CHAPTER II
I. The Mammary Gland

a) Structure of the mammary gland

i) Gross morphology

The work on the developmental aspects of the murine mammary gland was initiated by the turn of this century by Mayers (1919) and is still continuing. The in vivo morphogenesis of the prenatal mammary gland has been studied by a number of researchers (Turner and Gomez, 1933; Balinsky, 1950; Raynaud, 1961 and Hogg et al., 1983).

The mammary gland originates from a thickening of ectoderm which invades the underlying mesenchyme. Mammary parenchyma develops from this mass of epidermal epithelial cells to form a branching system of ducts which is present even in neonatal animals (Kratochwil, 1969; Anderson, 1978). Growth of the neonatal mammary gland from birth until puberty is isometric and allometric growth begins earlier than outward signs of puberty (Cowie, 1949; Sinha and Tucker, 1966).

The postnatal development of the mouse as well as rat mammary gland is characterized by the growth of a system of branching ducts in the mammary fat pad. Extension of the mammary ductal tree and the generation of its branching pattern occurs in three main ways. First, by linear lengthening of existing ducts, secondly by dichotomous branching of the
growing ductal tips and thirdly by the monopodial branching produced by the growth of collateral buds situated at the sides of existing buds (Cole, 1933; Turner and Gomez, 1933; Russo and Russo, 1978). The ductal tips called end buds are large and club shaped in the immature rat and mouse (Russo et al., 1982; Williams and Daniel, 1983). The ductal system develops under the influence of sex hormones. The most rapid ductal expansion has been observed between 4 to 7 weeks of age and is a result of intense mitotic activity in the end buds (Bresciani, 1965; Russo and Russo, 1978; Russo et al., 1982; Williams and Daniel, 1983). Williams and Daniel (1983) have shown that the end buds penetrate the fat pad at a rate of 0.5 mm/day. Adjacent ducts do not come closer than 0.25 mm to each other and preferentially grow into unoccupied fat pad region.

The end buds vary from 0.1 to 0.8 mm in prepuberal mice (Williams and Daniel, 1983) and from 0.4 to 1.7 mm in rat (Ormerod and Rudland, 1984). These end buds have been shown to be highly responsive to local tissue environment. As in embryonic development, they act in a variety of developmental phenomena as, epithelial stromal interactions, the penetration of one tissue by another, histogenesis, cytodifferentiation and the emergence of organotypic patterns through the regulation of turning, branching and growth rate (Faulkins and Deome, 1960; Russo and Russo, 1978; Williams and Daniel, 1983).

At the tip of the end bud is present a layer of undifferentiated and continuously proliferating cells called cap
cells (Daniel, 1975). Williams and Daniel (1983) have hypothesized that, since the cap cells are separated from stroma only by a basement membrane, the end bud response to chemical and physical cues may involve modulation of cap cell activities which would ultimately effect the direction, pattern and rate of growth.

ii) Cell types in the mammary gland

It was in 1972, that Radner started investigation of the cellular composition of the mammary parenchyma of rats of different ages. Bennet et al. (1978) and Rudland et al. (1980) postulated the existence of pluripotent progenitor cells in both embryonic and end bud tissues of normal gland. More recently thorough investigations have been conducted to identify different cell types in the in vivo mammary gland of mice (Williams and Daniel, 1983; Hilgers and Sonnenberg, 1985; Sonnenberg et al., 1986) and rat (Dulbecco et al., 1983; Ormerod and Rudland, 1984; Dulbecco et al., 1986) using morphological characteristics and immunological markers. Sonnenberg et al. (1986) have indicated that pathways of mammary differentiation for mouse are similar to those found in the rat.

The development of the mammary tree has been understood to be complex and involves a number of cell types. Dulbecco et al. (1986) and Sonnenberg et al. (1986) have ordered the various cell types in a developmental pathway based on the
marker distribution of various cell types. Basically three types of epithelial cells have been recognized.

