Lithospermum ruderale and the Incidence of Mammary Tumors in Mice.* (18340)

ELIZABETH M. CRANSTON, GRENAVIERE R. KUCERA, AND JOHN J. BITTNER.

From the Department of Pharmacology and the Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School.

Mammary tumors in mice are thought to develop "spontaneously" as the result of 3 concomitant factors: (a) an inherited susceptibility, (b) the milk agent and (c) hormonal stimulation(1). All 3 components are necessary for the development of such tumors. Therefore, any procedure which would interfere with the action of one of these factors should interfere with the development of mammary tumors by breaking the chain of events. *Lithospermum ruderale* is a plant which induces diestrous by inhibiting the action and/or formation of gonadotropic hormones(3-8). Thus supposedly, the formation of estrogenic hormones is inhibited. For this reason, a study of the effect of lithosperm on the incidence of mammary tumor formation in susceptible mice was undertaken.

Exp. I. C_3H female mice, which as virgins have a high incidence of mammary tumors, namely 63.6% at 402 days(2), were used. Twenty-two virgin females, about 3 months of age, were caged individually. Estrous smears were taken daily, except Sundays, by the lavage method. After a control period of 30 days, during which time regular estrous cycles were recorded in all mice, they were fed various concentrations of ground lithosperm mixed with ground Purina fox chow to determine the concentration of lithosperm necessary to cause diestrous. Six of 11 mice given 15% lithosperm for 35 days went into diestrous, five having occasional estrous cycles. Increasing the concentration of drug to 20% for 40 days had little added effect on estrous cycles. These mice were then used for other purposes. Another 11 mice received 30% lithosperm diet for 85 days. No estrous cycles occurred. Reducing the concentration to 20% for 30 days caused 3 mice to develop one or 2 estrous periods. They were then returned to a 30% lithosperm diet for the rest of their lives. None of the 11 went into estrous again and none developed mammary tumors. These mice died 3 to 14 months (average 11 months) after lithosperm diet was started, or at an average age of about 15 months. The antiestrous effect of lithosperm was not due merely to added bulk in the diet for neither 30% powdered cellulose (alphacel) nor 30% lithosperm marc (after extraction to remove the active principle) induced diestrous in C_3H mice.

Exp. II. Sixty-seven C_3H virgin female mice, 6 to 8 weeks of age, were individually caged and divided into 2 groups: 31 control mice, receiving ground Purina fox chow *ad libitum*, and 36 treated mice, receiving drug diet *ad libitum* which consisted of ground Purina fox chow plus ground lithosperm. The concentration of lithosperm was 15% for 4 weeks, 20% for 8 weeks and then 30% for the duration of the experiment. Mice were weighed once a week, at which time they were examined carefully for the presence of

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TABLE I. Incidence of Mammary Tumors in C_3H Mice.

Group	No. mice	No. dev. tumors	Avg tumor age in days	No. dying without tumors	Avg non-tumor death age in days	No. survivals
Control	31	18	413	12	483	1
Treated	36	1	378	32	483	3

TABLE II. Body Weights of C_3H Mice.

Age in days	Avg body wt in g		% diff.
	Control	Treated	
49	14.5	14.9	+ 2.7
119	21.1	19.8	— 6.1
189	23.6	19.9	—15.7
259	24.3	19.4	—20.1
329	25.2	19.4	—23.0
399	24.6	18.3	—25.6
469	25.9	17.6	—32.0

mammary tumors. The experiment was terminated after 90 weeks. Surviving mice were then 686 days old.

Results are recorded in Tables I and II. In the control group, 18 of 31 mice (58.1%) developed mammary tumors at 287 to 616 days of age, average tumor age being 413 days. Twelve mice (38.7%) died without tumors at an average age of 483 days. One mouse was alive and tumor free (grossly) at the end of the experiment. In the treated group, only one of 36 mice (2.8%) developed a mammary tumor at 378 days of age, thirty-one (88.8%) died without tumors at an average age of 483 days and three (8.3%) were alive and tumor free at the end of the experiment.

Daily food intakes of 18 treated and 14 control mice were recorded for a period of 10 days when the mice were 390 days old. At this time control mice, weighing an average of 26.0 g, ate an average of 4.51 g food per mouse per day, or 17.34 g per 100 g body weight per day. Treated mice, weighing an average of 18.85 g, ate 4.25 g diet, of which 30% was lithosperm giving a fox chow intake of 2.98 g per mouse per day, or 15.8 g per 100 g body weight per day. Thus this group of treated mice weighed 28% less than controls and ate 34% less fox chow on the basis of intake per mouse per day, or 9% less on the basis of intake per 100 g body weight. These figures disregard whatever food value was obtained from lithosperm.

