Subsequent studies presently in progress shall attempt to verify the above hypothesis, i.e. that reduction in the blastogenic response of PBL to PHA in the presence of DEP-S is due to an intrinsic nuclear alteration of DNA synthesis and to evaluated and correlated the level of estrogen in the systemic circulation with lymphocytic reactivity to PHA: a) in patients with prostatic cancer prior to and following estrogen therapy and b) in females prior to conception and during each trimester period in the presence of autologous or isologous serum.

Zusammenfassung. Nachweis, dass die Fähigkeit zur Blastogenese der Lymphozyten gesunder junger Männer nach Reizung mit Phytohämagglutinin im peripheren Blut unterdrückt wird, wenn die Lymphozyten zusammen mit Östrogen (Diethylstilbestrol-Diphosphat) kultiviert werden.

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Influence of Chronic Treatment with 2-Bromo-α-ergocryptine (CB-154) on the Reproductive and Lactational Performance of the C3H/HeJ Female Mouse[1]

2-Bromo-α-ergocryptine (CB-154) is an effective suppressor of pituitary prolactin secretion in mice[2,3], rats[4,5], certain domestic animals[6] and man[7–9]. A number of laboratories have provided convincing evidence that prolactin is critically involved in the development and growth of murine mammary tumors[10–13]. Thus, chronic CB-154-induced suppression of prolactin secretion has been shown to virtually prevent the appearance of spontaneous mammary carcinoma in mice[3] and promote regression of carcinogen-induced rat mammary tumors[14–16].

Because of the: 1. striking anti-mammary tumorigenic effects of the drug in rodents[3,14–16]; 2. possible significant role for prolactin in human breast tumorigenesis[17–19] and 3. current and contemplated use of the drug for prolactin suppression in women[8], it is imperative to determine the effects of the drug on other endocrine related processes. Thus, the purpose of this investigation is to determine the effects of chronic treatment with CB-154 on the reproductive and lactational activities of the C3H/HeJ female mouse.

Materials and methods. All animals used in this study were C3H/HeJ mice obtained from the Jackson Laboratories, Bar Harbor, ME. They were housed in either groups of 3 (3 females) or groups of 4 (3 females plus 1 male) in a temperature (24° ± 0.5 °C) and light (14 h/day) controlled environment and provided a diet of Wayne Lab Blox (Allied Mills, Inc., Chicago, IL) and water ad libitum.

Treatment of mice with CB-154 prior to mating. 24 nulliparous 2-month-old female mice were given s.c. injections of 0.1 mg CB-154 suspended in 0.9% NaCl solution daily, for 50 days. The CB-154[4] suspension (1mg/ml) was prepared by dissolving the drug initially in a minimal amount of ethanol and diluting to volume with 0.9% NaCl solution.

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Table I. Effect of daily treatment for 50 days of C3H/HeJ female mice with CB-154 prior to mating on reproductive performance

Treatment	No. of mice	No. and % of mice which became pregnant	Mean[a] latency period of parturition (day)	Mean[a] No. of pups per litter at weanling	Pups surviving to weanling (%)	Mean[a] weight of pups at weanling (g)
Controls	24	24 (100%)	35.0 ± 2.4	4.9 ± 0.7	53	9.5 ± 0.6
CB-154[b]	21	21 (100%)	28.1 ± 3.4	5.2 ± 0.3	62	8.4 ± 0.1

[a] Mean ± standard error. [b] CB-154, 0.1 mg/mouse/day.

Table II. Effect of daily treatment for 50 days of C3H/HeJ female mice with CB-154 during mating on reproductive performance

Treatment	No. of mice	No. and % of mice which became pregnant	Mean [a] latency period of parturition (day)	Mean [a] No. of pups per litter at weanling	Pups surviving to weanling (%)	Mean [a] weight of pups at weanling (g)
Controls	24	23 (96%) [c]	33.5 ± 2.6 [c]	5.2 ± 0.8	60	7.8 ± 0.7 [d]
CB-154 [b]	24	11 (46%) [c]	53.3 ± 2.7 [c]	7.0 ± 0.6	53	9.8 ± 0.5 [d]

[a] Mean ± standard error. [b] CB-154, 0.1 mg/mouse/day. [c] $P < 0.001$. [d] $P < 0.01$.

A second group of 21 2-month-old female mice was given daily s.c. injections of the diluent only and served as controls.

