During both experiments the guinea pigs exhibited no adverse symptoms. They all ate readily, gained weight, and were normally active while in the tank and when they were put back into the storage cage with their controls during the interval period. No differences between the normal and the experimental animals could be observed.

Comment. Using the intermittent exposure method, it is apparent that high oxygen tension does not cause a depression of erythropoiesis in guinea pigs in contradistinction to the marked stimulation of erythropoiesis produced by low oxygen tension. With the continuous exposure method Campbell[3] observed significant reductions in the erythrocyte count and hemoglobin values in various species of animals. Because of the importance of this observation we exposed 6 guinea pigs continuously to 50-60% oxygen. Three animals died of pneumonia during the experiment. After 30 days, the remaining 3 animals had an average decrease of 21% in the erythrocytes and 13% in the amount of hemoglobin. Whereas these changes are not as great as those reported by Campbell, they further suggest that long continued exposure to oxygen may depress erythropoiesis in a normal animal.

The interesting experiment of Reinhard[2] *et al.* shows conclusively that erythropoiesis is depressed in patients with sickle cell anemia exposed to high oxygen tension. As such an effect was not obtained in patients with polycythemia vera (Barach and McAlpin)[1] it might be inferred that the hemopoietic equilibrium in sickle cell anemia is especially sensitive to increased oxygen pressures. The effect of prolonged high oxygen tension on the rate of erythropoiesis in animals and normal human beings warrants further investigation.

Summary. Guinea pigs were intermittently exposed to 80-100% oxygen at atmospheric pressure for 57 days, and to 60-70% oxygen at 2 atmospheres pressure for 20 days. The periods of exposure covered 6 hours a day 6 days a week. There was no significant diminution in the erythrocyte counts and hemoglobin values in any of these animals.

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The Effect of *Lithospermum* on the Mouse Estrous Cycle.*

MARION L. DRASHER AND PAUL A. ZAHL.

From the Haskins Laboratories, New York City.

Introduction. Based on a report[1] that certain American Indians have employed the ingestion of the herb *Lithospermum ruderale* as a conception preventative, Cranston[2] performed a series of experiments on mice and found that a fluid extract of *Lithospermum* when mixed with normal diet would rapidly induce a suppression of the estrous cycles as well as a lowered birth incidence in breeding females. From inferential evidence Cranston concluded that the active factor in the herb operates directly on the pituitary gland, suppressing the formation or release of gonadotropic hormone.

Since this type of "anti-hormonal" action

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1 Train, P., Hendricks, J. R., and Archer, W. A., *Medicinal Uses of Plants by Indian Tribes of Nevada*, Part II, p. 102. Issued by the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D.C., 1941.

2 Cranston, E. M., *J. Pharm. and Exp. Therap.*, 1945, **83**, 130.

is pharmacologically unique, work was undertaken (1) to confirm the effect of the drug on the estrous cycles of at least 2 unrelated strains of mice, and (2) to determine the effect of the drug on the incidence and development of mammary tumors, the appearance of which, in high tumor strains, is known to implicate the pituitary-ovarian endocrine system.

The latter study is still in progress. The former constitutes the subject of this note.

Experimental. Data were collected on 2 strains of mice: C_3H high mammary tumor females bred in this laboratory from stock obtained originally from the National Cancer Institute, and a heterozygous line of low-mammary-tumor albino mice.

Powdered *Lithospermum* from the whole plant, collected and dried in Montana, was fed in pellet form at a 15% level, the remaining 85% consisting of powdered Rockland Mouse Diet. The 15% level was chosen by extrapolation from a curve based on toxicity tests with groups of Rockland mice receiving 5%, 10%, 20%, 30% and 40% *Lithospermum* for a period of one month during which time a careful weight record was kept. The 15% level was the maximum dosage not affecting the weight of the fed animals.

Vaginal smears were made by the lavage method, with the use of distilled water and a fine pipette. The smears were dried under heat and stained with aqueous methylene blue. Differential counts were made under high dry magnification and approximately 5 fields were counted for each determination.

Twenty-four C_3H females 6-8 months of age were placed on the experimental diet and vaginal smears were taken daily for one month, after which smears were made weekly. Smears of 5 normal-diet females served as control. The animals were maintained on the diet for 3-5 months, during which time estrus occurred only rarely and in only a few of the mice. Generally speaking, the response in the C_3H strain to *Lithospermum* was immediate and persistent, in contrast to a type of refractoriness to the drug developed by some of the Rockland mice, described below.