Exp. III. Twenty first generation hybrids (ZD_8F_1) of C_3H females mated with D_8 males were studied for the effect of lithosperm on estrous cycles. ZD_8F_1 virgin females have a high incidence of mammary tumors, namely 96.4% at 355 days(2). Twenty percent, 30% and 40% lithosperm drug diet did not alter the frequency of estrous in this strain of mice. Also subcutaneous injection of an extract of lithosperm which induced diestrous in C_3H mice had no effect in ZD_8F_1 mice in up to 4 times the dose effective in C_3H mice. Therefore ZD_8F_1 mice are less sensitive to the anti-estrous effect of lithosperm than C_3H mice.

Exp. IV. ZD_8F_1 virgin females, at 6 weeks of age, were divided into 2 groups: 30 were given control diet, ground Purina fox chow, *ad libitum*; 32 were fed *ad libitum* on ground Purina fox chow mixed with lithosperm, 15% for 4 weeks, 20% for 8 weeks and then 30% for the remainder of the experiment (448 to 497 days). Usually 5 mice were housed together in a cage. Body weights were recorded once a week, at which time the mice were examined carefully for the presence of mammary tumors. Results are recorded in Tables III and IV. Twenty-nine of 30 control mice (96.6%) developed tumors at an average age of 371 days (217 to 504). Seven of 32 treated mice (21.9%) developed tumors at an average age of 406 days (224 to 518). Again

TABLE III. Incidence of Mammary Tumors in ZD_8F_1 Mice.

Group	No. mice	No. dev. tumors	Avg tumor age in days	No. dying without tumors	Avg non-tumor death age in days	No. survivals
Control	30	29	371	0	—	1
Treated	32	7	406	3	413	22

TABLE IV. Body Weights of ZD_8F_1 Mice.

Age in days	Avg body wt in g		% diff.
	Control	Treated	
42	15.8	16.3	+ 3.1
112	22.2	21.1	— 4.9
182	22.9	19.0	—17.0
252	25.5	20.6	—19.1
322	26.0	19.4	—25.4
392	26.6	19.2	—27.8
462	23.8	19.3	—18.9

treated mice weighed less than control mice.

Discussion. The above experiments show that a ground lithosperm diet will markedly decrease the incidence of mammary tumor formation in two tumor-susceptible strains of mice. However, they do not prove that this decrease is due necessarily to drug administration.

Treated mice weighed approximately 18 to 28% less than control animals during the tumor developing period and consumed in the neighborhood of 34% less fox chow per mouse. The caloric value of lithosperm is not known but presumedly the plant has some caloric value and therefore decreased, to some extent at least, this difference in food consumption between treated and control mice.

Tannenbaum and Visscher *et al.*(9,10) have shown that underfeeding and caloric restriction inhibit the formation of mammary tumors in mice, as well as other types of tumors. For example, Visscher *et al.*(9) decreased the incidence of mammary tumors in C_3H virgin female mice, of the same subline as used in the present experiment, from 67% to 0 by restricting caloric intake by one-third. However their restricted mice weighed only 12 to 14 g as compared with 30 to 32 g for the *ad libitum* mice, a decrease of approximately 58%. Tannenbaum(10c) found that decreasing the daily caloric intake from 11.7 to 8.5 (a 27.3% decrease) in C_3H virgin females decreased body weight from 28 to 20 g (a 28.5% decrease) and lowered the incidence of mammary tumors from 57% to 0. The results with lithosperm on body weight and incidence of tumor formation in C_3H mice are in fair agreement with those of Tannenbaum with caloric restriction. The actual caloric intake of mice in the present study is not known.

Huseby *et al.*(11) think that caloric restriction reduces the incidence of mammary tumors in mice by producing a pituitary insufficiency, with resultant decreased ovarian secretion and a relative refractoriness of the mammary gland to estrogenic stimulation. Lithosperm has been shown to decrease the formation and/or action of gonadotropic hormones(5,6,8). In the present experiments it is impossible to determine the part played by lithosperm and that due to possible caloric restriction in decreasing the incidence of tumor formation. When the active principle of lithosperm is isolated, further studies along this line should be of value.

Summary. Ground *Lithospermum ruderale*, administered by drug-diet methods, lowered the incidence of mammary tumors from 58.1% to 2.8% in C_3H mice and from 96.6% to 21.9% in ZD_8F_1 mice. The exact roles played by the drug and by possible diet restriction were not determined.

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