1. Stem cells
2. Myoepithelial cells
3. Luminal cells

Stem cells have been reported to be present in the embryonic mammary gland and in the end buds of the mature gland. They are considered to be pluripotent cells which differentiate into myoepithelial and luminal cells (Williams and Daniel, 1983) lining the ducts. Though Dulbecco et al. (1983) could not find evidence for connection between myoepithelial and luminal cells, Ormerod and Rudland (1984) on the basis of morphological characteristics and Dulbecco et al. (1986) with the help of immunological markers have found intermediate type of cells. They have body in the luminal layer and appendages reaching the basement membrane. Dulbecco et al. (1986) have further suggested that myoepithelial layer may constitute an evolving system containing mammary stem cells, precursors of alveoli and mature myoepithelial cells so it should be called basal layer. A scheme for the evolution of cells types has been proposed by Dulbecco et al. (1986).

The mammary mesenchyme supports and surrounds the mammary parenchyma. Its role in the morphogenesis has been described (Grobstein, 1967; Krtochwil, 1969; Sakakura et al., 1976). Even the adult mammary cells have been shown to be stimulated
Scheme for the evolution of epithelial cell types during mammary development of rat (Dulbecco et al., 1986).
to grow after transplantation of embryonic mesenchyme into the mammary gland (Sakakura et al., 1979). Two types of mesenchymes have been identified by Sakakura et al. (1982) in the mouse mammary gland. One, made up of fibroblastic cells called fibroblastic mammary mesenchyme is described to be closely connected with mammary epithelial cells. The other is made up of fat pad precursor cells. In the embryo it generally increases in size and immediately after birth the whole area of fat pad precursor cells gets converted into typical adipose tissue. Fat pad is associated with the maintenance of the characteristic morphology of the mammary gland and the promotion of the mammary epithelial growth (Faulkin and Deome, 1960; Levine and Stockdale, 1984). Dulbecco et al. (1982) have suggested that local factors elaborated by mammary stroma, particularly adipocytes influence the growth of the mammary epithelial terminal end buds. Enami et al. (1983) have proposed the presence of a growth factor(s) produced by the stromal cells of mammary gland which plays an important role in the control of growth of normal and neoplastic mammary epithelial cells in vivo as well as in vitro.

b) Regulation of growth and development of mammary gland

i) Hormonal control

According to the definition given by Squartini (1983) the breast develops as a result of hormonal stimuli it
receives. In relation to puberty, sexual cycles, pregnancy and lactation this end organ of the endocrine system converts the hormonal stimuli into morphological structures and thus changes continuously its size, the gross and histologic structures, the amount and type of function.

Various studies have revealed that the prenatal as well as postnatal mammary gland responds to essentially the same group of hormones of the hypothalamo pituitary-ovarian axis (Nandi, 1959; Rivera, 1971; Mehta and Banerjee, 1975; Banerjee, 1976; Cowie et al., 1980).

It has long been known that the pituitary gland is necessary for mammary growth in rodents (Reece et al., 1936; Nathanson et al., 1939; Gardner, 1940; Nandi, 1958). These studies with hypophysectomized rats have shown that estrogen and progesterone fail to induce a growth response in the mammary gland in the absence of pituitary. Kleinberg et al. (1985) have described the pituitary to be absolutely essential for the normal mammary development of primates. In a classic study Lyons et al. (1958) have demonstrated the hormonal complement required by the rat mammary gland. Using triply operated (Ovariectomized-adrenalectomized-hypophysectomized) rats they have demonstrated that the animals required replacement of estrogen, progesterone, glucocorticoids, growth hormone and prolactin to stimulate the lobuloalveolar development attending the naturally occurring differentiation during pregnancy and lactation.
Hormonal actions are generally considered to be dependent on each other. Nandi (1959) has demonstrated in case of mice, the synergistic action of prolactin and growth hormone with ovarian steroids to stimulate mammary growth. Cell proliferation leading to alveolar growth in the mammary parenchyma in vivo is known to be stimulated by the synergistic action of prolactin and/or growth hormone, ovarian steroids and presumably insulin (Banerjee and Rogers, 1971; Anderson, 1974; Topper and Freeman, 1980; Topper et al., 1984; Schams et al., 1984; Nagasawa et al., 1986). Effects of progesterone on mammary tumorigenesis in relation to estrogen and prolactin have been studied in mice (Nagasawa et al., 1987) and in cultured breast cells of rats (Manni et al., 1987).

