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REVIEW ARTICLE

Influence of Ergot Alkaloids on Pituitary Prolactin and Prolactin-Dependent Processes

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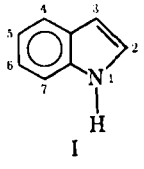
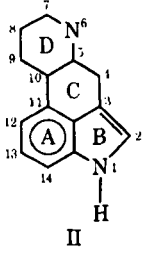
Keyphrases ☐ Ergot alkaloids—review of occurrence, structure, and general pharmacology, effect on pituitary prolactin, mammary tumors, and nidation inhibition ☐ Prolactin—review of secretion control and physiological activity, effect of ergot alkaloids on lactation and prolactin release, relationship to mammary tumors ☐ Pituitary hormone release—effect of ergot alkaloids on pituitary prolactin and prolactin-dependent processes, review ☐ Lactation and prolactin release—effect of ergot alkaloids, review ☐ Mammary tumors—relationship to prolactin, effect of ergot alkaloids, review ☐ Nidation—inhibition by ergot alkaloids, review

The ergot alkaloids occur in various species of claviceps including *Claviceps purpurea* (Fries) Tulasne (1, 2), other closely related fungi (3), and certain species of the Convolvulaceae, mainly in the genera ipomea, rivea, and argyreia (4).

The ergot alkaloids are all 1,4-substituted derivatives of indole (I), with the majority possessing the tetracyclic ring structure which has been designated ergoline (II).



I



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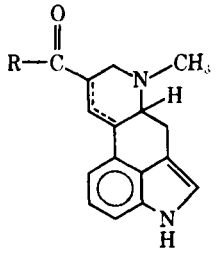

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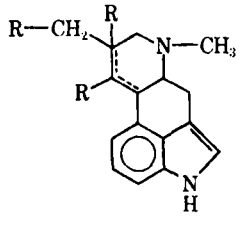
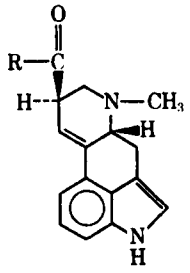


IV

The naturally occurring ergot alkaloids can be conveniently divided into two groups according to their chemical structure (1): the lysergic acid derivatives (III) and the clavines (IV). The clavines are substituted 6,8-dimethylergolines and include a few members, namely the chanoclavines, with a 6,7-*seco* D-ring.

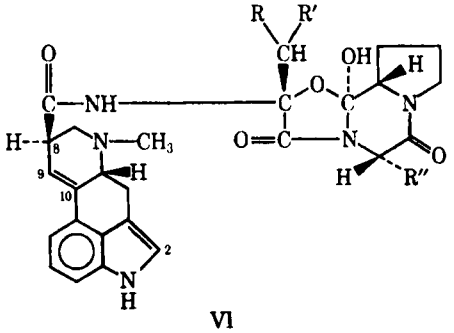
The lysergic acid derivatives can be further divided into the simple amide derivatives (V*a*–V*e*) and the cyclic peptides (VI*a*–VI*o*). All are derivatives of (+)-lysergic acid with the absolute configuration 5*R*,8*S* as shown. Stereoisomers at C-8 also exist in nature and are members of the (+)-isolysergic acid group.

The various clavines (VII*a*–VII*p*) are divided into three groups; $\Delta^{8,9}$, $\Delta^{9,10}$, and those containing a saturated D-ring. The tricyclic clavines, a relatively small group, consist of the isomeric chanoclavines and the interesting lactones, rugulovasine A and B (5).



V

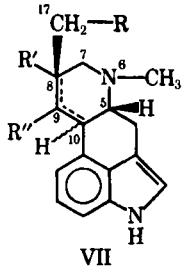
	R
V*a:* (+)-lysergic acid	—OH
V*b:* ergine	—NH_2
V*c:* (+)-lysergic acid α-hydroxyethylamide	—NH—CH(OH)—CH_3
V*d:* ergonovine (ergobasine, ergometrine)	—NH—CH(CH_2OH)—CH_3
V*e:* lysergic acid L-valine methyl ester	—NHCH($COOCH_3$)—$CH(CH_3)_2$



VI

	R	R′	R″
VI*a:* ergotamine	H	H	—CH_2—C_6H_5
VI*b:* ergotaminine*	H	H	—CH_2—C_6H_5
VI*c:* ergosine	H	H	—CH_2—$CH(CH_3)_2$
VI*d:* ergosinine*	H	H	—CH_2—$CH(CH_3)_2$
VI*e:* ergocristine	CH_3	CH_3	—CH_2—C_6H_5
VI*f:* ergocristinine*	CH_3	CH_3	—CH_2—C_6H_5
VI*g:* α-ergokryptine	CH_3	CH_3	—CH_2—$CH(CH_3)_2$
VI*h:* α-ergokryptinine*	CH_3	CH_3	—CH_2—$CH(CH_3)_2$
VI*i:* β-ergokryptine	CH_3	CH_3	—$CH(CH_3)CH_2CH_3$
VI*j:* β-ergokryptinine*	CH_3	CH_3	—$CH(CH_3)CH_2CH_3$
VI*k:* ergocornine	CH_3	CH_3	—$CH(CH_3)_2$
VI*l:* ergocorninine*	CH_3	CH_3	—$CH(CH_3)_2$
VI*m:* ergostine	H	CH_3	—CH_2—C_6H_5
VI*n:* ergostinine*	H	CH_3	—CH_2—C_6H_5
VI*o:* ergovaline	H	H	—$CH(CH_3)_2$

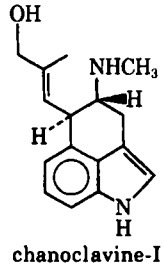
* epimeric at C-8; isolysergic acid series.



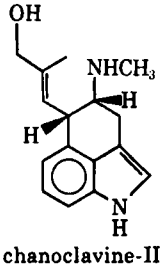
VII

	R	R′	R″
Δ^{8,9}-ergolenes			
VII*a:* agroclavine [10(*R*)]	H	—	H
VII*b:* elymoclavine [10(*R*)]	OH	—	H
VII*c:* molliclavine	OH	—	OH
Δ^{9,10}-ergolenes			
VII*d:* lysergene (Δ^{8,17} and Δ^{9,10})	—	—	H
VII*e:* lysergine	H	H	H
VII*f:* isolysergine*	H	H	H
VII*g:* lysergol	OH	H	H
VII*h:* isolysergol*	OH	H	H
VII*i:* setoclavine	H	OH	H
VII*j:* isosetoclavine*	H	OH	H
VII*k:* penniclavine	OH	OH	H
VII*l:* isopenniclavine*	OH	OH	H
ergolines (D-ring saturated)			
VII*m:* festuclavine [10(*R*)]	H	H	H
VII*n:* pyroclavine* [10(*R*)]	H	H	H
VII*o:* costaclavine [10(*S*)]	H	H	H
VII*p:* fumigaclavine A	H	H	CH_3COO—

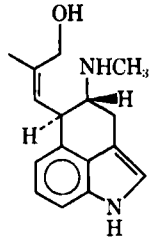
* epimeric at C-8, C-17 is α.



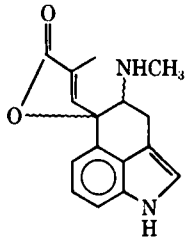
chanoclavine-I



chanoclavine-II (and enantiomer)



isochanoclavine-I



rugulovasine A and B

naturally occurring tricyclic clavines

GENERAL PHARMACOLOGY

The pharmacology of ergot and its alkaloids finds its beginnings in the myriad historical accounts from the Middle Ages of ergot toxicity in man and animals. This toxicity, called ergotism, was due to the ingestion of the grain from rye or products from the grain that had been infected with the ergot fungus, *Claviceps purpurea*. From the accounts of ergotism reviewed by Barger (6) and Bové (7), it is apparent that during the Middle Ages there were two types of ergotism in Europe. The form of ergotism referred to as St. Anthony's fire or ignis sacer was common west of the Rhine, particularly in France, where vasoconstriction of the peripheral blood vessels resulted first in intense burning pain in the extremities, with gangrene eventually setting in. East of the Rhine the poisoning usually resulted in CNS effects, with the most prominent symptom being convulsions. The basis for the occurrence of these two general types of toxic manifestations was attributed by Barger (6) to differ-

ences in diet, principally a deficiency in vitamin A being a factor in convulsive ergotism. However, the difference in toxic manifestations still remains one of the mysteries of history which has never been adequately explained.

What prompts the initial use of a toxic material as a drug is difficult to determine; however, in 1582, Adam Lonicer mentions in his Kreuterbuch the use of ergot to induce childbirth. For centuries it was used by midwives and physicians for this purpose until, in 1808, ergot moved from folkloric medicine to official status when an account of its use to quicken childbirth appeared in the *Medical Repository of New York*. Since this introduction into established medicine, the pharmacological effects of ergot and its alkaloids have been extensively documented (8, 9). The effects which have given ergot importance in medicine are related to its ability to stimulate both vascular and nonvascular smooth muscle. The effect apparently is direct, although the mechanism of action is not known. All natural alkaloids of the lysergic acid type have qualitatively the same effects on smooth muscle, but they exhibit differences in potency.

