

Proceedings  
of the  
Society  
for  
Experimental Biology and Medicine

VOL. 67

APRIL, 1948

No. 4

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SECTION MEETINGS

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| CLEVELAND                      |                   |
| Western Reserve University     | March 12, 1948    |
| MINNESOTA                      |                   |
| University of Minnesota        | March 10, 1948    |
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| Permanente Foundation Hospital | February 25, 1948 |
| SOUTHERN                       |                   |
| Louisiana State University     | March 2, 1948     |

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16322

Some Characteristics of the Anti-Estrous Factor in *Lithospermum*.\*

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Two laboratories have independently reported that the herb *Lithospermum ruderale* when mixed with normal diet abolishes the estrous cycle in mice.<sup>1,2</sup> The present note describes further studies on modes of administration, site of action within the body, differential strain susceptibilities, distribution of the factor within the *Lithospermum* plant, as well as some preliminary fractionation work.

*Experimental.* 1. *Absorption from Pellets.* Daily vaginal smears, using the method previously described,<sup>2</sup> were made from large

groups of Rockland Swiss strain virgin female mice between 2 and 4 months of age. Fifty of these mice showing least irregularity of estrus were selected. These were further segregated into 5 uniform weight groups. One lot was maintained as the control, each mouse receiving a subcutaneously implanted 50 mg beeswax pellet. Individuals of the second lot received similarly implanted pellets consisting of 25 mg of beeswax and 25 mg of the crude oil residue of the ether extraction of 100 g of powdered *Lithospermum*.<sup>†</sup> A neutral water extract of the same batch of *Lithospermum* was incorporated in similar proportions into other beeswax pellets, and implanted into a third group of mice. A fourth group received beeswax pellets con-

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\* The work reported in this paper is a portion of that being carried on under a grant from the National Advisory Cancer Council of the U. S. Public Health Service. Technical assistants on the project were M. L. Drasher and Andrew Nowak.

<sup>1</sup> Cranston, E. M., *J. Pharm. and Exp. Therap.*, 1945, **83**, 130.

<sup>2</sup> Drasher, M. L., and Zahl, Paul A., *Proc. Soc. Exp. Biol. and Med.*, 1946, **63**, 66.

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<sup>†</sup> *Lithospermum* powder was prepared by milling the whole plant, including roots, stems, floral parts, stalks, and leaves. Florescent plants were collected in mid-June in the deserts of western Montana, and dried in the shade.

taining 2.5 mg of *iso*-androliclactone acetate.<sup>‡</sup> A fifth group received beeswax pellets containing 10 mg of thiouracil.<sup>§</sup> A daily estrous record for each of the mice comprising these groups had been kept for 20 days previous to pellet implantation; and vaginal smear estrous assay was continued for 40 days following the implantation. In only one of the 5 groups was there observable suppression of estrus, *i.e.*, the group receiving the water-soluble *Lithospermum* fraction.

The mean weights of each group of mice were taken weekly, starting with the time of pellet implantation and continuing during the experimental 40-day period. The weight curves were normal except for that of the thiouracil group, and to some extent that of the *iso*-androliclactone acetate group, which dropped somewhat initially, but recovered within 3 weeks. The drop in the thiouracil weight curve is taken to indicate that an effective anti-thyroid level of the compound was being maintained by absorption of the pellet, without exertion of any pronounced anti-estrous effect. It is not clear how thiouracil under these conditions would effect a weight loss; one may assume either some kind of side action, or assume that absorption from the pellet was so rapid as to constitute a gross overdose. Whether the *iso*-androliclactone acetate was similarly toxic was not clear, due to the questionable significance of its weight curve aberrancy.

2. *Strain Differences.* It was desirable to obtain additional data on strain differences in respect to susceptibility to the action of *Lithospermum*. The strains to be compared were the Rockland Swiss and the C<sub>3</sub>H lines, the latter a high mammary tumor incidence strain. A set of 2 to 4 months old Rockland females was examined for estrous regularity, and all clearly irregular or persistently di-

estrous animals were discarded. The selected mice were divided into 2 lots, one fed stock Rockland diet, the other a 7% *Lithospermum* diet mixed with powdered stock Rockland diet, compounded into pellets. During the first week of feeding no vaginal smears were taken; thereafter for 2 months daily smears were made.

