studies using the slide cell technic to determine the effect of human C-reactive protein on normal human leukocytes, the following conclusions are indicated. 1. Purified human C-reactive protein in final concentrations higher than 0.005 mg/cc destroys normal human leukocytes. 2. Purified human C-reactive protein in lower concentrations stimulates the migration of normal human leukocytes. 3. C carbohydrate, sodium citrate, and normal human serum protein do not stimulate normal human leukocytes in the concentrations employed in this study. 4. The acute phase human sera tested caused increased migration of normal human leukocytes by virtue of their content of C-reactive protein. 5. Human serum diminishes at least in part the toxic effect of the C-reactive protein on normal human leukocytes.

Received April 5, 1951. P.S.E.B.M., 1951, v76.

Acute Toxicity of Veratrum Derivatives.* (18651)

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Interest in veratrum preparations has varied considerably over a period of years. Fruitful clinical application of the blood pressure lowering powers of veratrum derivatives depends on the use of materials of constant hypotensive power in the form of pure alkaloids or of mixtures standardized by their ability to lower mammalian blood pressure. The interrelationship between lethality of these substances and their hypotensive property has attracted interest as a possible method of assay(1). The results detailed below should do much to eliminate from further consideration lethality as a method of assay. While some data of the acute toxicity of veratrum derivatives have been published (1-3), strictly comparable toxicity data for the various substances have not been found. This study of the acute toxicity of veratrum preparations has been divided into (a) comparison of the acute toxicity of pure ester alkaloids or of alkaloidal mixtures from veratrum with their hypotensive potency(4). (b) Elucidation of influence of species of animal and of route of administration on the acute toxicity of a clinical veratrum preparation (Veriloid†).

Method. Albino Swiss mice (15-25 g) and albino Wistar rats (175-245 g) were weighed to the nearest 0.1 g. The solutions to be injected were prepared so as to hydrate the mouse at a rate of 0.01 ml and the rat at 0.005 ml per g of body weight. The injected animals were observed for 24 hours (actually all deaths occurred within the first hour). The New Zealand albino rabbits (2-3 kg), and the adult mongrel dogs (3-12 kg) were hydrated at the rate of 0.5 ml per kg and observed for 24 hours. The solutions of the pure alkaloids‡ were prepared by dissolving the alkaloids in a minimum amount of 5% acetic acid and diluting to the desired concen-

* Grateful acknowledgement is made for financial support of this work received from Riker Laboratories, Los Angeles, Calif.

1. Rowe, L. W., *J. Am. Pharm. A.*, 1925, v14, 24.
2. Christensen, B. V. and McLean, A. P., *J. Am. Pharm.*, 1936, v24, 414.
3. Krayer, O., Acheson, G. F., *Physiol. Rev.*, 1946, v26, 283.
4. Maison, G. L., Gotz, E., and Stutzman, J. W., *J. Pharm. and Exp. Therap. Proc.*, 1951, v101, 24.

† *Veriloid* (Trademark of Riker Laboratories) is a biologically standardized alkaloidal extract of *Veratrum viride* of uniform hypotensive potency.

‡ The substances utilized were kindly provided by the following sources: Eli Lilly and Company, Ind., Protoveratrine; Merck and Company, Rahway, N. J., Veratrine; S. B. Penick and Company, Detroit, Mich., Veratrine; Dr. S. J. Ringel, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md., Cevadine; Riker Laboratories, Los Angeles, Calif., Protoveratrine, Veriloid and Veratridine; Squibb Institute for Medical Research, New Brunswick, N. J., Germitrine, Neogermitrine, and Germidine.

TABLE I. Intraperitoneal Toxicity of Veratrum Alkaloids and Extracts.

Drug Lot # (MP °C)	LD_{50} C.L. 19/20 mg/kg	No. of animals tested	Standard hypotensive dose, μg/kg/min.*	LD_{50} in terms of standard reference potency†
Neogermitrine JF 115—C_5 (231-232°)	.51 (.41-.63)	40	.115	4.43
Protoveratrine (Riker) 7/18/50 (265°)	.44 (.30-.64)	40	.212	2.07
Protoveratrine (Lilly) P—49366 (246°)	.37 (.29-.48)	110	.218	1.72
Germidine JF 68—C 8 (230-232°)	10.0 (9.1-11.0)	29	.416	24.0
Veratridine 5/19/50 (171°)	1.35 (1.12-1.62)	70	2.13	0.63
Cevadine‡ (209°)	3.5 (2.7-4.4)	50	5.55	0.63
Standard reference powder (Veriloid)	3.75 (3.4-4.1)	390	1.00	3.75
Veratrine§ (Penick) (NF IV)	8.5 (7.5-9.6)	50	3.7	2.3
Veratrine§ (Merck) (145-155°)	7.5 (6.1-9.2)	30	3.7	2.5
Veratrone	2.45 (1.8-3.2)‖	48	0.85	2.9
Fluid extr. USP IX	182 (151.6-218.4)¶	30	42.3	4.3

* These data are from Maison, Gotz and Stutzman(4). Standard dose is an estimate of μg/kg/min. over 10-minute period required to produce average fall of mean arterial pressure of 30% in a large number of anesthetized dogs.

‖ Calculated on basis that 100 ml of Veratrone equals ¼ g.