Stimulation of the vascular smooth muscle causes constriction of the blood vessels in the vascular bed, and the resulting constriction of intracranial arteries is useful in the treatment of migraine. The intensity of pain in migraine headache has been related to the amplitude of pulsations of the cranial arteries, chiefly the branches of the external carotid. Relief of headache by ergotamine is attributed to a reduction in amplitude of cranial artery pulsations because of vasoconstriction. The sensitivity of the smooth muscle of the uterus to stimulation by ergot increases along with the stage of gestation, so the resulting contractions at the end of the third trimester may induce labor. The principal use of the ergot alkaloids, however, involves their administration postpartum to prevent hemorrhage. For this purpose, ergonovine and its semisynthetic derivative, methylergonovine, are the drugs of choice. They are more potent, may be administered orally, have a more rapid onset of action, and are less toxic than ergotamine.

Mention should also be made of the pharmacological effects that have minor importance in current therapeutics. One of these effects is α-adrenergic blockade, which was reviewed by Nickerson (9) and Nickerson and Hollenberg (10). The ergot alkaloids were the first adrenergic blocking agents discovered, and only those alkaloids with a peptide side chain have this activity. The 9,10-dihydro derivatives of ergotamine and ergotoxine, which is a mixture of ergocornine, ergocristine, and ergokryptine[1], are the most potent of these blocking agents; consequently, they have been used to control hypertension and in the treatment of peripheral vascular diseases. Unfortunately, at doses where these compounds are effective, the side effects which include nausea and vomiting and, occasionally, headache, dizziness, blurring of vision, rashes, anorexia, nasal stuffiness, and abdominal cramps are usually too great to allow for their general use in therapeutics.

[1] Many authors in the English language, including *Chemical Abstracts*, have used the spelling "ergocryptine," apparently in analogy to ergocornine and ergocristine. However, the ending -kryptine is derived from the Greek "kryptos" = hidden; therefore, ergokryptine is the correct spelling.

An additional neurohormonal effect exhibited by the alkaloids of ergot is serotonin antagonism. Takeo (11) reported that the clavine-type alkaloids including agroclavine, elymoclavine, and their dihydro derivatives had moderate antiserotonin activity *in vitro* and *in vivo*. In later work, Yui and Takeo (12) found an increase in antiserotonin activity by 1-methylation of the clavine-type alkaloids, and Fanchamps *et al.* (13) showed that 1-methylation of several lysergic acid derivatives resulted in an increased antiserotonin activity over their original compounds. Consequently, it is not surprising that 1-methyl-methylergonovine, methysergide, is the most potent serotonin antagonist of the ergot compounds. Methysergide is effective in preventing all types of migraine headache (common, classic, and cluster), but it is of no value in treating an acute attack of migraine. It has been suggested (14) that serotonin, together with other humoral agents such as noradrenalin, exerts a tonic vasoconstriction effect on cranial arteries. Consequently, the prophylactic effect of methysergide in migraine is probably due to the potentiation of serotonin vasoconstriction rather than serotonin antagonism. Serotonin antagonism has been implicated in a rather simplistic explanation of the psychotomimetic activity of lysergic acid diethylamide, a semisynthetic derivative of lysergic acid (15). While it is now apparent that the mechanism behind psychotomimetic activity cannot be explained in such a simple fashion, it is noteworthy that there is a structural similarity between serotonin and psychotomimetic agents such as lysergic acid diethylamide, psilocybin, and nitrogen-substituted tryptamines.

A new phase of ergot pharmacology was initiated in 1954 when Shelesnyak (16) reported that the systemic injection of ergotoxine into rats inhibited deciduoma formation. Since that time, Carlsen *et al.* (17) demonstrated that a single subcutaneous injection of ergocornine methanesulfonate terminated early pregnancy in mice, and Zeilmaker and Carlsen (18) found that ergocornine administered to lactating rats temporarily inhibited milk production, the effect being prevented by treatment with luteotropic hormone (prolactin). Lactation inhibition by ergot was first reported in 1676, when Dodart noted that agalactia occurred among nursing women suffering from gangrenous ergotism (6). By far the most exciting aspects of this new phase of ergot pharmacology are the reports by several groups that ergocornine and ergokryptine induced regression of carcinogen-induced mammary adenocarcinomas in rats (*e.g.*, 19–21) and inhibited the growth of spontaneous mammary tumors which appear in the postreproductive phase of life of female rats (22). All these effects of the ergot alkaloids can be related to an inhibition of prolactin secretion from the anterior pituitary.

PROLACTIN AND ITS CONTROL

Prolactin is a peptide hormone secreted by the adenohypophysis. It is generally assumed that a prolactin is present in the pituitary of all vertebrate species, and Bern and Nicoll (23) reviewed the comparative endocrinology of prolactin. The prolactins and the pituitary growth hormones comprise groups of protein hormones with similar structural features, homologous amino

acid sequences, and, in some species, overlapping biological properties, even though there is considerable species specificity within each group.

Prolactin has been isolated and purified from the pituitary glands of a number of species of mammals including the sheep, ox, pig, and rat. Li *et al.* (24) reported the complete amino acid sequence of ovine prolactin, and Seavey and Lewis (25) determined that bovine prolactin differed from ovine prolactin by only two amino acids in the peptide map. On the other hand, the identification and isolation of prolactin as a protein distinct from growth hormone in primates have been difficult to achieve. This difficulty, coupled with the fact that highly purified human growth hormone possesses substantial prolactin activity (26, 27), raised some doubt as to the existence of prolactin as a separate entity in man.

Because of the difficulties encountered in the isolation and purification of prolactin, studies on the physiological role of prolactin, which require quantitation of the hormone, have had to rely on bioassays. The most practical of these assays in terms of efficiency and sensitivity is the pigeon crop-sac assay. Columbiforme birds, such as pigeons and doves, secrete a special fluid into their crop which they use to feed their young. This secretion, called pigeon's milk, is under the control of prolactin, since injections of this hormone stimulate growth of the special cells involved in this process. The essential features of this assay, which has undergone various modifications since Lyons (28) first described it in 1937, are that a standard amount of prolactin is injected on one side of the crop gland while the material to be assayed is injected on the other side. In some cases, different birds are used for the control than for the unknown. A ratio of the degree of proliferation of crop cells of the control to the unknown is determined from a standard curve (29).

The recent development of radioimmunoassays has made it possible to measure blood levels of prolactin for the first time. These assays generally involve a double antibody method. Prolactin obtained from the animal species to be assayed is injected into another species of animal to prepare antiserum against the prolactin. Antiserum to the immunoglobulin G of the animal which manufactures the prolactin antiserum is prepared by using a different species of animal. Highly purified prolactin from the animal species to be assayed is radioactively tagged by treating it with radioactive iodine, *e.g.*, ^{131}I. Blood serum from the animal to be assayed for prolactin levels is incubated with prolactin antiserum and radioiodinated prolactin. The nonlabeled hormone from the test animal competes with the ^{131}I-labeled hormone for the antibody so that the ratio of bound to free labeled hormone decreases. When enough time has elapsed for maximum binding, immunoglobulin G antiserum is added to precipitate the prolactin–immunoglobulin G complex, which is then assayed for radioactivity. Various adsorbents such as charcoal and cellulose can also be used for this separation in place of the second antibody. Recent publications that described radioimmunoassays for prolactin are those of Niswender *et al.* (30), Johke (31), Neill and Reichert (32), Davis *et al.* (33), Hwang *et al.* (34), Raud and Odell (35), and McNeilly (36).

Recently, Frantz *et al.* (37) reviewed their previous works in which they determined unequivocally the presence of human prolactin separate from human growth hormone in plasma. These workers used an *in vitro* mammary gland culture system from a midpregnant mouse breast tissue and found high bioassayable prolactin activity in human plasma samples that had low immunoassayable growth hormone. They then demonstrated that antihuman growth hormone antiserum was incapable of neutralizing prolactin activity, whereas it completely neutralized the prolactin activity of growth hormone itself.

The isolation and purification of human prolactin in sufficient quantities for chemical and biological characterization are well on the way to being achieved. Guyda and Friesen (38) separated monkey prolactin from monkey growth hormone using affinity chromatography. Sepharose-coupled antibodies to human placental lactogen were able to remove more than 99% of monkey growth hormone present in incubation media or tissue homogenates of monkey pituitaries. The potency of prolactin in these fractions, however, remained unaltered or even increased after the immunoadsorption of monkey growth hormone.

Chrambach *et al.* (39), using polyacrylamide gel electrophoresis, identified human prolactin and determined several of its physical characteristics. They found that biologically active serum human prolactin is a single, homogeneous protein, which is distinct from the human growth hormone species hGH-β. Human prolactin is more highly charged than growth hormone but has the same molecular size. Lewis *et al.* (40) isolated human prolactin from pooled human pituitary glands (nearly 500 were used), using a combination of Sephadex chromatography and DEAE–cellulose chromatography. Their product had a potency of 22 I.U./mg. in the pigeon crop-sac assay for prolactin but only 0.4 USP unit/mg. in the tibial line assay for growth hormone. The product was less electronegative than growth hormone at pH 9.5 and had a molecular weight of about 22,000. Most recently, Hwang *et al.* (41), after gel filtration and ion-exchange chromatography of a discard fraction obtained in human growth hormone purification, obtained a preparation with 30.5 I.U./mg. of prolactin activity in an *in vitro* mouse mammary tissue culture assay. Human growth hormone contamination was less than 0.5% by radioimmunoassay. The authors claimed that the method of purification can easily be scaled up for larger batches of material so that prolactin of sufficient quantity and purity can be obtained for the determination of the primary structure of the molecule.

Since it is not within the scope of this article to provide references to the specific experimental literature documenting the current concept of prolactin action in the body, the reader is referred to the extensive review papers of Meites and Nicoll (42) and Meites *et al.* (43). It is the purpose to limit this discussion to the salient features of prolactin activity, with emphasis on the control of prolactin secretion.