Sixty days after the initiation of treatment (10 days after the last injection), all mice of both groups were mated with males for a period of 50 days. Care was taken to rotate the males through the cages so that each male was exposed once to each female, controls and experimentals for a period of 3 days. Percent pregnancies, mean latency period for parturition, (interval between the initial male contact and birth, mean litter size at weaning, percent pup survival at weaning and mean pup weight at weaning were determined for each group.

Percent pregnancies and percent pup survival were statistically analyzed by χ^2 analysis. Mean latency period of parturition, mean number of pups per litter and mean pup weight were analyzed by Student's t-test.

Treatment of mice with CB-154 during mating. 24 2-month-old female mice were treated daily for 53 days with CB-154 as described previously. An additional 24 2-month-old female mice were treated daily with the diluent only and served as controls. 3 days after the first injection, all mice were mated with males of the same age. The males remained with the females for 50 days and were rotated though the cages as previously described. Percent pregnancies, mean latency period for parturition, mean litter size at weaning, percent pup survival at weaning and mean pup weight at weaning were determined for each group.

Treatment of mice with CB-154 during lactation. 46 pregnant mice were randomly divided into 2 groups. Beginning on the day of parturition, the dams of 1 group were treated daily for 21 days with CB-154 as described previously. The dams of the other group were treated daily for 21 days with the diluent only. Number and percent of mice which raised litters to weaning, mean litter size at weaning, percent pup survival at weaning and mean pup weight at weaning were determined for each group.

Results. Treatment of female mice with CB-154 for 50 days prior to mating had no significant effect on subsequent reproductive performance (Table I). Number of mice which became pregnant, mean latency period of parturition, mean number, weight and percent of pups at weaning in the CB-154 treated group did not differ significantly from controls. 100% of the ergot-treated mice when mated after cessation of drug treatment, became pregnant and delivered normal offspring.

Treatment of female mice with CB-154 for 50 days during mating significantly impaired reproductive performance (Table II). 96% of the controls (23/24) became pregnant in contrast to only 46% (11/24) in the CB-154 treated group ($p < 0.001$). Furthermore, mean latency period of parturition was markedly lengthened in the ergot-treated group ($p < 0.001$). In the ergot-treated mice which eventually became pregnant, no effect of the treatment on litter size was observed. Mean weight of pups of the ergot-treated mice was slightly greater than in the controls.

Treatment of female mice with CB-154 for 21 days during lactation significantly impaired pup development (Table III). Pup survival ($p < 0.001$) and weight ($p < 0.01$) were significantly reduced in the ergot-treated groups. Surviving pups from dams chronically treated with the drug during lactation were, compared to controls, very weak.

Discussion. The results of this study provide evidence that daily treatment of female mice with CB-154, during mating and lactation, significantly impedes these activities, but upon drug withdrawal, normal fecundity is immediately reestablished. There is no evidence that chronic pretreatment with the drug results in irreversible inhibition of these reproductive processes.

CB-154 is an efficacious inhibitor of the secretion of prolactin in rodents[2–5], domestic animals[6] and in man[7–9]. The action of the ergot appears to occur primarily at the pituitary level[7,20], but may also be exerted at the level of the hypothalamus[21]. The role of prolactin in reproduction and lactation has been studied in a number of laboratories[22–24]. Normal secretory rates of the hormone appear to

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Table III. Effect of daily treatment for 21 days of C3H/HeJ female mice with CB-154 during lactation

Treatment	No. of mice with litters	No. and % of mice which raised litters to weanling	Pups surviving to weanling (%)	Mean [a] weight of pups at weanling (g)
Controls	24	16 (66%) [c]	57 [c]	8.6 ± 0.5 [d]
CB-154 [b]	22	6 (27%) [c]	25 [c]	5.9 ± 0.9 [d]

[a] Mean ± standard error. [b] CB-154, 0.1 mg/mouse/day. [c] $P < 0.001$. [d] $P < 0.01$.

be required for optimal ova implantation in rodents[23], a concept in accord with the findings of this study, in which we showed a greater than 50% reduction in pregnancies in female mice chronically treated with CB-154 during mating. Furthermore, mean number of pups per litter in the ergot-treated group was not significantly different from that in the controls, suggesting that the antifertility activity of CB-154 is not exerted by a direct effect on either the blastocyst or embryo, but by selective inhibition of ova implantation. Pregnancy was not, however, totally prevented in the ergot-treated mice but was significantly delayed. Nearly all of the mice in this group that eventually became pregnant, did so in the last 10 days of the 50-day treatment period. A slight adaptation to chronic treatment with the drug is, therefore, suggested by these results. The luteotrophic action of prolactin is the most probable mechanism by which this hormone influences ova implantation in rodents[22–24].