Cell differentiation involves the successive and synergistic action of estrogen and progesterone. Estrogen has been shown to be the hormone initially responsible for the differentiation and development of the ductal epithelium increasing mitotic activity and progesterone acts synergistically with estrogens on the distal part of the duct, favoring lobular development (Jacobsohn, 1961; Mauvais-Jarvis et al., 1986). Estrogen induction of the progesterone receptor synthesis provides a basis for the priming effect of estrogen in the preparation of target tissue for subsequent progesterone response (Leavitt et al., 1974; Leavitt et al., 1978). Estrogen and progesterone when secreted in an adequate balance permit the complete and proper development of the mammary gland.
Lyons and McGinty (1941) and Cowie et al. (1952) in castrated goats and Bassler (1970) in castrated rats have observed that estrogen given alone or in overdose in an estrogen/progesterone preparation results in cysts formation and overgrowth of mammary epithelium. In contrast when estrogen is administered with progesterone in a proper ratio, complete and proper development takes place. Biochemically the antiestrogenic activity of progesterone has been shown by the reduction of estrogen secretion in systemic circulation, inactivation of estradiol by metabolism at the target tissue and a lowering of estrogen receptors in these tissues and a direct effect on cell multiplication (Leavitt et al., 1978; Mester and Saulieu, 1984). Both progesterone and progestin have been shown to prevent the stimulation of mammary cell growth in culture media (Mauvais-Jarvis, 1986).

Progesterone participates mainly in the development of the lobulo alveolar system of normal mammary gland (Cowie et al., 1980), but, both estrogen and progesterone are essential for the manifestation of its effects. Further it has been demonstrated that treatment of nulliparous intact ewes with a combination of estradiol and progesterone is able to stimulate mammary development only when prolactin is present (Fulkerson and McDowell, 1974; Fulkerson et al., 1975; Schams et al., 1984). Suppression of prolactin in normal mammary gland induces marked regression and during the period of steroid treatment inhibits any stimulatory effect (Welsch
and Nagasawa, 1977; Nagasawa, 1982). The importance of prolactin for the formation and function of alveoli has been stressed in different species (Hooley et al., 1978; Hart and Morant, 1980; Nagasawa et al., 1985).

Indirect influence of prolactin is exerted through its effect on ovary. There is evidence that prolactin inhibits ovarian estrogen biosynthesis (Wang et al., 1980; Dorrington and Gore-Langton, 1981; Wang and Chan, 1982) and stimulates progesterone secretion (Nagasawa et al., 1985; Ota, 1986). The evidence for a direct mitogenic effect of prolactin on mouse breast tissue has been given by Nagasawa et al. (1985). But, Kleinberg and Newman (1986) have reported that prolactin is not essential for primate mammary development. In their experiments, chronic prolactin suppression did not prevent estradiol induced mammary development of monkey.

Estrogens exert a biophasic action. Its direct effect has been observed by increasing susceptibility of mammary epithelial cells, which enhances their susceptibility to the action of prolactin (Delowis et al., 1980; Nagasawa et al., 1986). However, high levels of estrogen decrease susceptibility which is counteracted by prolactin (Nagasawa and Yanai, 1972). Indirect effect is by triggering the release of prolactin from pituitary gland irrespective of the amount, by acting on both the hypothalamus and the pituitary (Chen and Meites, 1970; Schams and Karg, 1972; Nagasawa et al., 1986). Shyamala and Ferenezy (1984) by studying the effect
of estrogen on mammary fat pad have suggested that estrogentic regulation of mammary epithelium may be under the influence of mammary adipose tissue and connective tissue. They have found in castrated virgin mice that estrogen can augment the rate of DNA synthesis in both the mammary fat pad and mammary epithelium and response may be initiated earlier in the fat pad than in epithelium. In an earlier study (Haslam and Shyamala, 1981) have found estrogen receptors in the mammary fat pad which is devoid of epithelium.