Regulation of prolactin secretion from the adenohypophysis, as with the other anterior pituitary hormones,

is mediated in various ways through the hypothalamus. Prolactin secretion is unique in that it appears to involve hypothalamic inhibition, hypothalamic stimulation, and prolactin feedback control. There is evidence that the hypothalamus continually inhibits prolactin secretion by the adenohypophysis. This is exactly opposite to most pituitary hormones whose release is stimulated by hypothalamic secretions. It is presumed that the hypothalamus is the source of a continuously secreted substance that inhibits prolactin synthesis as well as release. Meites and coworkers (42, 43) called this hormone prolactin-inhibiting factor, PIF. Everett (44) demonstrated that pituitary transplants in hypophysectomized rats could secrete prolactin for months, as evidenced by maintenance of luteal function, and transplantation studies in certain teleosts and amphibians showed that their anterior pituitary can secrete prolactin autonomously while the secretion of other hormones is decreased (23).

Bilateral lesions placed in the median eminence of intact female rats, which destroyed the connection between the hypothalamus and the anterior pituitary, have resulted in a rapid increase in serum prolactin levels in rats (45, 46). This effect is similar to that seen after pituitary stalk section (47*a*). In this regard, Turkington *et al.* (47*b*) demonstrated elevated serum prolactin levels in patients subjected to surgical section of the pituitary stalk for metastatic breast cancer or for diabetic retinopathy. In tissue culture of human fetal pituitaries, Pasteels (48) showed that the secretion of prolactin increased with the length of the culture period; however, if crude hypothalamic extracts were added, there was a significant decrease in the amount secreted. At the present time the chemical structure of prolactin-inhibiting factor is not known. It may be a small polypeptide by analogy with the structures of the various hypothalamic-releasing factors. Experimental determination of prolactin-inhibiting factor activity must rely on bioassays, and Nicoll *et al.* (49) reviewed the several *in vitro* and *in vivo* assays employed.

To complicate the picture of prolactin secretion control, Nicoll *et al.* (49) discussed the evidence indicating that the hypothalamus may also secrete a prolactin-releasing factor (PRF). Early studies indicated that the avian hypothalamus exerts a stimulatory effect, rather than an inhibitory effect as in mammals, on prolactin release. Kragt and Meites (50) showed that an extract of pigeon hypothalamus stimulates prolactin secretion by the pigeon pituitary *in vitro*.

It is now apparent that prolactin-releasing factor is present in mammals. Nicoll *et al.* (49) presented evidence that there is both prolactin-inhibiting activity and prolactin-stimulating activity in rat hypothalamic extract when incubated with rat pituitary tissue. Tashjian *et al.* (51) obtained direct evidence that thyrotropin-releasing hormone stimulated the production of prolactin and inhibited growth hormone production in rat pituitary cell cultures. In this regard, Bowers *et al.* (52*a*), using a radioimmunoassay, demonstrated that synthetic pyroglutamyl-histidyl-prolinamide (thyrotropin-releasing hormone) stimulated the secretion of prolactin as well as thyrotropin in normal men and women. Tyson *et al.* (52*b*) found that synthetic thyrotropin-releasing hormone administered to parturient, lactating women and to women with normal menstrual cycles provoked a maximal rise in prolactin levels 15 min. after injection, with a return to preinjection levels within 60 min. Valverde-R. *et al.* (52*c*) presented evidence that there is a prolactin-releasing hormone distinct from thyrotropin-releasing hormone. By using Sephadex G-10 chromatography and testing in male rats previously sensitized with estrogen and progesterone, they were able to separate from a porcine hypothalamic extract a fraction with prolactin-releasing activity that was different from thyrotropin-releasing hormone.

The final aspect of prolactin secretion to be discussed is the matter of feedback control. MacLeod *et al.* (53) transplanted prolactin-secreting pituitary tumors into intact rats and found a decrease in pituitary prolactin content. Chen *et al.* (54) in the same type of experiment also found an increase in hypothalamic prolactin-inhibiting factor activity. Also, implantation of prolactin into the median eminence of rats was demonstrated by Clemens and Meites (55) to cause a decrease in pituitary and serum prolactin levels as well as an increase in prolactin-inhibiting factor activity. These investigations lend support to the postulation that prolactin inhibits its own secretion by the pituitary gland through influencing the hypothalamus to secrete more prolactin-inhibiting factor. Hökfelt and Fuxe (56) recently established that the tubero-infundibular dopamine neurons are involved in the regulation of prolactin secretion. These authors postulated that the tubero-infundibular dopamine neurons stimulate the release and/or synthesis of prolactin-inhibiting factor at the level of the median eminence and that they are a link in a feedback system by which serum prolactin levels influence prolactin-inhibiting factor secretion. Supporting data for this hypothesis come from the work of Lu and Meites (57), who found that L-dopa and various MAO inhibitors increased catecholamic activity in the hypothalamus as well as hypothalamic prolactin-inhibiting factor activity.

The physiological effect of prolactin on female mammals is complex, and many differences in effect are found between species (43). The principal functions of prolactin, however, are in the initiation and maintenance of lactation and in promoting the growth of the mammary glands. In rats, prolactin has an additional importance in inducing luteolysis of the previous corpora lutea during each estrus cycle and helping maintain corpora lutea together with luteinizing hormone during pseudopregnancy and early pregnancy.

In studies on the pregnant female rat, Voogt *et al.* (58*a*) showed that serum prolactin (except for the first 3 days), ACTH, and blood corticosterone levels are low during gestation, although Butcher *et al.* (58*b*) showed a semicircadian rhythm in plasma levels of prolactin during early gestation in the rat. The absence of lactation during pregnancy is believed to be due to an insufficiency of adrenal cortical hormones or prolactin or both. At the time of parturition there is an increase in blood prolactin, ACTH, and corticosterone and these are thought to initiate lactation. To develop this idea further, it is important to note that estrogen is a potent stimulator of prolactin release in mammals. Apparently estrogen can directly stimulate the anterior pituitary to secrete prolactin without intervention of the hypothala-

mus (59). Therefore, the low serum prolactin in the rat during pregnancy is probably associated with low estrogen secretion during gestation (60) and perhaps with the ability of progesterone to prevent the stimulating action of estrogen on prolactin secretion. Also significant is that an increase in pituitary prolactin content at the time of parturition was observed in humans by Frantz and Kleinberg (61). This effect may be due to a decrease in progesterone levels and an increase in estrogen level.

A definite stimulus for prolactin secretion during postpartum lactation is suckling. Ratner and Meites (62*a*) showed that stimulation of the numerous sensory nerves in the nipples and surrounding skin evokes a reflex release of prolactin from the pituitary by depressing prolactin-inhibiting factor activity. Johke (62*b*) reported that in lactating cows plasma prolactin levels rapidly increased and reached a peak 4–20 min. after the start of milking; in lactating goats, milking caused a 40–100-fold increase in prolactin release. Hwang *et al.* (34) demonstrated that serum prolactin increased by 10–20-fold 30 min. after the start of breast feeding in postpartum humans.

Prolactin is clearly one of the major hormones involved in mammary growth along with somatotropic hormone (growth hormone), and practically the entire endocrine system has some regulatory influence on the mammary gland. The ovarian hormones, interestingly, have little effect on mammary growth in the absence of the pituitary gland (43). This observation suggests that the ovarian hormones act in part *via* the pituitary, and it has been postulated that the ovarian hormones sensitize the mammary gland to the effects of prolactin and somatotropin (42).

INFLUENCE OF ERGOT ALKALOIDS ON LACTATION AND PROLACTIN RELEASE

As mentioned earlier, the knowledge that ergot alkaloids can inhibit lactation dates back several hundred years to Dodart, who noted that agalactia could be observed among nursing women suffering from gangrenous ergotism (6). This knowledge was reinforced repeatedly in the following centuries and its potential clinical usefulness was discussed (*cf.*, 63, 64). Animal work similarly indicated suppression of lactation by ingestion of crude ergot sclerotia or by some of the constituent alkaloids (65–71). Such studies have involved the effect in rats, mice, guinea pigs, dogs, sows, and cows. One of the first systematic studies was that of Sommer and Buchanan (69) in albino rats. They showed that 0.5–1.0 mg./kg. of several ergot alkaloids given twice daily during the second half of pregnancy significantly reduced the rate of weight gain of the litter after birth. Ergotamine was most effective in this respect, followed by ergotoxine, whereas dihydroergotoxine and methylergonovine showed little activity. Later studies showed the same effect in mice who were kept on a diet containing the clavine alkaloid, agroclavine (71). At the lowest dose tested, about 15 mg./kg. daily until 3–8 days before parturition, all animals completely failed to raise their litter. In addition, an almost complete inhibition of the hypertrophy of mammary tissue during pregnancy and lactation was observed (71), as was also noted by Sommer (72). A recent study in cows showed a slightly more differentiated picture. Administration of the prolactin inhibitor, 2-bromo-α-ergokryptine[2], to lactating cows, while drastically reducing the serum prolactin levels, had only little effect on the milk yield (73). However, treatment immediately before parturition effectively inhibited the onset of lactation (74).

An understanding of the mechanism of lactation inhibition by ergot alkaloids came about only gradually and it can, even today, not be said to be complete. While there were some indications in the literature of effects of ergotoxine on the pituitary gland (75, 76), early workers apparently did not suspect this to be the site through which the ergot effect was mediated. A "lack of normal maternal instincts" (67, 68), inadequate development of the mammary glands (65), and reduced food intake by the mothers (77) were suggested as the causes for impaired lactation following administration of ergot alkaloids. Grosvenor (78) found that daily administration of ergotamine during lactation interfered with the milk-ejection reflex in rats and later showed this to be due to blocking of the release of oxytocin in response to suckling (79). Zeilmaker and Carlsen (18) apparently were the first to establish a relationship between the inhibition of prolactin release from the pituitary and the inhibition of lactation by ergot alkaloids. They found that in rats the lactation inhibition caused by a single injection of 1 mg. ergocornine given after parturition could be overcome by simultaneously administering prolactin.