CB-154 has been previously shown to effectively inhibit lactation in rats[25], rabbits, pigs, dogs[4] and man[8], but has been shown to be essentially ineffective in suppressing established lactation in cows[6]. We have provided evidence in this study that the ergot is also effective in inhibiting lactation in mice. Pup mortality and rate of growth during lactation were markedly increased and decreased, respectively, in the CB-154 treated groups. The lactating dams treated with the ergot, as well as the other ergot-treated mice, showed no apparent ill effects of the treatment such as changes in body weight, activity, etc., when compared to controls. On the other hand, surviving weanling mice derived from the ergot-treated lactating dams were weak and stunted, indicating severely suppressed lactation.

The notable anti-mammary tumor effects of CB-154 in rodents[3, 14–16] and a potential role indicated for prolactin in human breast tumorigenesis[17–19], have prompted a number of clinicians to contemplate and/or actively use this drug for treatment of metastatic carcinoma of the breast[26, 27]. The drug has also been used, and appears to be potentially very effective, in suppressing non-puerperal galactorrhea in women[8]. The results of the present study provide evidence that chronic treatment with the drug in mice does not, upon drug withdrawal, induce sustained irreversible inhibition of fecundity and lactation. Whether or not use of the drug in primates will induce adverse irreversible effects on these processes remains to be determined. It is most unlikely that CB-154 would alter fecundity in primates as prolactin is not luteotrophic in these species[24].

Zusammenfassung. C3H/HeJ-weibliche Mäuse, die vor der Paarung dauernd mit CB-154, einem stark wirkenden Mittel zur Unterdrückung der Prolactinsekretion, behandelt wurden, wiesen keine bedeutsame Schädigung ihrer Fortpflanzungsfähigkeit auf. CB-154 Dauerbehandlung von weiblichen Mäusen sowohl während der Paarung als auch während der Laktation führte zu einer bedeutsamen Herabsetzung der Fruchtbarkeit und Milchabsonderung.

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Effect of Sulpiride on Prolactin Release by Rat Pituitaries in vitro

It has been recently demonstrated that N-(ethyl-1-pyrrolidinil-2) methylmethoxy-2-sulfamoyl-5-benzamide (sulpiride), a tranquilizing drug, can induce galactorrhea in humans[1]. It has also been demonstrated that sulpiride induced a decrease of serum gonadotropin levels in postmenopausal women[2]. In the rat, sulpiride modified serum gonadotropin and prolactin levels[3]. The aim of the present investigation was to demonstrate whether sulpiride can act directly on the pituitary gland. This possibility was investigated by incubating pituitary glands in vitro in the presence of sulpiride.

Materials and methods. Adult male rats of the Wistar strain were used as pituitary donors. They were killed by decapitation, the pituitary gland exposed, and the neural lobe was discarded. The pituitary gland was then cut into 2 approximately equal pieces, each being placed in a different beaker containing 5 ml of medium TC 199 (Difco). Each beaker contained 5 pituitary halves. The glands were preincubated for 1 h at 37 °C, being gassed continuously with a mixture of oxygen (95%) and carbon dioxide (5%) in a Dubnoff metabolic shaker. The medium was then discarded, being replaced by new medium. A small volume (0.2 ml) containing either ot the following solutions was added to the beakers: a) saline, b) 1 mg of sulpiride sulphate, c) 0.05 mg of sulpiride sulphate, d) a rat hypothalamic extract equivalent to 1.5 hypothalami, e) a rat hypothalamic extract equivalent to 1.5 hypothalami plus 1 mg of sulpiride sulphate, and f) a rat hypothalamic extract equivalent to 1.5 hypothalami plus 0.05 mg of sulpiride sulphate. 3 beakers for each treatment were used. The incubations were carried out for 4 additional h. At the end of the incubations, the media were separated frozen, and kept at −20 °C until assayed. The wet weight of the 5 pituitary halves contained in each beaker was recorded. Prolactin was assayed in the medium using the double-antibody radioimmunoassay described by Niswender et al.[4], with materials distributed by the National Institute of Arthritis and Metabolic and Digestive Diseases, Bethesda, USA. The results were expressed as ng of NIAMDD-Rat Prolactin-RP 1 per mg of wet pituitary weight and per ml of medium. The

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