The estrus data for the second strain were obtained from females of a group of several hundred C<sub>3</sub>H mice which had been placed on a *Lithospermum* diet shortly after weaning. Random samples from the control and experimental C<sub>3</sub>H reservoirs were selected for 2-week assays when about 4 months of age. In a second instance, the C<sub>3</sub>H females assayed were from a group which had been on a *Lithospermum* diet for about 8 months; and a third sampling was made of the C<sub>3</sub>H stand-by group and their normal diet controls when the animals were about 14 months of age (*i.e.*, having been on the *Lithospermum* diet for about 12 months). All of the C<sub>3</sub>H females, regardless of age, responded almost uniformly in showing a continuous anestrus, whereas only a partial inhibitory response characterized the Rockland females, confirming observations made earlier.<sup>2</sup> It is evident that the C<sub>3</sub>H strain is considerably more susceptible to the anti-estrous factor in *Lithospermum* than is the Rockland strain. Also, it appears that the cyclic refractoriness described for the latter strain in our earlier paper<sup>2</sup> is largely absent in the C<sub>3</sub>H strain.

Two groups of C<sub>3</sub>H females, one having been on *Lithospermum* diet for 8 months, and the other on normal diet for a similar period of time, were assayed for estrus. The *Lithospermum* group had been sampled throughout the 8 months period, and the animals were found to have been in a state of continuous diestrus. The diet of these two groups was reversed: those which had been on *Lithospermum* were placed on normal diet, and conversely. The group which for the preceding 8 months had been in a state of continuous diestrus, immediately returned to relatively normal cyclicity; and the normal animals previously on the stock diet, responded within 96 hours to the *Litho*-

<sup>‡</sup> *Iso*-androliclactone acetate was selected for test because it is thought to cause an accumulation of gonadotrophins in the anterior pituitary. The compound was supplied through the generosity of Dr. Gregory Pineus of the Worcester Foundation for Experimental Biology.

<sup>§</sup> Supplied by Dr. R. O. Roblin, Jr., of the American Cyanamide Company, Stamford, Conn.

