17310. Veriloid, a New Hypotensive Extract of Veratrum Viride.*

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The promising results of Freis *et al.*[1,2] in therapy of patients with essential hypertension suggested that a hypotensive extract of constant composition from *Veratrum viride* would be highly useful. Accordingly a series of fractionations was carried out.‡ An assay routine based on hypotensive properties in normal dogs under pentobarbital anesthesia was applied. Accessory screening technics included emetic and bradycardic potency estimations. After screening some 75 fractions, a highly potent, reproducible, stable and uniform extract was selected for further study. This material has been given the proprietary name "Veriloid."

While the physical and chemical properties of "Veriloid" will be treated *in extenso* elsewhere a brief outline is presented in Table I. Evidence exists that "Veriloid" is a mitxure and not a single alkaloid. None of the previously described potent alkaloids of *Veratrum viride* has been obtained by fractionation of "Veriloid." (See Krayer[3] for comprehensive review of literature). The relatively impotent rubijervine and isorubijervine have been identified as present in the mixture to the extent of 25%. For clinical use further purification at present was not warranted for logistic reasons. A series of 12 separate fractionations from crude root has shown inappreciable variations in product by both chemical and biological tests.

Biological effects of "Veriloid" are outlined in Table II. Full data will be published separately.

The data illustrate a high therapeutic ratio for hypotensive action. With increasing intravenous dosage in normotensive animals, the degree of hypotension increased only up to an average maximum fall of 40% of the pre-existing mean arterial pressure. As dosage was raised the duration of the reduced mean pressure increased. Usual duration at fully effective intravenous dosage was 30 to 90 minutes. The return to previous pressure was gradual. Preliminary data indicated that the role of the bradycardia in the hypotension was a secondary one. Hypotension still was elicited after vagotomy; bradycardia was not. The hemodynamic rearrangement seen during this hypotension would best fit a hypothesis that "Veriloid" caused dilatation of arterioles in skeletal muscle, splanchnic region and skin accompanied by constriction of venous vascular beds. Evidence was elicited that blood flow was not reduced with the hypotension. Systolic and diastolic blood pressures were both decreased and nearly equally so. Anesthesia did not alter the minimal hypotensive dose. Hypotension resulting from "Veriloid" was corrected by pressor amines such as epinephrine, phenylephrine and methoxamine.

Minimal bradycardic action from intravenous administration had a higher minimal effective dose, a longer latency and a shorter duration than did the hypotensive action. Pentobarbital anesthesia reduced the amount of drug required to produce bradycardia. As the dose was raised degree and duration of bradycardia increased. Lengthening of P-R interval, partial heart block, and transient A-V nodal rhythm were seen at 20 to 50 times the minimal dose. As the dose was raised still further ventricular tachycardia, venous and arterial hypertension resulted. A coarse ventricular fibrillation was observed occasionally at 200 to 500 times the minimal dose. Very high intravenous dosage in the unanesthetized

* Supported in part by a grant from the Coe Chemical Co., Los Angeles, Calif.

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1 Fries, E. D., and Stanton, J. R., *Am. Heart J.*, 1948, **36**, 723.

2 Fries, E. D., Stanton, J. R., Culbertson, J. W., Litter, J., Halperin, M. H., and Wilkins, R. W., *J. Clin. Inv.*, 1949, **28**, 353.

‡ In the laboratories at Los Angeles.

3 Krayer, O., and Acheson, G. H., *Physiol. Rev.*, 1946, **26**, 383.

TABLE I.
Physical and Chemical Properties of "Veriloid."

Appearance: Pale yellow amorphous powder.

Melting Point: Sinters at 102°-105°C.
Melts at 148°-155°C.

Optical Rotation: a $^{26°}_{D}$ in EtOH = —17.3°C = 0.5 g/100 ml.

Ultra Violet Absorption Curve: Peak at 2500 Å ln E = 3.30 (at 0.00008 g/ml).

Solubility: In water—very slightly soluble.
In dilute acid—soluble.
In benzene, alcohol, chloroform, propylene glycol, acetone—soluble.

Spot test reaction with c. H_2SO_4—Dark orange going to reddish orange to brown in 24 hours.

Nitrogen Content—2.9%.

TABLE II.
Dosage of "Veriloid" in mg/kg Required to Produce Physiological Alterations in Dogs.