Twenty Rockland females 2 months old were put on the 15% *Lithospermum* diet, with 10 females on normal diet serving as controls. Vaginal smears on the Rockland mice were taken daily for a period of 3 months. These smears were read differentially for percentage of cornified cells, nucleated epithelial cells and leucocytes. Four typical graphs of cornified cell levels are shown in Fig. 1. In contrast to the uniform response of the C_3H strain, the Rockland mice showed a great degree of individual variation, ranging from complete absence of response to full and continuous response. About 20% of the animals are in the former category, and 15% in the latter. The majority of the animals, however, (65%) went into an initial anestrus but came into estrus at about 30-day intervals with prolonged periods of anestrus between. Some of the mice at the end of several months developed a complete refractoriness to the drug, apparently recovering their normal cycles, though still on the *Lithospermum* diet, indicating either the development of an antagonist to the drug or an elevation of the organism's response threshold. Some evidence for the development of such a refractory state can be seen in 2 of Cranston's animals.[2]

Weights were taken on the Rockland animals at the conclusion of the experimental feeding. The control animals averaged 36.6 g and the *Lithospermum*-fed animals averaged 37.1 and 36.1 g, with no evidence of abnormal scatter. Cranston, using younger mice in experiments which involved administration of *Lithospermum* at rather higher dosage levels than employed by us, observed initial weight losses from which, however, the mice recovered while still on the diet.

The ovaries and uteri of females in *Lithospermum* anestrus showed marked atrophy, being about one-fourth normal size, with ovaries, especially in the C_3H strain, containing largely atretic follicles. Animals, however, which after long periods of anestrus became refractory to the drug and again developed estrus, had smaller than normal ovaries which, nevertheless, contained what appeared to be functional follicles.

