dant in the tops than the roots, but both preparations may be shown to be oestrogenic by direct application to the vaginas of ovariectomized rats. The oestrogen content varied from one extract to another, but 200 mg of lyophilized tops may contain up to 1 γ equivalent of stilboestrol, when assayed on the uterine weights of immature rats. The oestrogen content of lithosperm is a factor which may influence the gonadotrophic content of the pituitaries of treated animals and throws some doubt on the interpretation of previously published assays.

Summary. It has been shown that *Lithospermum ruderale* has an anti-oestrous effect on rats, the roots containing more active material than the tops. The subcutaneous injection of extracts of the plant is at least 10 times more effective than oral administration. Lithosperm does not inhibit the action of administered oestradiol. Experiments indicate that lithosperm contains a water soluble oestrogen which is found primarily in the tops.

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Value of Daphnia as an Assay Organism for Veratrum Alkaloids. (17667)

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Veratrum plants contain an unknown number of alkaloids of apparently similar but not identical physiological action, which are not amenable to chemical assay. The availability of a biological testing method would, therefore, be of decided advantage. Recently, *Daphnia magna* has been extensively publicized as a testing object. Daphnia have been recommended before for the assay of cardiotonic drugs(1). Concerning the adaptation of the test for Veratrum, the only information available is the statement that the criterion of potency consists in the arrest of the heart of *Daphnia magna*. It is evident that this possibly measures a toxic effect not necessarily proportional to the therapeutic value of the material. Another point is that any analogy between Daphnia and human being cannot be postulated. For the present study we prepared jervine, veratrosine, pseudojervine and veratramine following the methods of Jacobs and Craig(2). Having difficulty in obtaining *Daphnia magna,* our first experiments were performed with a non-identified Daphnia strain obtained from a New Jersey pond; later we repeated the work with *Daphnia magna.*

To make these tests, a capillary tube, bent slightly downward at the end, is fixed with Scotch tape to an object carrier with a ground hollow in such a way that the tip rests at the edge of the hollow. A small piece of absorbent cotton is placed into the hollow without touching the capillary. The latter is connected to a suction pump. Daphnias are caught with a wide-tipped medicinal dropper and placed in the center of the cotton bed. The excess of water is promptly removed by the suction but sufficient liquid remains in the cotton to cover the animals caught between the fibers, provided the capillary does not touch the cotton. It is feasible to observe 4 or 5 small Daphnias at a time but only one or 2 of the *magna* species. The medication, in proper dilution, is then dropped from a pipette on the cotton until the water is replaced by the solution. From this moment on the time of action is counted. Every 3 to 4 minutes one-half cc of the solution is flown through the cotton to prevent a change in concentration by evaporation. The normal heart rate of the Daphnia ranges from 250 to 600 beats per minute(1), which is beyond the possibility of counting. Under the influence of the alkaloids the rate

1. Boylor, E. R., *Biol. Bull.*, 1942, v83, 165.
2. Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, 1944, v155, 565; 1945, v160, 555.

TABLE I.
Response of Wild Daphnia to Veratramine at Varying Concentrations.

Veratramine conc.	pH	Total No.	Heart failure within 6 min.	12 min.	20 min.	21-30 min.	31-40 min.	40 min. +	Remarks
1:1000	5	13			2	1	2	8	Adults
1:1000	3	6	3	3					"
1:1000	4	57	0	7	31	11	7	1	" most bradycardia
		23	0	5	13	5			Young D. most dead
1:1330	4	35	0	3	12	8	6	6	Adults
		9	0	1	6	1	1		Young all dead
1:2000	4	6				4	2	2	Adults
1:3000	4	20					6	14	
1:5000	4	24					4	20	
1:50,000	4	9						9	

Neither veratrosine 1:500, jervine 1:500 nor pseudojervine 1:2000 (max. possible conc.) produced more than a moderate bradycardia within the time of observation, 40 to 60 min.

soon drops to 180 or less which permits counting with the aid of a stop watch. It was soon found that even the same alkaloid does not have a uniform effect on the Daphnia heart. Some hearts slow down gradually until they finally stop, others keep up a slow beat for an indefinite time; some come to a stop from a relatively fast beat, which stop may be final or followed by periods of resumed beating. Some hearts develop arrhythmias. All these signs may be considered as symptoms of heart failure.