The picture of these relationships has since been considerably refined, particularly by a recent study by Shaar and Clemens (80) which correlates the effect of a number of ergot alkaloids on serum prolactin levels, as measured by radioimmunoassay, with parameters like litter weight gain, body weight gain of mothers, and mammary tissue weight. The authors concluded that the inhibition of lactation is at least partly due to the suppression of prolactin release. The reduction in the body weight of the mothers is almost entirely accounted for by the reduction in mammary weight. Whether other factors, like the inhibition of milk ejection, contribute to the effect on lactation is not entirely clear. It seems likely that for some alkaloids such other factors contribute significantly to their overall effect on lactation, whereas with other alkaloids the effect on prolactin levels may be the main mode of action. Several pieces of circumstantial evidence support this view. For example, in the work of Shaar and Clemens (80) the ability of several alkaloids to decrease the weight gain of litter did not strictly parallel their effect on serum prolactin levels. A comparison of the ability of various alkaloids to inhibit lactation (69) with their activity as inhibitors of nidation (81) (see below) also points in that direction. For example, ergotamine is a good lactation inhibitor but has little activity in inhibiting nidation. Ergotoxine, on the other hand, is much less active in lactation inhibition but is very effective as a nidation inhibitor. This was studied systematically by Flückiger and Wagner (82) in a comparison of ergokryptine and 2-bromo-α-ergokryptine as lactation and nidation inhibitors. They found that the dose–response curves for lactation and nidation inhibition by ergokryptine converged toward one dose which com-

[2] CB 154, Sandoz.

pletely blocked both effects, suggesting a common primary effect. The same is true for ergocornine but not for 2-bromo-α-ergokryptine. Therefore, the latter must act in a somewhat different way than the other two. Whether the difference lies in a specific antifertility effect of the bromo derivative, as the authors suggested, or whether these compounds have different effects on oxytocin release, as suggested by Nicoll *et al.* (83), remains to be established. Another possibility is that differences in the pharmacokinetic properties could well account for the differences in the quantitative effectiveness of various ergot alkaloids in lactation and nidation inhibition. Finally, it should be mentioned that Edwardson carried out experiments in rats with cannulated mammary ducts and found that the alkaloid agroclavine did not inhibit the rise in intramammary pressure produced by oxytocin (84). Therefore, inhibition of the milk-ejection response can probably be excluded as a major factor in the mode of action of this alkaloid.

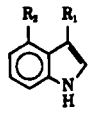
Most of the efforts in recent years have been directed toward understanding the mode in which ergot alkaloids influence prolactin levels. The work of Zeilmaker and Carlsen (18) with hypophysectomized rats bearing an autotransplanted pituitary under the kidney capsule showed that ergocornine still counteracts the prolactin effects, and thus presumably inhibits prolactin release, when the hypothalamic–pituitary connection is interrupted. As discussed later, their conclusion that ergot alkaloids inhibit prolactin release by direct action on the pituitary has been repeatedly challenged by other workers who felt that the action on the pituitary is mediated through the hypothalamus. More recent work seems to indicate that both sites may be involved. Wuttke *et al.* (85) found that pituitaries incubated *in vitro* with hypothalamic extract from ergocornine-treated rats released less prolactin and luteinizing hormone (LH) than ones incubated with hypothalamic extract from untreated animals. Ergocornine treatment, therefore, seems to increase the hypothalamic concentration of prolactin-inhibiting factor and decrease that of luteinizing hormone releasing hormone (LH-RH), suggesting an action *via* the hypothalamus. On the other hand, there is also overwhelming evidence for a direct action of ergot alkaloids on the pituitary.

Additional experiments with hypophysectomized, pituitary-grafted animals confirmed Zeilmaker and Carlsen's (18) earlier findings, both by following the influence of ergocornine on prolactin-dependent phenomena, *e.g.*, the luteolysis of nonfunctional corpora lutea (86), and by directly measuring serum prolactin levels using radioimmunoassay techniques (59, 80). Further *in vivo* evidence for a direct action of ergot alkaloids on the pituitary was obtained in rats in which the vascular continuity between the hypothalamus and the pituitary had been eliminated by median eminence lesions (87). The increase in serum prolactin levels resulting from the removal of the control of pituitary prolactin release by prolactin-inhibiting factor could be prevented by ergocornine but not by ovariectomy. *In vitro* studies with explanted rat (59, 83, 88) and human (89) hypophyses in culture fully confirmed these findings. Ergocornine (59, 88, 89), 2-bromo-α-ergokryptine (89), and ergotamine (83) all significantly inhibited prolactin release at concentrations in the range of 1–20 mcg./ml. No effect of ergotamine on the secretion of growth hormone was detected (83). Prolactin secretion can be restored by washing out the ergot alkaloid, indicating that the effect is not due to cellular destruction (89). According to Pasteels *et al.* (89), preliminary electron microscopic evidence suggests a direct action on prolactin cells to inhibit the excretion of secretory granules. Ergot alkaloids also inhibit prolactin release and prolactin formation in cultures of pituitary tumor cells without affecting the levels of growth hormone (90). Finally, a recent study indicates that ergot alkaloids also inhibit pituitary prolactin release in certain species of fish (91) in which prolactin is required to maintain plasma sodium levels when the animal is in fresh water. In this case the eta cells of the pituitary, which most likely are the source of prolactin, seemed to be inactivated as a result of administration of 2-bromo-α-ergokryptine.

Only a limited amount of information is available in the literature on relationships of the structure of ergot alkaloids and their prolactin-inhibitory or lactation-inhibitory activity. Particularly, quantitative information on structure–activity relationships is almost completely missing. Compounds that have been reported to have activity as lactation inhibitors include ergotamine (69, 80), ergotoxine (69), ergocornine (18, 80), ergokryptine (80, 82), dihydroergotoxine (weak) (69), dihydroergocornine (80), methylergonovine (weak) (69), ergonovine (weak) (80), 2-bromo-α-ergokryptine (82), agroclavine (71), and D-6-methyl-8-ergolinylacetamide (92). Activity toward inhibiting prolactin release *in vivo* or *in vitro* has been reported for ergocornine (59, 80, 85, 87–89), 2-bromo-α-ergokryptine (73, 74, 89), ergotamine (80, 83), lysergic acid diethylamide (93), ergokryptine (80), dihydroergocornine (80), and ergonovine (80). Also active are agroclavine and elymoclavine (94–96).

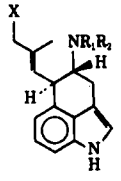
In a collaborative study (97) aimed at defining the minimal structural requirements for prolactin inhibition, the activity of a variety of simple ergolines and some of their analogs and derivatives was determined. The assay involved injecting 10 mcg. of the compound into reserpinized male rats (average body weight ~200 g.), which were killed 2 hr. later and assayed for their serum prolactin level using the rat prolactin radioimmunoassay kit (NIAMD). In each group of tests, ergocornine was included as a standard and the observed values were normalized according to the inhibition by ergocornine. The data, grouped according to structural types, are summarized in Table I. The figures in parentheses give the prolactin-inhibitory activity relative to ergocornine = 100. The actual inhibitions observed with ergocornine under these conditions ranged from 58 to 75%. Compounds having relative activities of less than 50 were considered marginally active or inactive. It can be seen that simple 3,4-substituted indoles, even if they have essentially all the carbon atoms of the ergoline system, as well as tricyclic clavines are completely inactive. Apparently, the complete tetracyclic ergoline ring system is required for activity. Quaternization of the nitrogen atom 6 completely abolishes activity, possibly because of reduced permeability. The double bond in the 8,9-position is not absolutely essential for activity, but shifting it to the 9,10-position reduces activity. A methyl

Table I—Ergolines and Related Compounds as Inhibitors of Prolactin Release



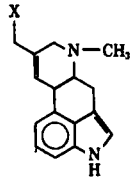
I: Substituted indoles

Compound	Activity
1. 1-(3-Indolyl)-2-amino-5-methyl-4-hexene acetate R_1 = —CH_2—CH(—$NH_3^+CH_3COO^-$)—CH_2—CH=$C(CH_3)_2$ R_2 = H	(−)
2. 4-(γ,γ-Dimethylallyl)-skatylformamidomalonic acid diethyl ester R_1 = —CH_2—C(—$CO_2CH_2CH_3$)—NHCHO—$CO_2CH_2CH_3$ R_2 = —CH_2—CH=$C(CH_3)_2$	(−)
3. 4-(γ,γ-Dimethylallyl)gramine R_1 = —CH_2—$N(CH_3)_2$ R_2 = —CH_2—CH=$C(CH_3)_2$	(−)
4. 4-(γ,γ-Dimethylallyl)tryptophan R_1 = —CH_2—CH(—NH_3)—COOH R_2 = —CH_2—CH=$C(CH_3)_2$	(−)



II: Clavines, tricyclic

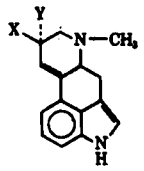
Compound	Activity
1. Chanoclavine-I R_1 = H, R_2 = CH_3, X = OH	(−)
2. *N*-Methylchanoclavine-I R_1 = R_2 = CH_3, X = OH	(−)
3. *N*-Methyldesoxychanoclavine-I R_1 = R_2 = CH_3, X = H	(−)



