025 mgm. per cc. with normal saline. The solutions were sterilized by passage through a glass filter and were stored in rubber capped vials. No loss of activity was apparent after storage at room temperature for more than two months.

A hypotensive dose was defined as the quantity of a given substance producing a reduction of the basal mean $\frac{(\text{systolic} + \text{diastolic})}{2}$ arterial pressure of at least 15 per cent. When a

[1] This investigation was aided in part by the Squibb Institute for Medical Research, New Brunswick, New Jersey, and was carried out in collaboration with Drs. Josef Fried, Howard L. White and O. Wintersteiner of the Squibb Institute, who supplied all of the fractions of *Veratrum viride* used in this study.

positive result was obtained, this was always confirmed by retesting in effective doses in two to three additional patients. On the other hand, before discarding a presumably inactive fraction, it was tested in doses six to eight times greater than the amount calculated to be present in an active dose of the crude drug, as judged by the percentage composition. For example, jervine composed 0.67 per cent of the crude drug. The mean effective dose of the crude root powder was 225 mgm. (table 1). Thus, if all of the hypotensive action of the crude drug were due to jervine, one would expect that a dose of 225 × .0067 or 1.5 mgm. of jervine would exhibit hypotensive activity. Nevertheless, this alkaloid was tested in doses up to 9 mgm. prior to discard.

RESULTS. All of the previously known crystalline alkaloids of *Veratrum viride* were inactive in the doses given. These included the benzene-extractable alkaloids jervine, veratramine, rubijervine, isorubijervine, germine (4–6), and the

TABLE 1

Progress of fractionation of total amorphous alkaloids

FRACTION	AVERAGE HYPOTENSIVE DOSE	CONCENTRATION IN CRUDE ROOT
	mgm.	*Per cent*
(1) Crude root powder	225	100
(2) Total benzene extractable alkaloids (ref. 6)	10	1.1
(3) Amorphous bases (fraction (2) minus crystalline alkaloids listed in text)	3.5	.36
(4) Tertiary bases from (3)	3.2	.26
(5) Combined plates 1–7 from 8-plate Craig distribution of (4)	1.8	.13
(6) Benzene-1% methanol eluate from alumina chromatogram of (5)	1.2	.065
(7) Fraction I (tube 15, K = 1.67, from 25-plate Craig distribution of (6))	.7	.045
(8) Fraction II (tube 6, K = 0.35, from 25-plate Craig distribution of (6))	5.5	.020

alcohol-extractable fraction containing the glucosidic alkaloids, pseudojervine and veratrosine (6). However, the amorphous material remaining after removal of the crystalline benzene-extractable alkaloids was active with 3.5 mgm. (mean effective dose in eight patients, range 2–5 mgm.). Table 1 illustrates the progressive purification of this active fraction up to the highly potent but still amorphous products (Fractions I and II) from which the crystalline alkaloids were isolated.

Following intravenous administration of the various active fractions the arterial pressure and heart rate began to decrease two to five minutes after injection of an effective dose (figure 1). Toxic effects such as substernal or epigastric burning sensation, tingling of the fingers and face, nausea and vomiting also occurred at this time. Arterial pressure and heart rate usually fell rapidly over a period of several minutes following which these values remained at low levels for three to ten minutes and then rose slowly over a period of one-half to four hours to the basal values (figure 1). Following oral administration, the arterial pressure and heart rate began to decline in one to two hours, reached a minimum in two to

three hours at which time toxic effects particularly nausea and vomiting and excessive salivation might occur, and then rose slowly over a period of two to

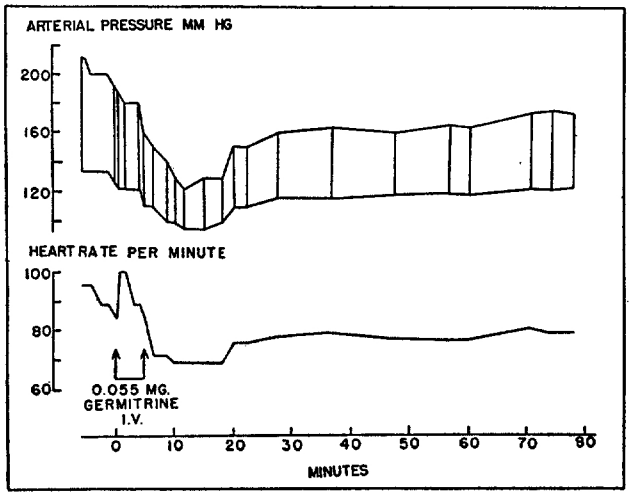

FIG. 1. Chart of patient J. U., 45-year-old male with essential hypertension illustrating the response of the blood pressure and heart rate to 0.055 mgm. of germitrine. The drug was given slowly over a period of five minutes intravenously, and injection was discontinued when a definite decrease in arterial pressure occurred. There was slight substernal and epigastric burning sensation from the second to the twelfth minute after the beginning of njection, but no other side effects.