Insulin is an important hormone required for the terminal differentiation of the mammary gland (Topper et al., 1984). In mouse and rat, the virgin animals show very little biological response to this hormone, while the cells in the pregnant animals are responsive (Friedberg et al., 1970; Bolander, 1983). Responsiveness is retained throughout lactation, but the cells revert to an insulin resistant state after cessation of lactation (Oka et al., 1974). Mammary tissue isolated from the virgin animal is insulin resistant initially and acquires the capacity to respond to the hormone sometime after explantation (Topper et al., 1984). Administration of prolactin for a few days to virgin mice (Oka and Topper, 1972) induced precoxious sensitisation to the epithelium for insulin. The mechanism by which the cells acquire the ability to respond to the hormone is not properly understood. Bolander (1983) has put forth the view that progesterone and/or prolactin might play a role in determining the responsiveness.
It has not been possible to assign an independent function to the growth hormone either in rodents (Nagasawa et al., 1985) or in primates (Kleinberg and Newman, 1986). Other hormones like thyroid stimulating hormone might be showing their effects through a general role in body metabolism and not by their direct action on the mammary gland.

Another hormone which has been obtained from human decidue, Relaxin, has been shown to act synergistically with other mammotrophic hormones (especially estrogens) to cause growth of ducts and lobulation (Trenin, 1951; Wada and Turner, 1959; Harness and Anderson, 1975). The mitogenic action of relaxin has been shown to be on the undifferentiated stem cells (Bani et al., 1985; Bani et al., 1986) thus resulting in the neoproliferation of both luminal epithelial and myoepithelial cells. When administered alone, relaxin has been shown to cause hypertrophy and when in combination with estrogen it acts synergistically to increase the hyperplasia and thus contribute to increase the overall growth of the mammary adipose tissue.

ii) Mammary DNA content and synthesis

DNA content has been extensively used as a measure of mammary growth (Wada and Turner, 1959; Nagasawa et al., 1967; Yanai and Nagasawa, 1971; Anderson, 1976; Welsch et al., 1984; Nagasawa et al., 1986). During allometric growth of the mammary gland, DNA synthesis is more abundant in the
terminal end buds than in ductal cells and at 5-6 months of age in virgins, proliferation of the mammary epithelium stops (Bresciani, 1968). In the end buds DNA synthesis rises sharply to a peak and then declines whereas in ductal epithelium it rises slowly and continuously. In virgin mice the S period for DNA is about 20 hours, it is reduced to 8-9 hours during pregnancy and can be again increased to 21 hours if the pregnancy tissue is transplanted into virgin mice (Banerjee and Walker, 1967). Banerjee and Rogers (1971) observed that DNA synthesis in the mammary epithelium of young virgin mice is abolished (3-5) days after ovariectomy, but daily treatment of estrogen and progesterone given to ovariectomized adult virgins have been found to increase mammary DNA levels similar to those in 18-20 days pregnant rats (Moon et al., 1959) and reduce the S period to 6-8 hours (Bresciani, 1965). Further, they have also found that in male rats estrogen alone is only slightly stimulatory but treatment of gonadectomized rats with both ovarian steroids significantly raise mammary DNA. Similar results have been obtained from female mice (Welsch et al., 1985).

c) Modulatory effects on the mammary gland

Various compounds have their modifying influence on the mammary gland either by their direct effect and/or by disturbing the delicate hormonal milieu of the mammary gland (Carroll, 1975; Welsch and Aylsworth, 1983; Faulkin et al., 1986).
The composition of diet has been shown to effect the growth of the murine mammary gland (Crecedo et al., 1952; Miyamoto-Tiaven et al., 1981; Abraham et al., 1984). The growth of the mammary ducts is faster and form more secondary and tertiary ducts in mice fed diets containing unsaturated than those fed saturated oils. Abraham et al. (1984) have observed an age dependent effect of dietary fat. They have shown that a diet devoid of unsaturated fat given to immature mice slows down the ductal growth. Similarly, Knazek et al. (1980) and Welsch et al. (1985) have observed a depressed growth of mammae in the absence of fat. Welsch et al. (1985) have further observed that such atrophied mammae are incapable of responding morphologically to a hormonal mammogenic stimulus. While the normal fat diet seems to be essential for normal mammary development, the contribution of high fat content does not appear signifant (Welsch et al., 1985; Aylsworth et al., 1986; Cohen et al., 1986). Faulkin et al. (1986) have not found any effect of fat on the growth of fine ducts and alveoli in the mammary glands of pregnant mice.