It was necessary to select somewhat arbitrarily the following criteria as end point; stop of the heart, regardless whether temporary or final; pronounced arrhythmias; drop of the heart rate to 75 or less. Another effect was observed in some rare instances: a gradual decrease in amplitude at sustained fast rate until finally only a vibration of the heart membrane remained. Since an endpoint in this case would be absolutely arbitrary, the few animals giving this reaction were not included in the compilation. *Daphnia magna* showed to some alkaloids reactions not seen in the wild Daphnia: a quick stop of the heart within a few minutes followed by resumed beating lasting some minutes and alternating stops and activity. In response to weak solution this behavior may last for more than an hour. A limitation was set to the tests by the low solubility of the alkaloids. A solution in dilute hydrochloric acid, neutralized to pH 5, remains clear, but the alkaloids have little effect in such solutions. The activity was manifest at higher acidity. Since Daphnias showed no deterioration in hydrochloric acid of pH 4 during one hour, all solutions were adjusted to that pH.

Table I shows the effect of veratramine on wild Daphnias. The other alkaloids tested at highest possible concentrations produced a moderate degree of bradycardia within 40 minutes but the signs of heart failure did not appear at all or only after a long and variable period of time. Table II shows the results obtained with *Daphnia magna*. The striking difference is the high potency of veratramine if the quick and temporary arrest of the heart is taken as an endpoint. It was obtained at dilutions about 100 times higher than those effective in the wild strain. The sudden, temporary stop seems to be produced by a different mechanism than the slow-developing bradycardia. It fails to appear if the concentration of the solution is increased gradually from subthreshold concentration to the supposedly active strength. Jervine, applied gradually in increasing dosage or at 1:2000 ten minutes preceding veratramine 1:2000, prevented the sudden arrest by the latter. Instead, bradycardia developed progressively to reach the limit of failure after 30 to 40 minutes. Jervine applied simultaneously with veratramine did not prevent sudden heart stop. That the time necessary to produce a certain degree of bradycardia is subject to wide variations may be judged from

TABLE II.
Response of Adult *Daphnia magna* to Four Veratrum alkaloids at pH 4.

Alkaloid and conc.	Total No.	Temporary heart stop		Heart failure in				Remarks
		6 min.	12 min.	20 min.	21-30 min.	31-40 min.	40 min. +	
Pseudojervine								
1:2000	13			1		4	8	
Jervine								
1:10,000	1						1	Bradycardia only; even those with temp. stop recover; none dead at 60'
1: 2,000	11				5	2	4	
1: 1,330	1						1	
1: 1,000	10				2	3	5	
1: 500	10	6	2		2			
Veratramine								
1: 10,000	1	1						
1: 50,000	12	10			2			
1:100,000	12	10		1	1			
1:150,000	10	7		1	2			
1:200,000	16	10	2	1			3	
1:400,000	12				2		10	
Veratrosine								
1:100,000	1						1	
1: 50,000	1						1	
1: 10,000	1						1	
1: 1,000	5						5	
1: 500	24	1			1	8	14	Moderate bradycardia

the following figures obtained with veratrosine 1:500, which alkaloid gives the most dependable responses: The heart rate of 5 Daphnias was determined every 3 to 4 minutes. A rate of 150 was observed at 4 to 8 minutes, 124 at 7 to 32 minutes, 100 at 9½ to 40 and 75 at 27 to 50 minutes.

Veratrum contains a number of other alkaloids not included in these tests. The present investigation leads to the conclusion that the effect of a crude extract, measured by the sudden heart stop, would essentially be caused by veratramine-type alkaloids. This sudden stop makes it impossible to use the bradycardia, that develops slowly, as a criterion for the assay of mixtures.

Krayer(3) has concluded from experiments in mammals that veratramine and veratrosine are of a potency to abolish adrenaline tachycardia, which is within the same range, the latter alkaloid being more potent and less toxic. This type of effectiveness of veratrosine is not likely to be noticed in the Daphnia test.

Conclusions. The Daphnia test actually measures toxicity. The assay of a crude extract would measure the presence of veratramine and of substances of similar action but would not reveal anything about the therapeutic value, if such should exist.

3. Krayer, Otto, *J. Pharm. and Exp. Therap.*, 1949, v97, 264.

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