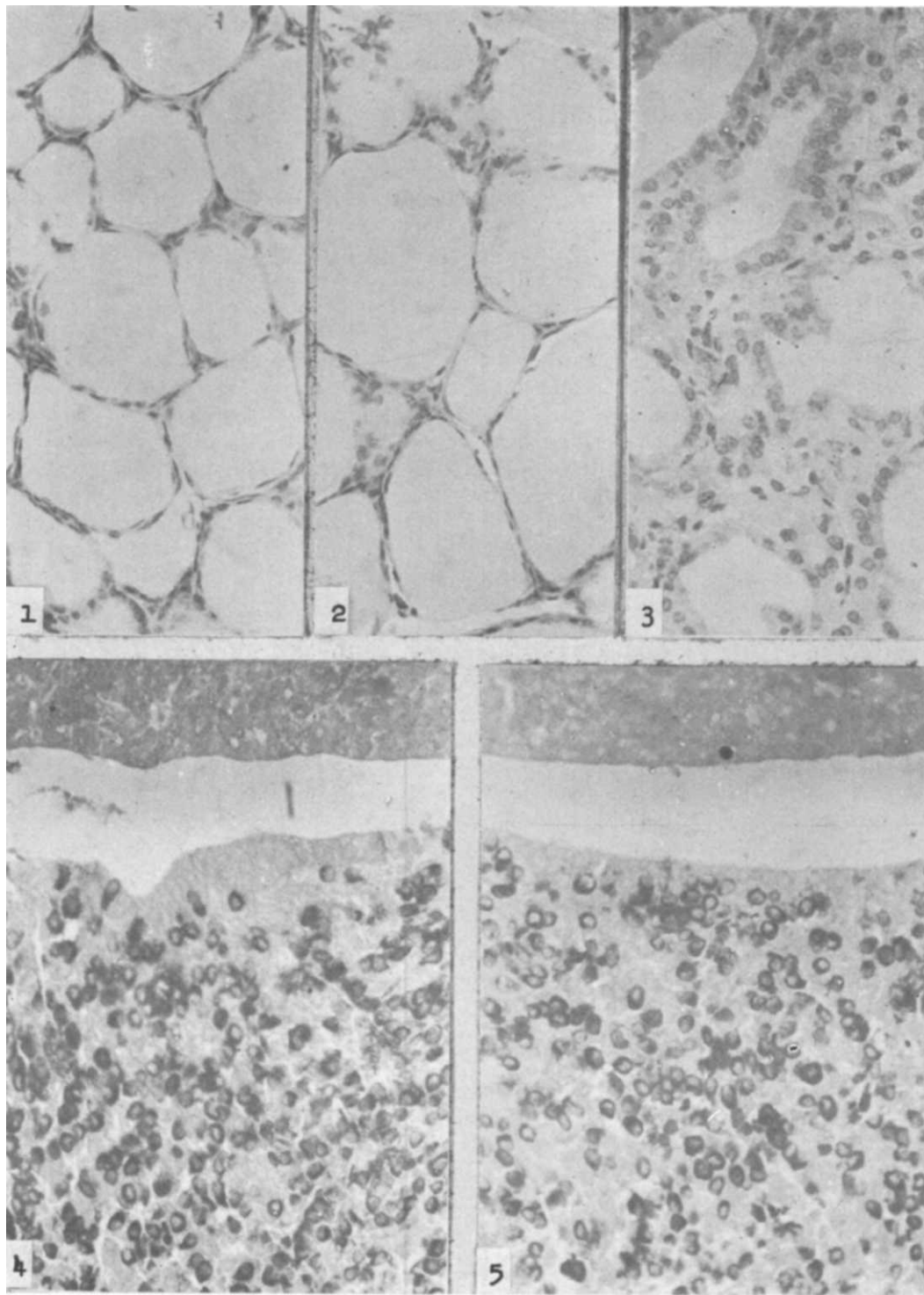


PLATE 1.

All photos taken under high-dry objective with  $\times 12$  ocular.

Figs. 1 and 4. Thyroid and anterior pituitary from 12-months-old  $C_3H$  female mouse which had been in continuous diestrus for 10 months, due to *Lithospermum* feeding.

Figs. 2 and 5. Thyroid and anterior pituitary from 12-months-old  $C_3H$  female mouse on normal diet.

Fig. 3. Thyroid from adult  $C_3H$  female mouse which had been on 0.1% thiouracil diet for 30 days.

*spermum*. The immediacy of the return to normal of the former group after being taken off the *Lithospermum* diet indicates that long exposure to the *Lithospermum* factor induces no permanent or irreversible changes within the body.

The weight history of female mice fed on *Lithospermum* diet from weaning, presents some points of interest. Such weight data were obtained each month beginning 5 months after the initiation of the experimental feeding. There was no significant weight difference between experimental and control groups. Cranston,<sup>1</sup> using larger doses—40% *Lithospermum* in the diet—reported that mice undergo an initial weight retardation from which they recover, however, in four months while still under treatment. Our weight data did not cover the age period between 2 and 7 months.

3. *Histology*. Studies were made of various tissues of C<sub>3</sub>H mice which had been on 7% *Lithospermum* diet for from 10 to 12 months. No changes were observed in the thyroid epithelium and follicles in animals which for almost a year had been in sustained diestrus due to *Lithospermum* feeding (Fig. 1 and 2). The anterior pituitary, fixed and stained according to a method used earlier,<sup>3</sup> appeared to be normal in both control and experimental animals, no marked qualitative or quantitative changes being observed in any of the cell types (Fig. 4 and 5). The uteri appeared somewhat atrophic and were clearly in the anestrus condition. The ovaries were somewhat smaller than normal and showed some follicular atresia. This atresia was similar to but not as marked as that reported for *Lithospermum*-fed Rockland Swiss mice, whose ovaries underwent pronounced atresia following prolonged *Lithospermum* administration.<sup>2</sup> The mammary tissues of the experimental C<sub>3</sub>H animals were rudimentary. It should be noted that control animals used for histological comparison with the experimental ones were sacrificed only when in true diestrus.

4. *The Thyroid*. The possibility that

*Lithospermum* acts by suppression of the thyroid rather than by affecting the gonadotrophin-producing tissues of the pituitary, seemed to justify further investigation, despite the failure of the pellet-administered thiouracil to show any anti-estrous effect. Accordingly, two sets of mice were fed on diets consisting respectively of 0.1% and 0.5% thiouracil. These diets were fed to C<sub>3</sub>H females with a history of normal estrous cyclicity. At these doses there was no disturbance of estrus. An observed body weight loss implies that enough thiouracil was absorbed to exert a systemic effect. Histologically the thyroids of mice so treated with thiouracil showed very marked hyperplasia (Fig. 3). These observations are consistent with the findings of Jones, Delfs, and Foote, who observed no change in the mating behavior of thiouracil-fed rats,<sup>4</sup> although gestation was often interfered with; but are at variance with those of Mann<sup>5</sup> who noted lengthening of inter-estrous periods in thiouracil-fed rats.