High fat diets have been shown to promote tumor development (Carroll and Khor, 1971; King et al., 1979; Rogers and Wetsel, 1981; Aylsworth et al., 1984; Cohen et al., 1986). Carroll et al. (1982) and Cohen et al. (1986) have proposed at least two factors required for the fat effect to manifest itself. 1) High total fat intake, and 2) a yet to be determined threshold level of linoleic acid, which acts as a rate
limiting factor in mammary tumor development. Cohen et al. (1986) have also pointed out that high fat intake is a necessary but not sufficient condition for mammary tumor promotion by dietary fat and the type of fat is a key determinant of the fat effect.

In contrast to the effect of fats, retinoids have been shown to suppress the mammary ductal and alveolar development (Moon et al., 1979; Radcliffe and Moon, 1983; Aylsworth, 1986). Gandilhon et al. (1982) and Aylsworth et al. (1986) have observed inhibitory influence of retinyl acetate on the growth of DMBA-induced rat mammary carcinomas. A dose dependent response to retinyl acetate has been observed by Afanasev and Israfilova (1984). While at nontoxic doses vitamin A has stimulatory effect on body weight, mammary weight, RNA and total proteins the toxic doses inhibit the activity of glandular cells.

There have been fewer and conflicting reports on the effect of proteins on mammary gland. High protein diet has been shown to increase the tumor incidence relative to animals getting low protein diet (Tannenbaum and Silverstone, 1949; Nakagawa et al., 1974; Hawrylewicz et al., 1982), but Clinton et al. (1979) have not observed any effect of dietary proteins on tumorigenesis and relate the tumor incidence directly only to the fat content. Sanz et al. (1986) have observed direct and specific effect of proteins on the growth of mammary epithelium. The low protein diet has been shown to retard
the growth and differentiation of the ducts in immature and mature rats. Sexual maturity is delayed and there is decrease in serum estrogen, progesterone and prolactin activities (Pyska and Styczynski, 1979). Rats fed high protein diet have an earlier than normal sexual maturation.

II. Biological Activities of Plant Products Chosen for the Present Study

Numerous plant products have been used by human beings as part of their diet or as drugs over the centuries. A number of plants have been and are being reported for their pharmacologically active components and their medicinal uses (Chopra et al., 1956; Chopra et al., 1969; Wealth of India (1948-1956); Medicinal Plants of India, 1976).

Betel nut (Areca catechu)

The use of betel nut as a vermifuge, antihelminthic and with other drugs was known to the chinese as early as sixth century and the nut is still employed for these purposes.

About 10% of the world's population has been reported to indulge in the habit of betel chewing especially in the oriental countries (Fendell and Smith, 1970). The nut is not chewed alone and is usually taken along with other ingredients wrapped in Piper betel leaf forming the quid. However, a good number of studies have been carried out with the total
extracts of betel nut to investigate their possible role in the genesis of cancer and the earlier works on this have been reviewed by Arjungi (1976).

Ranadive et al. (1976) and Kapadia et al. (1978) observed that following subcutaneous administration of betel nut extract to the mice and rats, transplantable fibrosarcomas develop at the site of injection. Bhide et al. (1979) have reported the induction of tumors of gastrointestinal tract in 58% swiss mice and 25% in C17 mice by aqueous extract of betel nut. Shivapurkar et al. (1980) from their studies on mice have reported about the tumorigenic principles present in the betel nut. Rao (1984) has studied the short term and long term effects of betel nut on buccal pouch of hamster and observed both preneoplastic and neoplastic lesions in the long term treatment group. Sinha and Rao (1985) have found the teratogenic effects of aqueous extract of betel nut on the mouse fetuses reflected by stunted and abnormal fetuses and a dose related fetal weight reduction.

Garg and Garg (1971) have found oxytocic anti-fertility effect of various extracts of betel nut. It has been found to have effect on the cardiovascular system, isolated ileum and Uterus of mouse (Dhawan et al., 1980).