III: Clavines, tetracyclic, $\Delta^{8,9}$

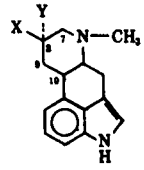
Compound	Activity
1. Agroclavine X = H	(+)
2. Agroclavine methiodide	(−)
3. Elymoclavine X = OH	(+)
4. Dihydroelymoclavine	(+) (82)
5. Elymoclavine-*O*-acetate	(+) (80)
6. Elymoclavine-17-carbamate	(+) (65)
7. Elymoclavine-17-benzoate	(+) (65)
8. Elymoclavine-β-D-fructoside	(+) (60)
9. Elymoclavine-*N*-oxide	(−)
10. Elymoclavine methiodide	(−)

Table I—(*Continued*)



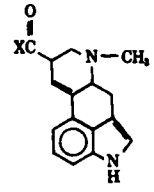
IV: Clavines, tetracyclic, $\Delta^{9,10}$

Compound	Activity
1. Lysergine X = CH_3, Y = H	(−) (24)
2. Isolysergine X = H, Y = CH_3	(−)
3. Isolysergol X = H, Y = CH_2OH	(+) (52)
4. Lysergene X, Y = CH_3	(−)
5. 6-Methyl-8-acetoxymethylene-9-ergolene X, Y = CHOCOCH$_3$	(+) (48)



V: Clavines, tetracyclic

Compound	Activity
1. 6-Methyl-8-aminoergoline	(+) (52)
2. Dihydrolysergic acid lactam X, Y = CH_2, 7-keto	(−)
3. Dihydrolysergic acid dihydrolactam X = CH_3, Y = H, 7-keto	(−)
4. Dihydroelymoclavine X = CH_2OH, Y = H	(+) (82)



VI: Lysergic acid derivatives

Compound	Activity
1. Ergocornine	(+) (100) control
2. Lysergic acid α-hydroxyethylamide X = —NHCH(CH_3)OH	(+) (74)
3. Dihydrolysergic acid azide·HCl X = N_3	(−)

group seems to be the minimal size substituent in the 8-position for activity, and a somewhat larger substituent seems to be advantageous particularly with a $\Delta^{9,10}$ double bond. A keto function in the 7-position completely eliminates activity. This defines some of the structural elements needed for activity, and further studies are underway to refine this picture.

Since the systematic study of lactation and prolactin inhibition by ergot alkaloids is only relatively recent, few reports of clinical trials or applications are found in the literature. Various ergot alkaloids have been repeatedly and successfully used in the treatment and prevention of puerperal mastitis in humans (*e.g.*, 98, 99) or of milk-fever (agalactia toxemia) in sows (100).

Their usefulness in these disorders has usually been ascribed to their sympatholytic and spasmolytic activity, but the possibility that their lactation-inhibitory effect may be a contributing factor certainly deserves consideration. A recent study reports experiences with 2-bromo-α-ergokryptine in the treatment of nonpuerperal galactorrhoea in three women. In all three cases, the galactorrhoea was terminated (101*a*). Even more recently, the same group (101*b*) extended this study to 14 women with galactorrhoea with good results. In a double-blind trial, they found 2-bromo-α-ergokryptine to be as effective as diethylstilbestrol in terminating lactation in women and they reported that they now use the compound commonly for that purpose (in 60 successful treatments so far).

RELATIONSHIP BETWEEN PROLACTIN AND MAMMARY TUMORS

It has been established that mammary cancer in humans is hormone responsive and susceptible at least temporarily to treatment by removal of the ovaries, testes, adrenals, or pituitary or by administration of steroid hormones (102*a*,*b*,*c*). Pearson *et al.* (103) and Pearson and Ray (104) found that a pituitary factor is probably involved in the estrogenic stimulation of mammary tumor growth in humans, since after hypophysectomy-induced remission the administration of estrogen to the patient did not reactivate tumor growth, whereas it did following an oophorectomy-induced remission.

Turkington *et al.* (47*b*) studied the effect of pituitary stalk section on human serum prolactin levels in 11 patients with metastatic carcinoma of the breast. Seven patients showed substantial elevations in the serum prolactin activity while four showed no increase. Eight of these patients evidenced objective remissions for periods ranging from 7 months to 12 years. Five of these eight patients had elevated prolactin levels during this period. Among the three patients who showed no objective remission, two had elevated prolactin levels. The authors concluded that prolactin does not promote the growth of mammary carcinoma during remission but perhaps is involved in "reactivation" of the disease.

A number of studies have been carried out with model tumor systems in mice and rats. These studies have provided a reasonably clear picture of the factors involved in the induction and maintenance of mammary tumor growth in these animals (105–108).

A series of publications (109–114) has appeared which establish that hypophyseal isografts produce a stimulatory effect on the mammary glands of mice, leading to mammary tumors in mice carrying the mammary tumor virus. Mühlboch and Boot (115) extended this research and found that multiple subcutaneous hypophyseal isografts in most mammary tumor virus-free mice also induced tumor formation. These grafts were found to produce prolactin continuously, and development of the mammary tumors was ascribed at least in part to the action of prolactin along with progesterone and/or estrogen. Boot and coworkers, in a series of publications, reported the effects of site of implantation, age and sex of donor (116), size of the growth (117), and steroid hormones on the growth and thereby indirectly the amount of prolactin on the production of mammary tumors (118–121). As a result of these studies, Boot (106) concluded that prolactin is the hormone most directly involved in mammary tumor formation in mice. Yanai and Nagasawa (122) also found that prolactin continuously secreted by isografted pituitaries promoted the maintenance and growth of mammary hyperplastic nodules in adreno-ovariectomized mice. In a later study (123), these same workers reported that mammary tumors were produced in about 51% of the adreno-ovariectomized mice by placement of pituitary isografts, and they concluded that prolactin is the primary hormonal factor in spontaneous mammary tumorigenesis in mice.

Prolactin along with estrogen is also involved in the development and growth of mammary tumors in rats. Spontaneous mammary tumors occur in old female rats and are stimulated by administration of estrogen (124) and inhibited by ovariectomy (125). It has been established that estrogen can promote the synthesis and release of prolactin by action on both the hypothalamus and pituitary (42). The spontaneous mammary tumors, which occur generally as a single benign adenoma, differ considerably from the multiple adenocarcinomas induced by carcinogens. Early induction and increased incidence of the spontaneous mammary tumors occurred on grafting multiple pituitaries (126) or on placing lesions in the median eminence of the hypothalamus (46, 127). Both of these treatments have been shown to result in increased serum prolactin levels.

A somewhat different pattern has been established in the induction and maintenance of carcinogen-induced mammary adenocarcinomas in rats. Dao (107, 108) established that pregnancy and lactation inhibit induction of mammary cancer by the carcinogen 3-methylcholanthrene. Stimulation of an increase in prolactin levels before administration of 7,12-dimethylbenz[*a*]anthracene results in an inhibition of the development of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors in the rat. Placement of lesions in the median eminence in rats prior to a single dose of 5 mg. of 7,12-dimethylbenz[*a*]anthracene significantly inhibited induction by the carcinogen (128, 129). Complete inhibition was effected if rats with bilateral lesions were also castrated. These results were subsequently confirmed by Klaiber *et al.* (130). Prolactin-secreting pituitary homografts (131), reserpine (132), or mestranol–norethynodrel[3] treatment (133) also inhibited tumor induction by 7,12-dimethylbenz[*a*]anthracene. This method of inhibition apparently involves the induction of mammary hyperplasia by prolactin prior to carcinogen administration. It appears that the functionally active mammary gland is protected from carcinogens.

Following the development of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors, an increase in prolactin secretion results in a definite enhancement of tumor growth, in contrast to the situation prior to induction. At this stage, a decrease in prolactin secretion results in inhibition of mammary tumor growth. Thus, after tumor development placement of lesions in the

[3] Enovid.

median eminence (128, 129), grafting of multiple pituitaries (131) or treatment with reserpine (132) or mestranol–norethynodrel[3] (133) stimulated the growth of 7,12-dimethylbenz[*a*]anthracene-induced mammary cancers. Kim and Furth (134) and Kim *et al.* (135) also found that prolactin was important in promoting and maintaining the growth of mammary tumors in rats.

Pearson *et al.* (105) used rats bearing 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors to show that the development of such tumors was dependent on the presence of prolactin in the serum. Their data suggested that prolactin may be the only hormone of significance in maintaining tumor growth. Further evidence for the role of prolactin in the development of such tumors was obtained in experiments in which antibodies to rat prolactin were injected into rats bearing 7,12-dimethylbenz[*a*]anthracene-induced mammary carcinomas, leading to a decreased growth of the tumors and an increased incidence of tumor regression (136).

It is apparent on the basis of the preceding discussion that prolactin and estrogen are the primary hormones controlling the maintenance and growth of mammary tumors in the rat, mouse, and probably the human. The exact mechanisms by which these two hormones effect carcinogenesis is not completely elucidated (107, 108); however, several reports dealing with this subject have appeared in the literature (138–142*b*).