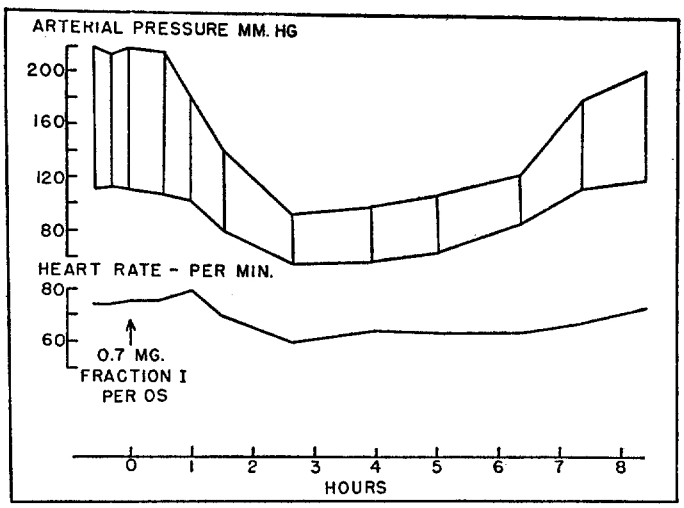

FIG. 2. Chart of patient E. M., 43-year-old female with essential hypertension illustrating the response of the blood pressure and heart rate to an oral dose of 0.70 mgm. of Fraction I. This patient exhibited no toxic side effects.

sixteen hours to basal levels (figure 2). In general the duration of the hypotensive response and frequency of side effects were related to the degree of hypotension

produced although in a few cases striking reductions in arterial pressure occurred without side effects (figure 2). Marked hypotensive reactions could be achieved in almost all patients by increasing the dosage but this also increased the severity

TABLE 2

Effect of intravenous administration of the pure alkaloids of Veratrum viride and album on the blood pressure and heart rate of hypertensive patients

ALKALOID	DOSE	PATIENT	CONTROL		AFTER DRUG		SIDE EFFECTS
			Blood Pressure	Heart Rate	Blood Pressure	Heart Rate	
	mgm.		*mm. Hg*	*per min.*	*mm. Hg*	*per min.*	
Fraction I	0.05	W. J.	160/98	90	130/80	68	Epigastric burning
Fraction I	0.09	W. C.	220/128	95	168/110	78	Epigastric burning
Fraction I	0.07	A. R.	230/130	104	192/105	82	0
Fraction I	0.06	G. S.	190/110	88	146/94	68	0
Fraction II	0.50	W. J.	176/108	92	132/88	72	Epigastric burning
Fraction II	0.54	G. S.	185/112	88	145/90	74	0
Fraction II	0.26	M. C.	168/110	80	132/88	68	Epigastric burning
Fraction II	0.60	J. S.	215/98	70	174/78	62	0
Germitrine	0.06	W. J.	190/120	96	110/78	56	Nausea and vomiting
Germitrine	0.05	B. M.	150/100	80	110/72	68	0
Germitrine	0.05	E. A.	190/100	84	80/40	74	0
Germitrine	0.055	J. U.	190/120	88	122/90	68	0
Germidine	0.12	G. S.	205/120	90	180/105	78	0
Germidine	0.15	W. J.	180/115	84	156/102	72	Epigastric burning
Germidine	0.09	M. C.	220/135	80	160/104	64	Epigastric burning
Germidine	0.14	J. F.	224/145	84	172/120	40	0
Germidine	0.23	E. K.	144/100	74	132/88	62	0
Germerine	0.15	G. S.	190/115	96	140/88	72	0
Germerine	0.28	J. S.	180/94	72	154/68	56	Tingling of skin
Germerine	0.10	M. C.	185/115	76	140/90	56	Substernal burning
Germerine	0.33	J. G.	240/150	96	180/118	74	0
Germerine	0.38	F. S.	150/96	96	110/75	88	Nausea
Germerine	0.30	W. K.	135/90	56	115/75	50	Epigastric burning
Protoveratrine	0.12	F. D.	175/120	64	115/70	52	Tingling of skin
Protoveratrine	0.15	J. S.	178/98	68	148/80	60	Substernal burning
Protoveratrine	0.15	G. W.	190/130	64	165/110	56	Epigastric burning
Protoveratrine	0.11	W. J.	175/100	84	140/88	60	Epigastric burning
Protoveratrine	0.10	M. C.	200/130	80	84/50	44	Tingling of skin
Protoveratrine	0.15	F. S.	145/98	80	98/60	56	Nausea, tingling

of the side effects. As observed following use of the crude drug (1), there was little relationship between body weight and size of an effective dose.