**Chemical composition of betel nut**

The betel nut chemically contains polyphenols, carbohydrates, fats and alkaloids. Mathew et al. (1963) have reported
the chemical composition of betel nut (composition of tender and ripe betel nuts is shown in Table 1).

Arecoline

Arecoline is a major alkaloid of betel nut. Many workers have studied the structure activity relationship of arecoline. It has been shown to be a muscarinic cholinergic agonist in both rat and mouse. Cholinergic stimulation has been shown to block the diurnal surge of plasma prolactin in ovariectomized, estrogen treated rats (Subramanian and Gala, 1976; 1977). Though the cholinergic system does not normally operate to inhibit prolactin release, but the inhibitory effect on prolactin release following the activation of cholinergic system and the existence of cholinergic receptors in the pituitary suggest its role under certain conditions. Subramanian and Gala (1977) have observed that atropine sulphate and atropine methylnitrate which are cholinergic blockers, are capable of blocking the inhibitory action of arecoline. Vale et al. (1976) have hypothesised the presence of cholinergic component in pituitary, as the cholinergic agonists inhibited the prolactin release from cultured pituitary cells and atropine could overcome this effect.

Arecoline has been reported to induce chromosomal aberrations as well as sister chromatid exchanges in the bone marrow cells of mice (Panigrahi and Rao, 1983). Arecoline has been
Table 1. Chemical composition of tender and ripened nuts.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Tender Nut (%)</th>
<th>Ripe nut (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extractives</td>
<td>75</td>
<td>20-30</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>40</td>
<td>11-18</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>0.0-0.1</td>
<td>0.20-0.24</td>
</tr>
<tr>
<td>Fat</td>
<td>1-4</td>
<td>10-15</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.5-6.7</td>
<td>decrease</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>1-2</td>
<td>15</td>
</tr>
<tr>
<td>Mineral matter, water soluble and water insoluble ash</td>
<td>1.1-3.8</td>
<td>decrease</td>
</tr>
</tbody>
</table>
Fig. 1. Structures of betel nut alkaloids.
seen to inhibit the synthesis of nucleic acids and proteins in mouse fetuses (Sinha, 1984, thesis). Sinha and Rao (1985) have demonstrated genotoxic effects of arecoline as indicated by the production of abnormal sperms and repair synthesis of damaged DNA.

Arecoline has been shown to facilitate memory in human and other animals under certain conditions (Sitaram et al., 1978; Bartus et al., 1980). It has also been shown to have behaviourally depressant effects (Pradham and Datta, 1970). Molinengo et al. (1986) have shown a dose dependent effect of arecoline in the central nervous system. Tolerance development to the behaviourally depressant effects of arecoline have been shown (Meltzer and Rosecrans, 1982; Overstreet and Jamal, 1986).

Arecoline has been shown to be a monofunctional alkylating agent in the biological systems (Boyland and Nery, 1969). It has binding reactions with nucleic acids and proteins (Nery, 1971). It gets metabolically converted into arecaidine in liver (Nieschulz and Schmersahl, 1967) but has been shown by Nery (1971) to be metabolically converted into a more reactive intermediate such as arecoline 1 oxide.

Caffeine

Caffeine (1,3,7-trimethylxanthine) is a plant alkaloid obtained from coffee and tea. It has a profound effect on a number of physiological and biochemical processes in
mammalian tissues (Abbott, 1986).

The oral administration of caffeine leads to 99% absorption within 15-45 minutes. In the rats, the half life of caffeine has been observed to be less than 3 hours (Burg and Werner, 1972), but is 3.0-7.5 hours in human subjects (Levy and Zylber-Katz, 1982). The major primary metabolite of caffeine has been shown to be paraxanthine while the minor primary metabolite is theobromine (Arnaud, 1984).

The acute ingestion of caffeine increases the metabolic rate, elevates the plasma free fatty acid level and slightly increases the cortisol levels (Abbott, 1986).

The studies on the neuroendocrine effects of caffeine (Spindel et al., 1980; 1983) have suggested that caffeine can influence the hormone secretion directly i.e. by increasing glandular adenosine, 3,5-monophosphate (cAMP) or indirectly by affecting brain neurotransmitters and subsequently hypothalamus releasing factor. It has been shown to inhibit thyroid stimulating hormone and growth hormone, but has little effect on serum prolactin level.