5. *Distribution of Factor within the Plant*. In all the preceding *Lithospermum* experiments, as well as in those of Cranston, the whole plant was used in the experimental diets. It was of interest to investigate the relative abundance of the anti-estrous factor in the various parts of the plant. Accordingly, dried *Lithospermum* plants were divided into (1) flowers and seeds, (2) roots, (3) leaves, (4) stems. These fractions were pulverized and made up to 15% with normal powdered Rockland mouse diet. Four groups of about 10 female Rockland mice each, on the stock diet, were assayed for estrous regularity for 10 days, then placed on the special diets. Daily estrous assay was continued during a 20-day experimental-diet period. The data are presented in Fig. 6, together with mean body weight curves. It is seen that the factor is practically absent from the stems, and maximally concentrated in the flowers and seeds. Return to normal diet resulted in almost immediate return to normal estrous cyclicity.

<sup>3</sup> Zahl, Paul A., *Z. f. Mikro-Anat. Forsch.*, 1937, **42**, 303.

<sup>4</sup> Jones, G. E. S., Delfs, E., and Foote, E. C., *Endocrinology*, 1946, **38**, 337.

<sup>5</sup> Mann, C. W., *J. Psychol.*, 1945, **20**, 91.

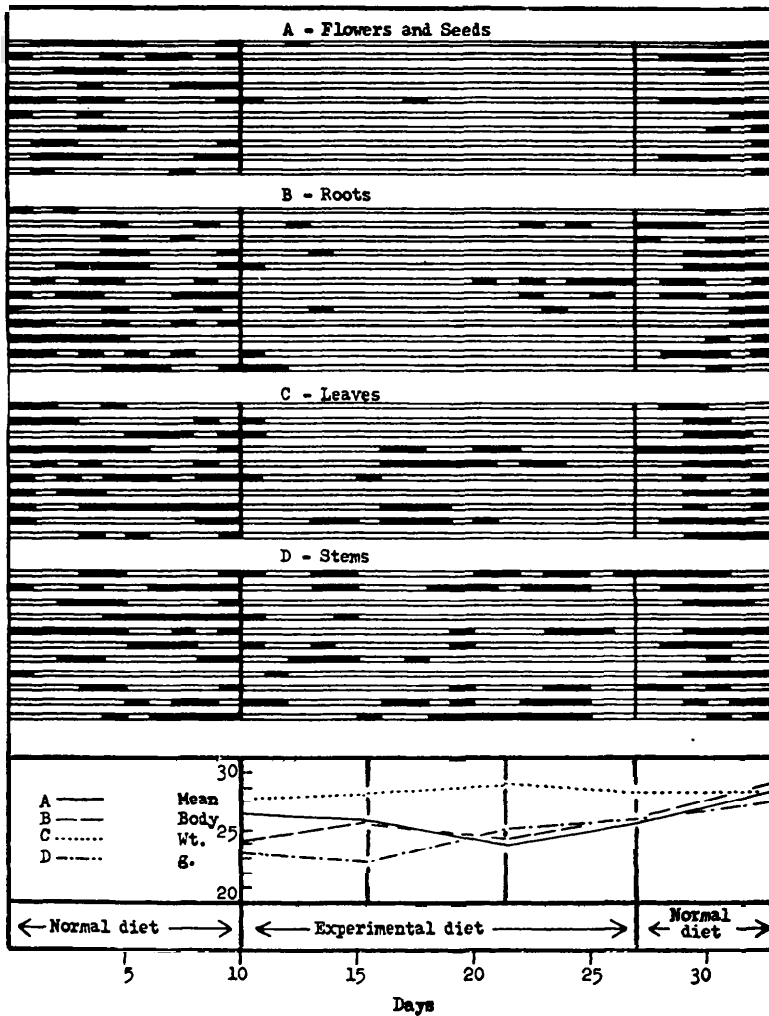


FIG. 6.

