The Action of Lithosperm[1] in Mice

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Interest in the anti-gonadal effects of the plant *Lithospermum ruderale* was stimulated by the initial experiments of Cranston (1), Drasher and Zahl (3), and Drasher (2). These investigators demonstrated that the feeding of dried overground parts of the plant inhibited the estrus cycles of mice and that the animals usually lapsed into extended periods of diestrus. It was necessary in these experiments, however, to feed the plant material in considerable quantity, approximately 30% of the diet. Treatment was often accompanied by weight losses which complicated the interpretation of the results since it was suggested that the observed diestrus might be a result of poor nutrition. Extracts of the plant were not effective since they rapidly lost activity and frequently were toxic. Consequently, Drasher (2), Noble et al. (4), and others attempted to extract the active principle from the plant and to produce a stable preparation. These attempts were not very successful but their work clarified certain phases of the problem: namely, that lithosperm actually was anti-gonadal in its action and that it probably inactivated anterior pituitary hormone LH, the luteinizing hormone.

Materials and Methods

Female white Swiss mice approximately 2½ months of age were used in these experiments. Five animals were kept in each cage in air-conditioned animal quarters. Each animal was ear-marked and vaginal smears were taken by the lavage method for from 12 to 14 days before the beginning of the experiment. Vaginal smears were stained mith aqueous-methylene blue and allowed to air-dry after which they were read. We were thus able to establish the cycle of each mouse and to allot experimental animals to groups according to the reproductive cycles which were demonstrated during the pre-treatment period.

The animals were treated with the lithosperm preparation in two ways: they were injected subcutaneously with 0.1 ml. daily, or were given the lithosperm orally also in 0.1 ml. daily. The control animals were administered distilled water orally or subcutaneously. An apparatus comparable with that which is sometimes used for oral administration of drugs to rats was used for feeding lithosperm to the mice. A small piece of polyethylene tubing with an outside diameter of 0.060 inches was slipped over the end of a long 22 gauge injection needle. The

[1] The term lithosperm is used as a generic designation for the hormone inhibiting principle, or principles, present in the genus *Lithospermum*.

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needle was attached to a 1 ml. tuberculin syringe graduated in 0.01 ml. so that accurate amounts of preparation could be administered. Paraffin was placed about the junction of the polyethylene tubing and the needle in order to avoid loss of fluid by back pressure. The mouth of the mouse was kept open by hooking the incisor teeth over a V-shaped mounting bar and the polyethylene tube was then inserted into the stomach. If the tongue is placed below the lower bar the insertion of the tube into the esophagus and stomach can be made without difficulty.

The lithosperm preparation used in this study was a spray-dried powder which was made from a cold water extract of dried, ground plant material. We are indebted to the Smith, Kline and French Laboratories for the preparation of this material. The spray-dried powder has been used in numerous experiments in this laboratory and has remained stable and active for more than a year. The powder was mixed with distilled water before administration and the dosages used were on the basis of milligrams of powder per milliliter of solution.

Results and Discussion

Since all of the animals used in the experiments were selected from animals with known estrus cycles determined by pre-treatment obser-

TABLE 1

Effect of Lithosperm on the Estrus Cycle of Mice

Amount of Lithosperm and method of administration	Amount of Estrus smears		No. of Metestrus I Smears			
	observed	% expected	observed	% expected	Totals	% expected
0.4 mg/0.1 ml. subcutaneously	30	88	19	119	49	98
0.4 mg./0.1 ml. tube fed	20	54	9	69	29	58
0.6 mg./0.1 ml. subcutaneously	31	79	13	81	44	80
0.6 mg./0.1 ml. tube fed	13	39	15	125	28	62
0.8 mg./0.1 ml. subcutaneously	22	69	16	55	38	62
0.8 mg./0.1 ml. tube fed	23	56	6	46	29	54
1.0 mg./0.1 ml. subcutaneously	26	42	11	23	37	34
1.0 mg./0.1 ml. tube fed	30	44	16	36	46	41
Totals	195		105			
% Expected		56		55		

vations of smears, it was possible to estimate how many periods of estrus would be expected to occur during the 20 day treatment period if lithosperm were not effective. A slight change in the usual procedure of evaluating the cycles was employed. It was felt that a more accurate picture of cycles would be obtained if metestrus I stages as well as estrus stages were counted because it might be possible to miss estrus stages from time to time. Table I presents the results of the experiments in which lithosperm was administered both by subcutaneous injection and by tube feeding.

The data clearly demonstrate several important points relative to the action of the spray-dried lithosperm preparation in mice. The administration of the plant extract by feeding was more effective than by subcutaneous injection. Even the lowest dosage of 0.4 mg. per day had slight inhibitory effect. The highest dosage, 1.0 mg. per day, was the only one in which the subcutaneous treatment appeared to be slightly more effective. The totals on the right of the table illustrate these differences in response according to the route of administration and likewise demonstrate that there is an increase in inhibition of the estrus cycles as the dosage of lithosperm is increased.

It is also of interest to note the results represented by the totals of vertical columns. The percentage of inhibition as indicated by the number of estrus and metestrus I smears is almost identical; 56% compared with 55%. We believe that this supports the idea that the two stages afford an excellent method of characterizing the estrus cycles. It will be recalled that lithosperm constituted as much as 30% of the diet in earlier experiments, but in these experiments dosages of 1.0 mg. per day of the powder and less had inhibitory effect, furthermore, the mice did not lose weight. We believe the inanition is not a complicating factor in these experiments and that very small amounts of the spray-dried preparation are potent.

Since it has been suggested that lithosperm inactivates the luteinizing hormone, or perhaps prevent its secretion, a histological study was made of some of the ovaries of control mice and lithosperm treated mice. All ovaries were removed from the mice when they were in diestrus. The number of corpora lutea in the ovaries were counted and this was correlated with the number of estrus smears which had been observed in these mice. These results are given in Table 2.

There is a clear difference between the number of corpora lutea and the number of estrus smears in the control and treated mice. Only mouse number 53 of the injected mice was comparable with the controls. The 14 ovaries represented in the controls had a total of 35 corpora lutea, whereas there were only 7 corpora in the 16 treated ovaries and 5 of these appeared in mouse number 53. These data support previous suggestions by other workers that the administration of lithosperm prevents the action of the luteinizing hormone. At present, however, the evidence is not adequate to explain whether inactivation of the hormone, prevention of its secretion by the anterior pituitary, or both factors are involved in the mechanism of inactivation.

TABLE 2

Number of Corpora Lutea in the two ovaries of lithosperm treated and control mice

Treatment	Mouse	Corpora Lutea	Estrus Smears
Lithosperm—Daily 1.0 mg./0.1 ml.			
Subcutaneous injection	2	0	0
	12	1	0
	27	0	1
	53	5	5
Tube fed	47	0	2
	1	0	0
	15	1	0
	30	0	0
Controls	14	3	0
	35	9	3
	46	3	1
	60	5	2
	28	2	3
	41	5	3
	55	8	2

Summary

Administration of a spray-dried preparation of *Lithospermum ruderale* (lithosperm) by subcutaneous injection or by tube feeding inhibited the estrus cycles of mice. Tube feeding was more efficacious than was subcutaneous administration and as little as 0.4 mg. of powder per day had a demonstrable effect. The number of corpora lutea in the ovaries also was significantly fewer in the lithosperm treated mice than in the controls.

References

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