EFFECTS OF ERGOT ALKALOIDS ON MAMMARY TUMORS

Yanai and Nagasawa (143, 144) studied the effects of ergocornine and 2-bromo-α-ergokryptine on the formation of hyperplastic alveolar nodules of the mammary gland in female C3H/He multiparous mice. It has been established (145) that hyperplastic alveolar nodules represent the preneoplastic state in spontaneous mammary tumor formation in mice. Ergocornine and 2-bromo-α-ergokryptine were found to have a significant effect, involving inhibition of development and growth of hyperplastic alveolar nodules. This effect was ascribed to the measured ability of these substances to inhibit pituitary prolactin secretion. These same workers (123, 146*a*) investigated the effect of subcutaneously implanted pellets of ergocornine methanesulfonate and 2-bromo-α-ergokryptine on the appearance of spontaneous mammary tumors in C3H/He strain mice. Mammary tumor development was enhanced by a subsequent (4 weeks after pellet implantation) pituitary isograft (122). The appearance of mammary tumors was significantly diminished in mice treated with the ergot alkaloids, with treated mice showing a mammary tumor incidence of 10 and 16% (2-bromo-α-ergokryptine) and 20 and 23.5% (ergocornine) compared to a 74% incidence in the controls. The mammary tumor appearance in a few of the treated animals was attributed to small, nonpalpable tumors already in existence at the start of the experiment and to loss of the implanted pellet during the experiment. Recently, Singh *et al.* (146*b*) reported the effect of ergocornine on transplanted Dz-mammary tumors in BALB/C mice. They found that the growth of tumors was decreased by ergocornine at doses ranging from 20 to 100 mg./day for 5 weeks. There was no significant regression of the tumors at these doses.

Several groups have studied the effects of ergot alkaloids on 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors in rats. Nasr and Pearson (147) studied the effect of ergocornine on 7,12-dimethylbenz[*a*]anthracene-induced mammary carcinomas and found that a dose of 2 mg. daily produced a 60% reduction in tumor size within 18 days. Henson *et al.* (148) studied the effect of 2-bromo-α-ergokryptine on this same tumor and observed a 67% reduction of tumor growth as a result of injecting 2-bromo-α-ergokryptine at a daily dose of 3 mg./kg. for 3 weeks followed by a 6-mg./kg. dose for an additional 3 weeks. Concurrently, Nagasawa and Meites (19) found that ergocornine inhibited the growth of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors in rats when injected daily for 15 days at a dose level of 0.6 mg./day. In a subsequent paper, Cassell *et al.* (20) reported a study involving a longer treatment period (28 days) using both ergocornine and 2-bromo-α-ergokryptine. At the doses used, both ergocornine and 2-bromo-α-ergokryptine suppressed tumor growth; however, ergocornine produced complete disappearance of some tumors and was more effective than 2-bromo-α-ergokryptine in inhibiting growth. Termination of treatment with ergocornine resulted in a marked increase in the size and numbers of mammary tumors. Quadri and Meites (22) also reported that these same drugs were equally effective in inducing regression of spontaneous mammary tumors in old female rats. These spontaneous tumors appear in the postreproductive phase of life and, unlike the carcinogen-induced mammary adenocarcinomas, they are usually benign fibroadenomas and occur as a single tumor per rat[4].

Stähelin *et al.* (149) tested the effect of 2-bromo-α-ergokryptine on the 7,12-dimethylbenz[*a*]anthracene-induced rat tumors by treatment at the time of tumor induction. The previous studies reported treatment after the tumors had reached a certain size. In this study, injections of 6 mg./kg. of 2-bromo-α-ergokryptine were given daily (except Sunday), beginning on the day of the second 7,12-dimethylbenz[*a*]anthracene application. The experiment was extended for 243 days, with the result that mean tumor size in the 2-bromo-α-ergokryptine-treated group was less than 50% of that of the 7,12-dimethylbenz[*a*]anthracene control up to at least 119 days. Tumor inhibition was minimal at a dose of 1 mg./kg./day for 80 days. However, at the higher dose, 2-bromo-α-ergokryptine clearly delayed tumor appearance and increased survival time in addition to reducing tumor growth. When treatment was terminated after 80 or 100 days, tumor growth was initiated as would be expected on the basis of the hypothesis that 2-bromo-α-ergokryptine depresses mammary tumor growth by reversibly depressing prolactin secretion. These workers also reported that 2-bromo-α-ergokryptine had no effect on sarcoma 137 (a hormone-nondependent transplantable tumor in the mouse), had no cytotoxic effect in cell cultures, and did not inhibit multiplication of P-815 mastocytoma cells *in vitro*.

In a recent paper, Clemens and Shaar (21) reported a similar study involving the effects of ergocornine on

[4] Both papers (20, 22) frequently refer to "ergokryptine," one of them even in the title. However, it is apparent that the compound actually used was 2-bromo-α-ergokryptine.

7,12-dimethylbenz[*a*]anthracene-induced mammary tumors as a function of tumor size. In this study, rats were treated with ergocornine before and after mammary tumor appearance. Treatment of rats with ergocornine methanesulfonate and ergocornine hydrogen maleate in corn oil at a dose of 0.4 mg. daily significantly inhibited the induction of mammary tumors in rats treated with 7,12-dimethylbenz[*a*]anthracene. Ergocornine administration after tumor appearance caused a consistent and rapid regression of tumors of a size up to about 1.8 $cm.^3$ to the point that they disappeared to palpation. This incidence of complete tumor regression was not obtained in other studies and is significant. The authors suggested that the more rapid and complete regression (62% of the established tumors) in this study was perhaps due to the mode of administration of the compound (suspension in corn oil *versus* saline plus alcohol or aqueous dimethyl sulfoxide). Tumors approximately 14.1 $cm.^3$ and over did not respond to the treatment, suggesting that as the tumors grow larger they lose their hormone dependence—a very important factor to consider in planning the optimum therapeutic usage of these compounds.

Quadri *et al.* (150) also studied the effect of ergocornine, ergonovine, and 2-bromo-α-ergokryptine on rat prolactin and growth hormone-secreting pituitary (MtW15) tumors. Daily injections of ergocornine (0.1 and 0.2 mg.) or ergonovine (0.2 mg.) for 3 weeks induced significant regression or inhibition of tumor growth. Ergokryptine (0.3 mg.) had no significant effect on this tumor. At the end of the 3 weeks, tumors in the control animals showed a 33.6% gain in average diameter, while those in rats injected with 0.1 and 0.2 mg. ergocornine showed a 14.8 and 30.6% loss in average diameter, respectively. Ergonovine was less effective at the same dose but did reduce tumor growth.

To date, only one clinical trial has been reported (151). In this study, 2-bromo-α-ergokryptine was given orally in dosages of 6 mg./day for 2 days, 9 mg./day for the next 2 days, 12 mg./day for the next 2 days, and 15 mg./day for an additional 36 days. Nineteen patients with advanced breast cancer were treated and no objective remission was observed.

NIDATION INHIBITION BY ERGOT ALKALOIDS

In the course of studies on the mechanism of egg implantation, Shelesnyak (152–154) examined the effect of various drugs on deciduoma formation in the uterus of pseudopregnant rats. The formation of deciduoma, an artificial maternal placenta, is a convenient model system for the exploration of the processes of ova implantation and decidua initiation. It has two essential requirements: a proper hormonal sensitization of the uterus, *i.e.*, a specific estrogen–progesterone ratio, and a "nonspecific" stimulation (traumatization) of the endometrium, which may be brought about by mechanical, electrical, or chemical means. Experimentally, pseudopregnancy is induced in adult rats by electrical stimulation of the cervix, followed on the 4th day of the diestrual phase by chemical (histamine) or mechanical (clamping, scratching) traumatization of one or both horns of the uterus. The weight of deciduomata is determined 96 hr. after uterine stimulation (*cf.*, 81). Using this system, Shelesnyak (152) observed that while a variety of compounds inhibited deciduoma formation when instilled into the uterus, only one compound, ergotoxine, was effective when administered parenterally (16). A single subcutaneous dose of 0.25 mg. ergotoxine at the time of uterine traumatization effectively inhibited the development of deciduomata. This observation was the starting point for extensive investigations by Shelesnyak's group and by others into the effects of ergot alkaloids on reproductive physiology.

It was found that the inhibition of deciduoma formation by ergotoxine could be reversed with progesterone (16). However, it was concluded that ergotoxine does not act as an antagonist or competitor of progesterone, since the minimum effective dose of the latter was independent of the ergotoxine concentration and since progesterone did not protect against the toxic effects of the alkaloid (158). Rather, the inhibition of decidual growth is the result of a disturbance in the hormonal balance (estrogen–progesterone) caused by the alkaloid (154). The same paper reported the finding that single injections of ergotoxine into pregnant rats, in a dose of 0.5 mg./animal or higher during the first 6 days postcoitus, terminated pregnancy in all animals with reappearance of the estrus cycle. Injections of 3 mg./animal on Days 7 and 8 postcoitus terminated pregnancy in six out of seven and one out of seven cases, respectively, and were ineffective on later days. In all cases where pregnancy had not been terminated, the animals delivered normal litters. A dose of 0.25 mg. injected on Day 5 was effective in 17 out of 20 cases, whereas 0.1 mg. was ineffective. The alkaloid was also found to terminate pseudopregnancy with reestablishment of the normal cycle (154). The basic finding that ergot derivatives can terminate early pregnancy was subsequently confirmed by a number of laboratories using both rats (82, 84, 92, 155, 156) and mice (17, 157) as experimental animals.