† Calculated from: LD_{50} value divided by the hypotensive potency in standard dose.

§ Veratrine is a mixture of alkaloids (predominantly cevadine and veratridine) obtained from the seeds of sabadilla.

¶ Calculated on basis that 1 ml of extract equals 1 g crude root.

‡ Tiglic acid ester of cevine.

tration with physiologic saline. The various purified extracts of veratrum were dissolved in a minimum of a solution of equal parts of propylene glycol and alcohol§ and diluted to the desired concentrations with acidified physiologic saline. All determinations were calculated by the method of Litchfield and Wilcoxon(5). Care was taken to utilize enough animals in each determination to produce statistical validity as reflected in the Chi^2 value.

Results. The acute toxicities of several pure veratrum alkaloids and their hypotensive potencies are listed in Table I. Fig. 1 depicts toxicity versus depressor power for a series of veratrum extracts of the same general nature at various stages of purification. Each point represents the lethal dose 50 (LD_{50}) determination for a different extract. Table II presents the acute toxicity values for *Veriloid*† standard reference powder in various species as well as by different routes of administration. Also included in this table is a demonstration of the validity of the method shown by repeated determinations of the toxicity of a particular powdered extract.

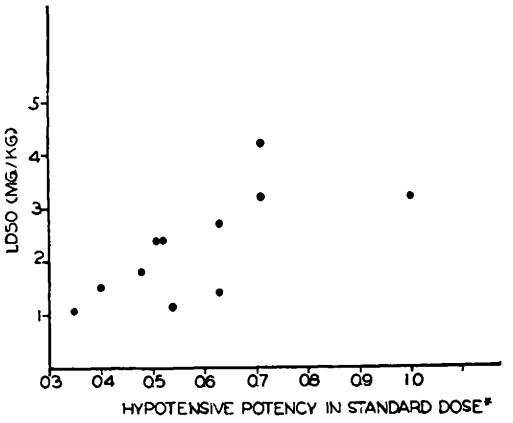

FIG. 1.
Various purified extracts of *Veratrum viride.*

§ Final concentration of alcohol in injected solution never exceeded 2%.

5. Litchfield, J. T. and Wilcoxon, F., *J. Pharm. and Exp. Therap.,* 1949, v96, 99.

* Standard dose is an estimate of μg/kg/min. over a 10-minute period required to produce an average fall of mean arterial pressure of 30% in a large number of anesthetized dogs.

TABLE II. Acute Toxicity of Veriloid Standard Reference Powder.

Animal	Route	LD_{50} 19/20 C.L., mg/kg	Date	No. of animals tested
Mice	Oral	4.5 (3.0-6.84)		40
"	I.P.	3.4 (2.6-4.6)	4/3/50	60
"	"	3.45 (2.83-4.21)	8/18/50	40
"	"	2.95 (2.54-3.42)	8/17/50	30
"	"	3.3 (2.8-3.83)	8/23/50	40
"	"	3.0 (2.5-3.57)	8/30/50	40
"	"	4.4 (3.7-5.2)	1/8/51	60
"	"	4.3 (3.5-5.2)	1/8/51	60
"	"	4.25 (3.6-4.9)	1/9/51	30
"	"	4.1 (3.2-5.2)	1/10/51	60
"	I.V.	0.95 (0.81-1.12)	—	40
Rat	Oral	12.2 (10.9-13.8)	—	291
"	I.P.	1.69 (1.26-2.26)	—	25
"	I.V.	0.44 (0.39-0.48)	—	60
Rabbit	Oral	18.7 (15.4-22.6)	—	63
Rabbit	I.V.	0.27 (0.24-0.31)	—	53
Dog	"	Emesis occurred—no death		

Discussion. Mean lethal dose (M.L.D.) values (intraperitoneal mice) were determined for *Veratrone*¶ in mice by Rowe(1) and by Christensen and McLean(2). Their results fall within the confidence limits obtained in this investigation. The nine determinations of the acute toxicity of *Veriloid*† standard reference powder (Table II) show a greater consistency of results than was to be expected from the data of previous workers. A seasonal variation was observed, with the less toxic results being obtained in the winter months. The oral lethal dose for the dog was not obtained because emesis occurred.

The hypotensive potencies of the various substances as revealed by the dog assay method have been utilized for comparative purposes in this study. While there may be argument as to the limit of accuracy of these data they do offer the best estimate available for the purpose.

The results presented in Table I suggest that no correlation existed between the acute toxicity and the hypotensive potencies of the pure veratrum alkaloids. Although a rough correspondence between hypotensive potency and acute toxicity existed for the extracts reported in Fig. 1, predictability of hypotensive power was not indicated for the acute toxicity method.

Summary. (1) The acute toxicity in mice by the intraperitoneal route for several pure veratrum alkaloids and several alkaloidal mixtures from veratrum are presented. (2) The results suggested that predictive correlation did not occur between acute toxicity and hypotensive potency. (3) The influence of route of administration and species of animal on lethal dosage was demonstrated. Oral administration did not produce lethality in species capable of emesis.

¶ Veratrone (Trademark Parke Davis and Co.) is a nonalcoholic solution equal to ¼ the strength of fluid extract Veratrum viride by alkaloidal assay (chemical).

Received April 10, 1951. P.S.E.B.M., 1951, v76.