Fraction I, when administered orally, exhibited hypotensive activity in four patients in doses of 0.6 to 0.7 mgm. (table 3). In the same patients Fraction II

exhibited activity in doses of 4.2 to 6.0 mgm. By the intravenous route Fraction I produced a significant decrease in arterial pressure and heart rate in doses ranging between 0.05 and 0.09 mgm. (mean 0.067 mgm.), while Fraction II produced a response in doses of 0.4 to 0.60 mgm. (mean 0.51 mgm.) (table 2). Germine, the parent crystalline alkaloid obtained after hydrolysis of these amorphous fractions was inactive in doses of 8 mgm. orally.

At this point it is necessary to review the present status of the chemical work on the crystalline alkaloids derived from Fractions I and II, and to make reference to some important new facts recently brought to light by the Squibb investigators which considerably add to, and in some respects alter, the picture as it was presented in their preliminary communication (3). In that note they announced the isolation from *Veratrum viride* of two new crystalline alkaloids, germitrine and germidine, obtained by crystallization of Fractions I and II, respectively. Both compounds were found to be esters of the known alkaloid germine, $C_{27}H_{43}O_8N$. The acids present in the ester groups of germitrine were identified as α-methylbutyric acid and methylethylglycolic acid, and those of germidine as acetic acid and α-methylbutyric acid. The analytical findings and other observations suggested that the alkaloid named germitrine was a triester of germine containing one α-methylbutyryl and *two* methylethylglycolyl radicals. However, it has now been established by the Squibb investigators (7) that this compound is actually the known alkaloid *germerine*, an α-methylbutyrate- *mono*-methylethylglycolate of germine which had been isolated in 1937 by Poethke (8) from *Veratrum album*, the white or European hellebore. It was furthermore ascertained that germerine does not occur as such in Fraction I, but arises from a labile precursor (which consititues most of this fraction) when the amorphous product is crystallized from aqueous methanol. This precursor, which has now been also obtained in crystalline form, is a *triester* of germine the acidic components of which include acetic acid in addition to the two acids present in the germerine moiety. The Squibb investigators will propose in their forthcoming detailed publication (9) to transfer the name germitrine (denoting a triester of germine) from the compound now identified as germerine to this genuinely new ester alkaloid, and it should be understood that the term is used with this meaning henceforth in this paper.

Thus three crystalline ester alkaloids were available for our study, namely, the triester germitrine and the diesters germerine and germidine. Germitrine showed about the same activity as the amorphous Fraction I, from which it is derived, by either route of administration (effective dose 0.05–0.06 mgm. intravenously, and 1.0 and 1.2 mgm. perorally). Its degradation product, germerine, had to be given at levels about twice as great (0.10–0.15 mgm. intravenously, and 2.5 to 3.0 mgm. perorally) to elicit the same response. The activity of germidine is of the same order as that of germerine (0.10–0.38 mgm. intravenously, and 2.5–3.5 mgm. perorally). It should be noted that in this case the crystalline compound was considerably more effective than the amorphous Fraction II from which it is derived.

It was of interest to compare the active alkaloids obtained from *Veratrum*

viride with protoveratrine, a crystalline alkaloid derived from *Veratrum album* (8) which has been found by Meilman and Krayer (10) to exhibit hypotensive activity on intravenous injection in hypertensive patients. A sample of this compound made available to us by the Squibb workers was active in doses varying

TABLE 3

Effect of oral administration of the pure alkaloids of Veratrum viride and album on the blood pressure and heart rate of hypertensive patients