The effectiveness of ergotoxine and ergocornine as fertility-control agents was tested on a larger scale in rats. In these trials, as a result of 1303 coital contacts only 23 pregnancies occurred. The fertility index was decreased to between 1.5 and 4% from a normal 96.5% in the same colony (158). As in the inhibition of decidual growth, the termination of early pregnancy by ergotoxine was reversed by progesterone, although a higher dose of the hormone was required and the effect was not complete (159). The treatment with ergot alkaloids had little or no effect on the subsequent fertility of the animals (157, 158). At this point it had become fairly clear that ergotoxine terminated early pregnancy by preventing nidation as a result of disturbing the hormonal balance rather than by any direct effect. However, the nature of the primary action of the alkaloid was completely unknown and Shelesnyak directed his attention to this question. He (160) showed that adrenalectomy did not interfere with the effect, indicating that the the action of ergotoxine was not mediated through the adrenal gland. Ergotoxine was found to have no estrogenic or gonadotropic activity (161), and a single injection of the alkaloid had no effect on the gonadotropin and prolactin content of the pituitaries of pregnant or

pseudopregnant rats (162). A limitation in the interpretation of the latter experiments is that the production of these hormones may have been influenced but, because of altered excretion into the system, this did not manifest itself in the hormone content of the gland (162). Injection of prolactin, on the other hand, counteracted the effect of ergotoxine and sustained pregnancy, indicating that the alkaloid did not interfere with the ability of the ovary to produce progesterone (163). These experiments also indicated that ergotoxine did not act by direct competition with prolactin (163).

After having thus ruled out many possible modes of action, Shelesnyak (81) speculated that ergocornine might affect the metabolism of progesterone. A preliminary study in women, in which the change in urinary levels of various steroids in response to ergocornine was measured, resulted in the observation that ingestion of a single dose of 2 mg. ergocornine in the immediate postovulatory phase markedly decreased urinary levels of pregnanediol and estrogens and increased those of ketosteroids and hydroxycorticosteroids (164). This finding, which was confirmed by Sterba (165), led to the more specific suggestion that the alkaloid might act by blocking 3β-hydroxysteroid dehydrogenase (164). However, later experimentation did not bear out this proposal. After administration of ergocornine to rats, the enzyme was still demonstrable histochemically (166, 167) and by assay in homogenates (168). In the ovaries of pseudopregnant rats, a drop of progesterone content following ergocornine injection was only observed after a latency period of 12 hr. and at the same time the 20α-hydroxypregn-4-en-3-one content increased. This change in the ratio of the two steroids is similar to that observed during normal estrus; it can account for the termination of the pregnancy but is clearly not the result of an inhibition of β-hydroxysteroid dehydrogenase (169).

Finally, an extended reinvestigation measuring the plasma and urinary levels of a number of steroids in normal and ergocornine-treated women failed to reproduce the dramatic decreases in urinary pregnanediol levels observed earlier and provided no evidence for a specific blockade of the Δ^5-3β-hydroxysteroid dehydrogenase system (170). The results of these studies (168–170) were, however, in accord with the emerging view that the action of the alkaloid was in some way mediated by the pituitary.

While Shelesnyak's early results seemed to rule out the pituitary as being involved in the effect of ergot alkaloids on ova implantation (162), a study by Zeilmaker and Carlsen (18) specifically pointed to this possibility. Their work with hypophysectomized rats carrying transplanted pituitaries suggested that ergocornine administration leads to a temporary inhibition of prolactin release by the pituitary, which brings about irreversible changes in the corpus luteum. As a result the progesterone level decreases, preventing the development of decidual tissues and leading to the onset of estrus. Their paper also quotes experimental evidence to the effect that the change in the pituitary function is not the indirect result of an influence of the alkaloid on the hypothalamus. This picture of the sequence of events has, for the most part, stood the test of time. Lobel *et al.* (167) observed that the corpora lutea showed necrosis and dissolution of cells 24 hr. after ergocornine administration, and Varavudhi *et al.* (171) confirmed the results with hypophysectomized rats carrying autotransplanted pituitaries. Further experiments indicated that ergocornine did not interrupt gestation in the presence of an extra source of luteotropic activity, *e.g.*, in the presence of fetal placenta (172) or the pseudopregnancy induced by ectopic trophoblast tissue (173). Finally, it was found that treatment of rats with ergocornine induced 20α-hydroxysteroid dehydrogenase, giving rise in the corpora lutea of pregnancy to a 100-fold increase in enzyme activity, and that this action was prevented by exogenous prolactin (174). The enzyme 20α-hydroxysteroid dehydrogenase appears in the late diestrus in newly formed normal corpora lutea but is absent in the corpora lutea of pregnancy or pseudopregnancy. Ergocornine acts to increase 20α-hydroxysteroid dehydrogenase activity when given during the first days of pregnancy when the corpora lutea are mainly under the control of the pituitary but not at later stages when the chorionic secretion of luteotropic activity plays a significant role (175). This indicates, as does other evidence (176–178), that the alkaloids of ergot inhibit release of luteotropic activity from the pituitary but not from the placenta and that the interruption of early pregnancy is most likely mediated through the pituitary.

These experiments indicate in which manner prolactin influences progesterone levels and how the drug-induced prolactin deprivation irreversibly leads to a change in the luteal cell which results in decreased progesterone secretion. While these subsequent results thus largely confirm the later steps in the sequence of events as postulated by Zeilmaker and Carlsen (18), their suggestion that the first step is a direct action of ergocornine on the pituitary to inhibit prolactin release has been repeatedly challenged. Experiments in which ergocornine was placed directly on the uterus, the ovaries, or the pituitary showed that the drug was inactive under these conditions (179), leading to the consideration that the effect of the alkaloid on the pituitary might be mediated by the hypothalamus.

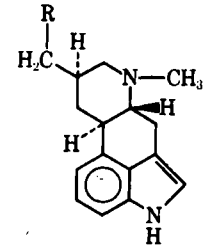
Later publications also discussed the possibility that the alkaloid acts by way of the CNS (169, 171, 180). Seda *et al.* (181*a*), in a recent study in rats using the nidation-inhibiting synthetic ergot derivative 6-methyl-8-cyanomethylergoline, noted an increase in gonadotropic [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)] secretion in addition to inhibition of prolactin release. This is supported by observations that lactation-inhibiting ergot alkaloids restore the estrus cycle or normalize it in the case of disturbances both in animals (181*b*) and in humans (101*b*). Seda *et al.* (181*a*) felt that this effect on these three hormones could hardly be explained by a direct action of the alkaloid on the pituitary but would more logically seem to be the result of a stimulation of the hypothalamus to release LH- and FSH-releasing and prolactin-inhibiting factors. Since the nidation inhibition by ergot alkaloids is overcome by prolactin alone (163, 178, 182), the effect on the release of this hormone seems to be at least the main mode of action. Therefore, the question comes down to the problem, which has been already discussed,

whether ergot alkaloids inhibit prolactin release directly at the level of the hypophysis or *via* the hypothalamus, or both.

Whether nidation inhibition by ergot alkaloids is solely due to their effect on pituitary prolactin release is not entirely clear. There seems to be a reasonable empirical correlation between lactation inhibition and nidation inhibition, in the sense that all ergot alkaloids which are active as nidation inhibitors also seem to inhibit lactation or prolactin release. However, a detailed study comparing dose–response curves of two compounds, ergokryptine and 2-bromo-α-ergokryptine, for nidation and lactation inhibition in rats raised doubt as to whether both responses depend entirely on the same primary effect (82). While the ED_{50} of 2-bromo-α-ergokryptine for nidation inhibition (0.75 mg./kg. s.c.) is smaller than that of the parent compound (1.15 mg./kg.), the ED_{50} for lactation inhibition of the halogenated derivative (3.5 mg./kg.) is larger than that of the parent compound (0.7 mg./kg.). As would be expected for a common mechanism, in the case of ergocornine and ergokryptine the dose–response curves for nidation and lactation inhibition converge toward one dose which completely inhibits both responses. However, this is not the case with 2-bromo-α-ergokryptine and, therefore, at least for this alkaloid the assumption of a common primary effect does not seem to be valid (82). As mentioned earlier, this conclusion was criticized by Nicoll *et al.* (83), who pointed out that a difference between these two drugs in their ability to depress oxytocin release could account for a difference in their potency as lactation inhibitors; therefore, the result would not necessarily disprove that nidation inhibition is due entirely to the inhibition of prolactin release. Furthermore, as mentioned earlier, differences in the pharmacokinetic properties could account for this result.

Only a limited amount of information is found in the literature on the relationship between the structure of ergot alkaloids and their activity as nidation inhibitors. Most of this information relates to the peptide-type ergot alkaloids. In early studies, Shelesnyak (81) found little activity for ergotamine (VI*a*) and its 9,10-dihydro derivative, for ergonovine (V*d*) and methylergonovine (lysergic acid butanolamide), for tochergamine, a synthetic analog belonging to the ergotamine group, and for ergocristine (VI*e*) and its 9,10-dihydro derivative. The active components of the ergotoxine complex were found to be ergocornine (VI*k*) and ergokryptine (VI*g*), of which the latter was more active. Their 9,10-dihydro derivatives were less active than the parent compounds. Dose–response curves established in later work indicated an ED_{50} of 335 mcg./rat (subcutaneous, average body weight 167 ± 7.4 g.) for ergocornine (183, 184), compared to 175 mcg. for ergokryptine, and values of 505 and 945 mcg. for ergosine (VI*c*) and ergovaline (VI*o*), respectively, two new alkaloids found to be active (184). Another newly tested alkaloid, ergostine (VI*m*), had only weak activity (184). As already mentioned, substitution of ergokryptine in the 2-position with bromine leads to a compound with somewhat lower ED_{50} for nidation inhibition (82). The main advantage of this substitution is a marked decrease in toxicity.