Discussion. That a factor in *Lithospermum*

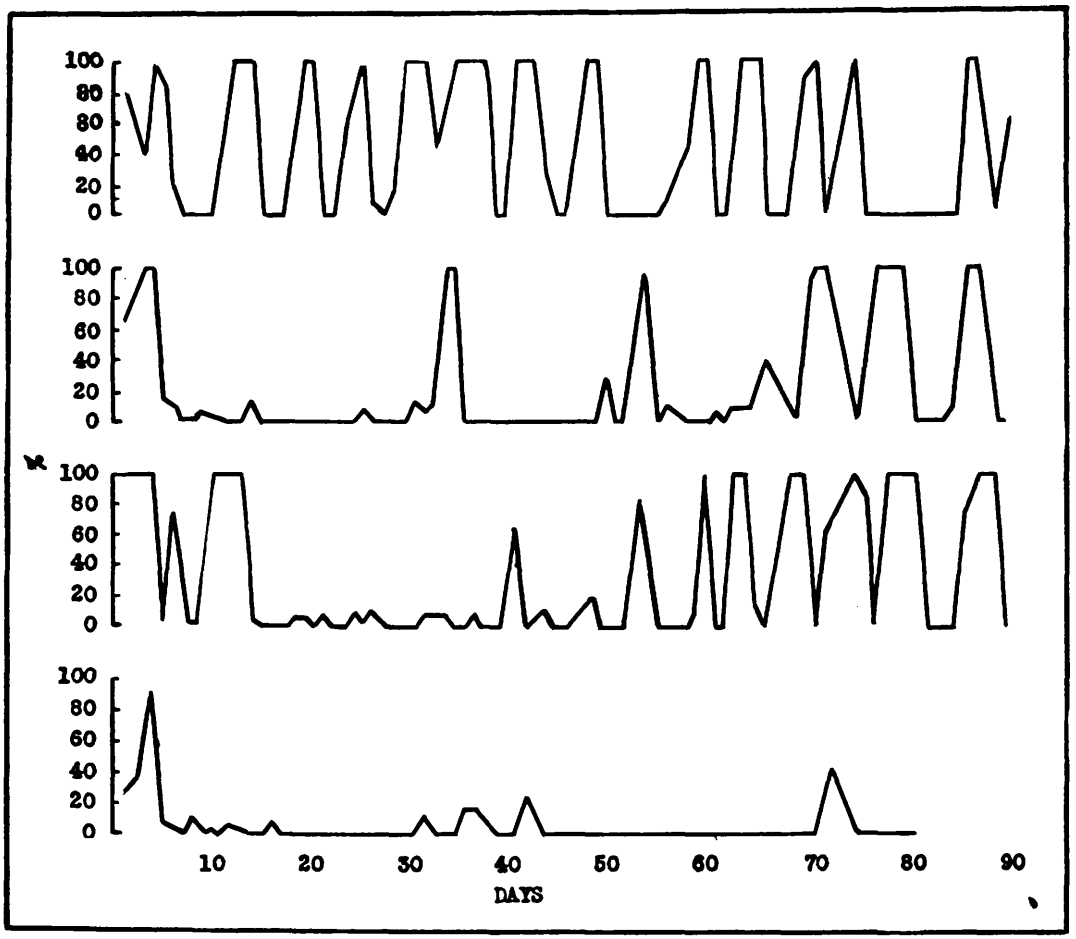

FIG. 1.
Graphs showing the percentage of vaginal cornified cells in female Rockland mice, over a period of about 90 days. Smears were made every 24 hours by the lavage method. The 4 sets of curves are: (top) of typical control mouse on standard diet; (two center) 2 examples of initial anestrus, followed by refractoriness in mice on a 15% *Lithospermum* diet; (bottom) example of persistent anestrus in mice on a 15% *Lithospermum* diet.

induces a suppression of estrous cycles in mice is confirmed. It is of interest to inquire into the nature of the mechanism of this action.

It is well known that a similar suppression of estrous cycles may result from a number of conditions, such as, inanition, deficiency of almost any of the vitamins, the action of injected "anti-hormone" substances, injected estrogens, androgens, progesterone, etc. It is doubtful, however, that *Lithospermum* operates through any of these media. General inanition may more or less be ruled out because of the fact that the *Lithospermum* effect can be achieved at dosages which do not affect body weight or general metabolic activity.[2] That a vitamin deficiency is involved is also doubtful. Vitamin A deficiency prevents the appearance of normal estrus and the condition is characterized by a persistent cornification of the vagina,[3] a symptom not present in animals in *Lithospermum* anestrus. Evans and Simpson[4] have produced permanent anestrus in rats by feed-

[3] Evans, H. M., and Bishop, K. S., *J. Metab. Research*, 1922, **1**, 335.

[4] Evans, H. M., and Simpson, M. E., *Anat. Rec.*, 1930, **45**, 215.

ing a Vitamin B deficient diet. This anestrous condition yielded to implants of normal pituitary tissue, indicating that such a deficiency results in decreased gonadotropic potency of the pituitary. The exact mechanism by which vitamin E deficiency produces sterility is still subject to controversy; however, there is no doubt but that its effects are irreversible. The fact that a refractoriness to *Lithospermum* may develop in mice would seem to negate any hypothesis proposing that *Lithospermum* activity is dependent upon the induction of a deficiency of vitamins B and/or E.

The effect of estrogens and androgens in interfering with gonadotropic action cannot be considered to be similar to the *Lithospermum* effect because the latter is entirely free of estrogenic or androgenic potency.

That the mechanism of *Lithospermum* activity involves the thyroid has been doubted by Cranston[2] who found no significant change in the weights of the thyroid glands or in basal metabolic rate after *Lithospermum* treatment. This view has been confirmed by us through histological examination of the thyroids of normal Rockland mice and those of mice on a ration of 30% *Lithospermum* for a period of 5 months. In both cases the alveolar epithelium was low to cuboidal with a normal amount of colloid storage, the experimental animals showing a slightly greater amount of interstitial tissue. The question may be raised as to whether such a histological picture might be expected if *Lithospermum* acts in the manner of some of the goiterogens of plant origin, specifically the seeds of the *Brassica* genus. Griesbach[5] has shown that a brassica seed diet induces hyperplasia of the thyroid follicles accompanied by typical goiterogenic changes in pituitary histology, *e.g.*, increase in the number of basophils with hyalinization and vacuolization, and a decrease in the number of acidophils. After 56 days, however, this effect becomes less pronounced at which time colloid is again stored in the thyroid follicles. This might indicate the appearance of a refractoriness to the brassica seed diet similar to the refractoriness developed to *Lithospermum*. Since the histological material examined by us was taken at a time when refractoriness had undoubtedly developed, it is impossible to state with certainty that the thyroid is not involved. However, the bulk of the evidence would seem to point against its primary involvement.

Cranston[2] by indirect experimental procedures has quite convincingly ruled out specific action of *Lithospermum* on the other endocrine glands except the pituitary, concluding that the action is directly on this gland, which in her experiments underwent marked weight loss during *Lithospermum* feeding and the resulting anestrus.