ALKALOID	DOSE	PATIENT	CONTROL		AFTER DRUG		SIDE EFFECTS
			Blood Pressure	Heart Rate	Blood Pressure	Heart Rate	
	mgm.		*mm. Hg*	*per min.*	*mm. Hg*	*per min.*	
Fraction I	0.7	E. M.	220/110	76	95/50	60	0
Fraction I	0.7	M. S.	235/125	84	85/58	52	Nausea
Fraction I	0.6	F. D.	160/115	75	148/100	60	0
Fraction I	0.65	G. P.	160/85	72	140/74	56	0
Fraction II	6.0	E. M.	170/98	74	136/88	60	Nausea and vomiting
Fraction II	5.5	M. S.	212/98	64	115/68	46	Nausea and vomiting
Fraction II	4.2	F. D.	170/110	64	135/78	52	Nausea
Fraction II	4.5	G. P.	180/95	74	130/74	58	Nausea and vomiting
Germitrine	1.2	J. M.	190/108	110	160/98	88	0
Germitrine	1.2	E. D.	170/105	86	152/86	72	0
Germitrine	0.9	B. M.	165/110	80	75/55	54	Nausea and vomiting
Germitrine	1.0	J. M.	190/100	104	160/100	74	0
Germidine	3.25	J. G.	212/140	88	90/50	68	Nausea and vomiting
Germidine	3.5	F. S.	160/100	76	120/90	70	Nausea
Germidine	2.2	M. C.	185/118	80	122/84	68	0
Germidine	2.8	J. S.	205/88	70	170/70	60	Nausea
Germerine	2.5	J. U.	170/125	100	150/100	68	0
Germerine	3.0	B. M.	185/125	88	140/100	68	0
Germerine	3.0	E. D.	162/85	88	135/80	64	0
Protoveratrine	1.1	J. S.	188/80	60	155/65	56	Nausea
Protoveratrine	0.9	M. C.	170/100	76	140/90	56	Nausea
Protoveratrine	1.5	F. S.	160/100	80	125/85	64	Nausea

between 0.1 and 0.15 mgm. (table 2), thus confirming the observations of the previous investigators. The side effects observed were similar to those seen after the alkaloids of *Veratrum viride*. In addition, protoveratrine was found to be active orally in doses varying between 0.9 and 1.5 mgm. (table 3).

DISCUSSION. Previous attempts at chemical separation of the alkaloids of

Veratrum viride failed to extract purified compounds from the amorphous base fraction (5, 6). In the present work the guidance obtained by assaying the fractions of the amorphous bases for hypotensive activity in man proved invaluable in guiding the chemists and eventually led to the isolation of two previously undescribed potent hypotensive ester alkaloids, germitrine and germidine. The alkaloid exhibiting the greatest activity was germitrine which exhibited significant hypotensive effects in some patients after intravenous doses of as little as 50 micrograms and was followed in descending order of activity by protoveratrine, germidine, and germerine. All of these compounds are ester alkaloids and those derived from *Veratrum viride* yielded germine on hydrolysis, the latter being inactive in man. Thus, the ester linkages seemed to be essential in determining the hypotensive potency of these alkaloids in man.

Despite the differences in hypotensive potency, the action of each ester alkaloid was quite similar to the others in the character of the response of the blood pressure and heart rate, the type of side effects observed, as well as the time of onset and duration of these responses after both intravenous and oral administration. Whether the frequency and severity of the toxic effects varies with the different compounds cannot be decided by this limited study. The effective oral dose was approximately eight to twenty times greater than the effective intravenous dose.

The results of this investigation suggest that the side effects observed in hypertensive patients receiving *Veratrum viride* may not be separable from the hypotensive principles of the crude drug. The present evidence indicates that the nausea and vomiting are central in origin since such effects may occur following parenteral administration of the alkaloids. Nevertheless, the availability of pure crystalline materials should be of benefit in that it will permit investigation of single pure alkaloids both acutely and on chronic administration in clinical and experimental investigation. Such studies are now in progress.

SUMMARY AND CONCLUSIONS

1. Systematic assay of the various chemical fractions of *Veratrum viride* in hypertensive patients revealed that the cardiovascular effects of the crude drug in man are due largely to the presence of two hitherto undescribed crystalline ester alkaloids, germitrine and germidine. Both these compounds exhibit high hypotensive activity in man on intravenous as well as on peroral administration.

2. Germerine, a third crystalline ester alkaloid which probably does not occur as such in the root but arises by partial hydrolysis of germitrine, exhibits similar hypotensive properties. This compound is not new, but has been previously reported as an alkaloidal constituent of *Veratrum album*.

3. These crystalline compounds are comparable in their effects on the circulatory system to the ester alkaloid protoveratrine derived from *Veratrum album*, although each alkaloid differs somewhat from the others in potency.

4. Partial hydrolysis of germitrine to germerine results in a reduction in hypotensive potency, while complete hydrolysis of either germitrine, germidine

or germerine to germine results in loss of hypotensive action. Thus, the presence of ester groups seems to be essential for hypotensive activity.

5. All of these alkaloids were shown to be active after oral administration, the effective dose by mouth being approximately eight to twenty times greater than the effective intravenous dose.

6. The toxic effects of *Veratrum viride* do not appear to be due entirely to contaminating substances present in the drug, since similar reactions may occur following administration of the pure amorphous and crystalline alkaloids.

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