More recent work has shown that the peptide portion of the alkaloids is not required for activity. The groups of Semonsky *et al.* (92) and of Rezabek *et al.* (156) prepared D-6-methyl-8-cyanomethylergoline (VIII*a*) and a series of amides of D-6-methylergoline-8-acetic acid (VIII*b*) and found these to be very effective nidation as well as lactation inhibitors. Compound VIII*a* inhibits nidation when given orally to rats in a single dose of 10 mg./kg. or in five daily doses of 1 mg./kg. during the first 7 days of pregnancy (92), as does apparently its $\Delta^{9,10}$-unsaturated analog (five times 5 mg./kg.) (185). This compares to an LD_{50} of 110 mg./kg. p.o. for VIII*a*. The figures are particularly impressive for the amide of 6-methylergoline-8-acetic acid (VIII*c*), which interrupted pregnancy in all animals when given in a single oral dose of 5 mg./kg. at any time between the 1st and 7th day of pregnancy or as a single oral dose of 0.4 mg./kg. on Day 6 or 7. This compound has an acute LD_{50} of 93 mg./kg. given intravenously and about 1 g./kg. upon oral administration (92). Very recently the same group reported additional structure–activity relationships (181*b*). Lysergic acid α-hydroxyethylamide (V*c*) was tested in mice and found to terminate pregnancy in all cases in a daily dose of 250 mcg./animal given orally (157). Finally, it was found that not only lysergic acid derivatives possess this activity but also clavine-type alkaloids. Agroclavine (VII*a*), a known lactation inhibitor (71), also inhibited nidation effectively in mice (250 mcg. daily orally during Days 3 and 4 or 5 and 6 of pregnancy) (157) and rats (1.5–2.5 mg. daily orally on Days 1–5 of pregnancy) (84, 182). As in the case of ergotoxine, the nidation inhibition by simpler ergolines was overcome by simultaneously administering progesterone (84) or prolactin (92, 178, 182). Interestingly, while the ergotoxine-type alkaloids are active both orally and when injected systemically (158), agroclavine is completely inactive when injected intraperitoneally (84) or subcutaneously (157). The question has been examined whether the ergot alkaloids themselves are the active entities or whether they require some kind of metabolic activation. In this study (186), ergocornine was perfused through a liver *in vitro* before administering it to rats or was directly introduced into the liver *in vivo* through the portal system. The results did not indicate any activation but strongly suggested metabolic inactivation of the alkaloid in the liver.



VIII*a*: R = CN
VIII*b*: R = $CONR_1R_2$
VIII*c*: R = $CONH_2$

Some consideration has been given to the question whether ergot alkaloids might have a teratogenic effect. Several studies on nidation inhibition in rats or mice reported that in the cases where pregnancies had not been terminated as a result of administering ergot alkaloids, normal litters were born (17, 154, 156). It was also shown that ergocornine in doses which are

effective in inhibiting decidualization and nidation does not have a direct toxic effect on the blastocyst of pregnant rats (187). However, at least one study reported very pronounced effects of ergocornine on fetal morphology (176). A dose of 1 mg. of ergocornine given to rats on the 8th day of pregnancy, while no longer effective in terminating pregnancy, caused a very significant increase in the number of fetal malformations. No such effect was seen when the same dose was given on the 12th day, and the detrimental effect on the 8th day could be averted by simultaneously administering progesterone. The authors concluded that the teratogenic effect of ergocornine was a result of the progesterone deficiency it induced.

It is evident from the above discussion that ergot alkaloids have been quite instrumental in the studies on the mechanism of nidation. Likewise, they have been and continue to be useful tools in the study of the complex hormonal relationships during the estrus cycle. It has already been mentioned that the termination of pregnancy or pseudopregnancy by ergotoxine or ergocornine induced the appearance of estrus and usually resulted in a new ovulation within 3–4 days of treatment with the alkaloid (154, 188, 189). While ergot alkaloids can thus stimulate ovulation, Kraicer and Strauss (190) reported that ergocornine can also act as an ovulation blocker under certain circumstances. A single dose of 1 mg./rat inhibits ovulation when given during the time period of 50–10 hr. before normal ovulation, with a maximum of effectiveness when given during proestrus at about 14 hr. before ovulation. The mechanism of this ovulation inhibition is not really understood, and in a more recent study by another group, the investigators did not observe an ovulation block by ergocornine (191). The discrepancy may, however, be due simply to differences in methodology. In other studies, ergot alkaloids were instrumental in securing evidence that prolactin, in addition to its luteotropic activity during early pregnancy, also has a luteolytic function during the estrus cycle in rats (192–194). Blocking the secretion of prolactin during the estrus cycle with ergocornine (193) or 2-bromo-α-ergokryptine (194) led to the accumulation of corpora lutea which failed to undergo normal involution. From this it was suggested that the normal surge of blood prolactin on the day of proestrus serves to induce luteolysis of the previous crop of corpora lutea formed during each cycle.

The crucial question in evaluating the potential of ergot alkaloids as fertility control agents is, of course, whether the effective nidation inhibition observed in rats and mice also holds true in other animals and in man. Unfortunately, there is only a very limited amount of information on this point in the literature. As discussed earlier, there is no doubt that the inhibition of pituitary prolactin release is a general phenomenon. However, the question is whether in other animals and in man prolactin plays a similar essential role in the process of ova implantation as it does in rats. Two studies have indicated that ergocornine is not effective in inhibiting implantation in rabbits (195) and guinea pigs (196). However, a more recent investigation in sows reported that doses of 125–250 mg. of ergocornine can terminate pseudopregnancy in swine with regression of the corpora lutea and reappearance of estrus (197). The limited studies in women (164, 165, 170, 198, 199) have so far not produced entirely conclusive results. All the studies agree that ergocornine has no effect on the basal body temperature curve. One study (170), in contrast to earlier results from the same group (164), found no significant changes in serum progesterone levels or urinary pregnanediol excretion, whereas other groups observed at least some decrease in urinary pregnanediol levels following ergocornine (165, 198, 199). In only one out of five women treated during early pregnancy did spontaneous abortion occur after oral administration of 10 mg. of ergocornine (198). It is apparent that this question cannot yet be considered settled.

FUTURE DEVELOPMENTS

It is probably too early to judge whether and to what extent the effect of ergot alkaloids on pituitary hormone release will turn out to be clinically useful. Ergot alkaloids, by virtue of this activity, have been and continue to be very useful tools in the study of prolactin-dependent physiological functions. Beyond this, Rezabek *et al.* (200), in a recent discussion of their work, suggested the following clinical applications in which ergot alkaloids might be utilized:

1. For decreasing milk production in puerperal mastitis or in painful milk retention and breast swelling.
2. For decreasing the prolactin secretion in the treatment of breast tumors, mastopathy, premenstrual mastodynia, and galactorrhoea.
3. For shortening or interrupting the luteal phase of the menstrual cycle.
4. For promoting follicle-stimulating hormone and luteinizing hormone secretion and for induction of ovulation.
5. In exceptional cases for the suppression of the initial phase of pregnancy.

One might add to the list the possibility of preventive treatment of breast cancer in high risk women, for example in the form of slow-release implants.

In addition, the same authors consider a number of potential applications in zootechnical practice:

1. For the induction and synchronization of estrus.
2. For suppression of lactation and for provocation of estrus and ovulation to obtain a higher frequency of litters.
3. For suppression of undesired pregnancies, *e.g.*, in bitches.
4. For suppression of clucking in the fowl and for stimulation of egg laying.

Obviously, the exploration of the practical and clinical usefulness of the pituitary effects of ergot alkaloids has only just begun and most of these potential applications still remain to be investigated in detail. At the moment the most promising compounds for clinical applications seem to be 2-bromo-α-ergokryptine, D-6-methyl-8-cyanomethylergoline-I, and D-6-methyl-ergolin-I-yl-8-acetamide (Compounds 6605 VUFB and 6683 VUFB of Semonsky). However, there is certainly room for further developments in this area.

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RESEARCH ARTICLES

Pharmacology of Mono- and Disubstituted Chlorpromazine Metabolites

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Abstract □ Chlorpromazine and 10 of its mono- and disubstituted metabolites were investigated for their effects on the CNS of male rats and mice. All of the compounds decreased motor activity and respiration, and all but two of the compounds decreased heart rate in rats. The most potent metabolite in depressing spontaneous motor activity of mice was 3,7-dihydroxychlorpromazine, with an ED_{50} of 5.1 mg./kg. i.p. in comparison to chlorpromazine which had an ED_{50} of 2.0 mg./kg. i.p. However, the 3,7-dihydroxy derivative was approximately twice as toxic as chlorpromazine in mice. 7,8-Dihydroxychlorpromazine did not alter forced motor activity, but it did induce a dose-related depression of spontaneous motor activity; 7-hydroxy-8-methoxy derivative also produced a marked decrease in spontaneous motor activity with minimal effects on forced motor activity. None of the compounds demonstrated anticonvulsant activity, and only 7-hydroxy-chlorpromazine elicited effects suggesting possible antidepressant activity. Barbiturate sleeping time was potentiated by all of the compounds, mainly due to their CNS depressant properties.

Keyphrases □ Chlorpromazine and 10 mono- and disubstituted metabolites—effects on CNS of rats and mice □ CNS depression—effects of chlorpromazine and 10 mono- and disubstituted metabolites, rats, mice □ Motor activity, spontaneous and forced—effects of chlorpromazine and disubstituted metabolites compared, rats, mice

Fishman and Goldenberg (1) identified several metabolites of promazine and chlorpromazine in human urine including 3-hydroxyphenothiazine and 7-hydroxychlorpromazine. These and other monohydroxylated and methoxylated derivatives have been investigated and found to be pharmacologically active (2–7); it also