Estrous history of mice fed, respectively, on A—Flowers and Seeds; B—Roots; C—Leaves; D—Stems, of the plant *Lithospermum ruderale*. Pulverized, these materials were made up to 15% with normal powdered mouse diet, then compounded into pellets. The experimental feeding was preceded and followed by periods of normal diet feeding. The black horizontal areas indicate duration of estrus. The mean body weight curve for each group of mice is plotted below.

**Discussion.** It is clearly established that the *Lithospermum* factor, presumably in the water-soluble fraction, produces a pronounced anestrus effect in mice. Our observations that neither the thyroid nor the pituitary exhibit histological changes following protracted *Lithospermum* feeding, do not necessarily militate against the suggestion by Cranston that the effect may be directly on the gonadotrophic elements of the

anterior pituitary. It is certainly possible that an inhibitory effect may occur which is not of sufficient magnitude to elicit observable morphological changes. The fact that mice after 8 months of *Lithospermum*-induced diestrus immediately reestablish normal cyclicity when returned to normal diet, suggests that no permanent changes have occurred in any of the tissues involved. That the effect of *Lithospermum* results from a direct action

on the ovaries has been doubted by Cranston on the grounds that estrone injection immediately reestablishes cyclicity in *Lithospermum*-fed animals. It is possible that the *Lithospermum* effect is a threshold one of just sufficient magnitude to disrupt cyclicity, but insufficient to produce irreversible changes in the tissues involved in estrous cycles. Perhaps the use of a more potent concentrate of the active material would clarify this matter. There is the possibility, too, that the factor interacts with circulating gonadotrophins, although we have neither positive nor negative evidence of such action.

**Summary.** (1) The anti-estrous factor of *Lithospermum* appears to reside in the neu-

tral water fraction. (2) The anti-estrous factor is most abundant in the flowers and seeds of the dried plant, and almost wholly absent from the stems. (3) Interference with estrus is stopped almost immediately, regardless of the length of treatment, when *Lithospermum* is withdrawn and animals returned to normal diet. (4) The C<sub>3</sub>H strain is more sensitive to *Lithospermum* than is the Rockland Swiss strain. (5) The action of *Lithospermum* is unlike that of thiouracil. (6) The factor induces no observable changes in the anterior pituitary, thyroid, suprarenals, or pancreas; some atresia is observed in the ovaries, and some atrophy of the uteri.

## 16323

***In vivo* Observations on the Distensibility of the Femoral Venous System.\***

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The marked engorgement of a vein when its central end is occluded tends to create the impression that veins are highly distensible structures. This illusion is probably created by the filling of partially collapsed veins<sup>1</sup> rather than by a true distension of the venous walls, since studies of segments of large veins<sup>2,3</sup> have revealed a relative lack of distensibility over the physiological range of venous pressures. The question arises as to whether observations on the large veins suited to direct distensibility measurements are necessarily typical of the venous system as a whole. Hochrein and Singer, for example, found the femoral vein considerably more distensible than the inferior vena cava.<sup>2</sup> Should the smaller veins and venules be

highly distensible, alterations in venous pressure would be of considerable significance in altering the partition of the circulating blood volume between the venous system and other channels of the circulatory system.

Clark<sup>4</sup> attempted an estimate of the distensibility of the entire venous system of the forearm *in vivo* by employing a plethysmographic method. Her results were difficult to interpret, however, because of the inability to differentiate changes in venous volume from concomitant changes in tissue fluid volume. A more direct approach to this problem in the experimental animal is suggested by the simple observation that if the venous outflow from an organ is watched at the time the outflow pressure is suddenly lowered, the fall in pressure will be observed to produce a transient gush of excess blood. This excess blood obviously represents a volume passively flushed out of the system by

\* Supported by a grant from the Life Insurance Research Fund.

<sup>1</sup> Ryder, H. W., Molle, W. E., and Ferris, E. B., Jr., *J. Clin. Invest.*, 1944, **23**, 333; Duomareo, J., Rimini, R., and Predari, F. N., *Rev. Argentina Cardiol.*, 1946, **12**, 333.

<sup>2</sup> Hochrein, M., and Singer, B., *Arch. Exp. Path. Pharmacol.*, 1927, **125**, 301.

<sup>3</sup> Green, H. D., *Medical Physics*, Otto Glasser, Editor, Year Book Publishers, Chicago, 1944, 213.

<sup>4</sup> Clark, J. H., *Am. J. Physiol.*, 1933, **105**, 418.