The fact that some mice, especially of the Rockland strain, develop a refractoriness to the herb action suggests either that the active factor is antigenic and that the organism builds up a protective antibody mechanism, or that the pituitary (if it, in fact, be the tissue affected) builds up a rising threshold of reactivity. That the refractoriness, however, may come and go in an almost cyclic manner (Fig. 1) suggests the existence of an obscure mechanism which with the data at present available cannot be illuminated.

The assumption that the *Lithospermum* action is via the pituitary gland is the basis for the further study now in progress of the effect of the drug on mammary tumor development. It is established that in high mammary tumor strains ovarian hormone activity is essential to the development of tumors. The milk factor apparently cannot operate without an adequate hormonal substrate. In suppressing the ovarian endocrine tissues by negating the pituitary gonadotropins, one might expect to break a link in the chain leading to mammary tumor development. Experiments are now in progress to determine whether such a rationalization is valid.

Summary. Female mice of C_3H strain when fed on a diet of 15% *Lithospermum ruderale* go into an immediate and persistent anestrus. Female mice of Rockland strain fed similarly also go into anestrus which,

[5] Griesbach, W. E., *Brit. J. Exp. Path.*, 1943, **22**, 245.

however, may be followed by varying degrees of developed refractoriness to the drug, as evidenced in some mice by a reappearance of normal estrous cycles. The anestrous condition in both strains is accompanied by atrophy of the ovaries and uteri, and atresia of the follicles. Possible modes of action of the *Lithospermum* are discussed.

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Anesthesia. XXI. Anesthesia and the Steroid Hormones.*

DECAMP B. FARSON, C. JELLEFF CARR, AND JOHN C. KRANTZ, JR.

From the Department of Pharmacology, School of Medicine, University of Maryland, Baltimore, Md.

Selye[1-4] reported that the steroid hormones injected into certain species of laboratory animals produced anesthesia. In the generalization enunciated by Selye and referred to as "the fundamental law of steroid hormone anesthesia," this investigator states that "All compounds having a steroid-hormone action are capable of producing anesthesia while no compound devoid of hormone action possesses this power."[1] Indeed, Cashin and Moravek[5] had previously reported that they had anesthetized cats by injecting cholesterol suspensions intravenously. These experiments, in our opinion, appear to be inimical to the validity of Selye's generalization. In our studies on the effect of cholesterol on ether and pentothal sodium anesthesia, it appeared desirable to study further Selye's generalizations.

Experimental. Twenty-five rabbits received 2.5 cc/kg of 2% cholesterol suspension in a 25% solution of polyethylene glycol (Carbowax 1500) intravenously. Most of these animals appeared depressed. Six of them became unconscious and died promptly of acute pulmonary edema.

Fifteen dogs treated in a similar manner all manifested depression. Ten of these animals lost consciousness and died within 5 minutes.

Our attention was directed next to the hormonal steroids. Using the dosage schedules established by Selye we used the following compounds: progesterone, methyl testosterone, stigmasterol, estrone, benzestrol and diethylstilbestrol. Owing to the fact that in the "cat assay" of digitoxin, this glycoside in toxic quantities, produces a depression of the animal so that the anesthetic (ether) may be discontinued, we also used digitoxin in the series. In doses of 50 mg per rat weighing 92-205 g (average 158 g) not any of the foregoing compounds except progesterone and digitoxin produced "anesthesia."

Twenty-six animals, 8 male and 15 female, were used. None of the animals receiving digitoxin recovered from the comatose state produced by the drug. Three of the 9 animals receiving progesterone died.

Neither in the animals which died of progesterone nor with those that recovered was there ever a period in which the animal did not respond promptly to pain stimuli (pinching tail with hemostat). This syndrome cannot correctly be referred to as anesthesia when the sensory pathways are not blocked. In the rat, 6 mg/kg of

* The expense of this investigation was defrayed in part by a grant from the Ohio Chemical and Manufacturing Company of Cleveland, Ohio. The hormones used in these studies were furnished by Parke, Davis & Co.; Merck & Co., Inc.; Abbott Laboratories; Ciba Pharmaceutical Products; the Charles E. Frosst Co.; and Schieffelin & Co.

1 Selye, H., *J. Pharm. and Exp. Therap.*, 1941, **73**, 127.

2 Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 116.

3 Selye, H., *Endocrinology*, 1942, **30**, 437.

4 Selye, H., *Anes. and Anal.*, 1943, **22**, 105.

5 Cashin, M. F., and Moravek, V., *Am. J. Phy.*, 1927, **82**, 294.