Effects	Anesthetized (Pentobarbital sodium) Intravenous	Unanesthetized	
		Intravenous	Oral
Hypotension (reduction of at least 10 mm Hg)	ED50 0.0021 ± 0.0001* (200 trials)	ED50 *ca* 0.002 (26 trials)	Irregular
Bradycardia	ED50 0.0021 ± 0.0003 (75 trials)	ED50 0.0055 ± 0.0004 (75 trials)	Not obtained at 0.05 (20 trials)
Emesis—Fasted	Varies with depth of depression. Rare		ED50 0.0136 ± 0.0017 (50 trials)
Not fasted		ED50 0.0197 ± 0.001 (50 trials)	ED50 *ca* 0.025 (30 trials)
Apnea (30 to 90 sec. duration)	Irregular but never below 0.02 (200 trials)	ED50 *ca* 0.1 (15 trials)	Never obtained (Tried to 5.0)
Hyperirritability (15 to 20 sec. duration)	Never obtained	Above 0.01 (45 trials)	Never obtained
Displacement of cardiac pacemaker	Approximately 0.1 (20 trials)	Never below 0.05. Occasionally at 0.1. Common at 0.2 to 0.5	Seen at 1.0
Hypertension	ED50 *ca* 0.05 (30 trials)		
Lethal	Varies 0.05 to 0.4 (30 trials)	LD50 *ca* 0.5 (6 trials)	Never obtained 5.0 tolerated (5 trials)

* Standard error (See Miller and Tainter, PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 261).

animal produced similar effects except that fibrillation has never been observed. Transient A-V nodal rhythm has also been obtained after oral administration of 1 mg/kg in the dog.

Tachyphylaxis to the bradycardic and hypotensive actions has not been seen. Similarly, tolerance did not develop in 2 dogs following the intramuscular administration of 5 times the minimal effective dose twice a day

for 5 weeks.

Like other Veratrum derivatives "Veriloid" is a potent emetic by any route. Oral dosage required to produce vomiting was at least doubled by the presence of food in the stomach. Thus reduction of clinical side effects would be expected to result from administration during meals.

Oral administration in dosage up to 0.075 mg/kg to the unanesthetized dog has only irregularly resulted in hypotension. The same dose did cause a fall of mean arterial blood pressure when placed in the upper jejunum of animals anesthetized with pentobarbital. Clinically, the effective single oral dose has been found to vary from 0.03 to 0.08 mg/kg in hypertensive patients.[4]

Comparison of the effects of intravenous and intestinal administration in the anesthetized dog showed that the drug was more effective in producing fall of blood pressure by the intravenous route. In order to delineate the effect of slow absorption prolonged infusions were made. Slow intravenous administration became ineffective in the anesthetized dog below ¼ γ/kg/min. Effective doses by this method produced graded maintained hypotension. Comparison of the effects after splenic and femoral intravenous administration has not demonstrated inactivation of "Veriloid" by the liver.

Limited clinical trial of "Veriloid" has been accomplished by Freis and Wilkins. This will be reported separately. Certain individuals required as little as 2 mg or as much as 6 mg for single oral dose to produce blood pressure fall. Latency for full hypotension to be established was 2 hours.

Summary. A stable, reproducible and highly potent extract of *Veratrum viride* has been described. The name "Veriloid" has been given to this preparation. Methods for biological control of potency have been developed. In normal dogs the extract was hypotensive, bradycardic and emetic. Pharmacologic results suggest that the hypotensive action of the extract deserves trial in the treatment of human hypertension.

[4] Wilkins, R. W., and Freis, E. D., personal communication.

Received June 21, 1949. P.S.E.B.M., 1949, **71.**

17311. A Comparison of Desoxyribonucleic Acid Content in Certain Nuclei of Normal Liver and Liver Tumors.

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Direct chemical analyses of actively growing tissues, particularly tumors, for desoxyribonucleic acid have been reported by several investigators with, however, scant agreement. The percentage of desoxyribonucleic acid in nuclear material was found by Dounce[1] to be the same for Walker carcinosarcoma 256 and normal liver; in hepatoma 31 the percentage of desoxyribonucleic acid was lower than the normal. Brues, Tracy and Cohn[2] observed that the phosphorus and nitrogen content of hepatoma 31 and normal liver was the same per unit weight. On a similar basis, Davidson and Waymouth[3] and Schneider[4] recorded values for desoxyribonucleic acid that were higher in hepatomas than in normal liver.

Using the light absorption of fields of Feulgen stained nuclei, Stowell[5] also found

* Fellow of the Jane Coffin Childs Memorial Fund for Medical Research. This investigation was aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research.

[1] Dounce, A. L., *J. Biol. Chem.*, 1943, **151**, 235.

[2] Brues, A. M., Tracy, M. M., and Cohn, W. E., *J. Biol. Chem.*, 1944, **155**, 619.

[3] Davidson, J. N., and Waymouth, C., *Biochem. J.*, 1944, **38**, 379.

[4] Schneider, W., *Cancer Res.*, 1945, **5**, 717.

[5] Stowell, R. E., *Cancer Res.*, 1946, **6**, 426.