Caffeine has been reported to produce a significant degree of fetal loss and a decrease in birth weight in rats when given at low doses (Gilbert and Pistley, 1973; Dunlop and Court, 1981). Intraperitoneal administration of caffeine (20 mg/kg/day) during pregnancy in rat has been shown to be accompanied by a 20% reduction in litter size, with 8.7% decrease in fetal weight (Gilbert and Pistley, 1973). The
caffeine intake of the order of 10 mg/kg/day has been implicated in fetal loss or pregnancy complications by premature birth in human studies by Weathersbee et al. (1977). Nagasawa and Sakurai (1986) have not found any change in litter size and weight by chronic ingestion of caffeine, but they have shown a reduction in rearing rate. Zetterberg (1959) in Drosophila and Weathersbee et al. (1975) in hamsters have observed a change in sex ratio favouring the female offsprings.

Caffeine has been reported to influence neoplastic processes in experimental animals. In *in vitro* studies, caffeine has been shown to both inhibit (Kakunaga, 1975) and enhance (Donovan and Dipolo, 1974; Ledinko and Evans, 1973) neoplastic mammalian cell transformation by chemical carcinogens. Welsch et al. (1983) have reported that the administration of caffeine via drinking water to female rats after giving the carcinogen treatment results in a slight but significant dose related increase in mammary carcinoma incidence and pointed out that the caffeine influences the promoting but not the initiating phase of the carcinogenic process. Minton et al. (1983) have reported that the caffeine administered to rats fed on a high fat diet or a standard laboratory chow results in a significant decrease or increase respectively, in latency period of mammary carcinoma appearance induced by DMBA. In *in vivo*, the alkaloid has been shown to inhibit (Rothwell, 1974; Nomura, 1976; Theiss and Shinkin, 1978) as well as enhance (Hoshino and Tanooka, 1979;
Fig. 2. Structure of caffeine.

Fig. 3. Structure of vasicine.
Gurkalo and Zabezhinski, 1982; Denda et al., 1983) tumorigenesis in a variety of organ sites. Takayama and Kuwabara (1982) have studied the long term effect of caffeine in Wistar rats and did not find it carcinogenic.

Vasicine

Vasicine is an alkaloid obtained from plant Adhatoda vasica. Vasicine and its derivatives have been reported to have bronchiodilatory and oxytocic activities (Chopra, 1925; Atal, 1980 and Gupta et al., 1982).

Vasicine gets absorbed in various tissues. The half life of the compound varies (5-7 minutes - 2 hours) depending upon the route of administration. The highest concentration has been found in the uterus. Atal (1980) has reported that vasicine is excreted mainly through urine and in about 24 hours half of the vasicine gets eliminated. The major metabolites of vasicine are vasicinone, deoxyvasicine and other three unidentified products.

Atal (1980) has reported the effects of vasicine on the uteri from rats, mice, rabbits and hamsters under different conditions. He has used uteri at different stages of estrous cycle and also estrogen and progesterone dominated uteri and has shown marked dose dependent increase in spontaneous rhythmic movements. Vasicine has been shown to have abortifacient activity and this effect increased with the advancement of pregnancy. This has been related to the priming influence
of estrogens on the uterus which are produced more towards later stages of pregnancy (Vane, 1971). Atal (1980) has also shown the enhancement of abortifacient activity of vasicine after prior treatment with estradiol dipropionate (EDP). Naylor and Poyser (1975) have shown that estrogens enhance the synthesis of prostaglandins in the uterus so the abortifacient action of vasicine has been attributed to synthesis and/or release of prostaglandins (Atal, 1980). Lal and Sharma (1981) in a study on rat isolated uterus have not observed any release of prostaglandins with small doses of vasicine (less than 2.5 - 50 μg/ml) as prescribed by Gupta et al. (1977). So, they have hypothesised that the potentiation could be by the increase in affinity of prostaglandins for specific receptors.

Diosgenin

Diosgenin is a steroidal sapogenin obtained from plant Dioscorea. It is one of the most abundant plant sapogenins. It has been commercially used to synthesize pregnenolone and progesterone.

Diosgenin has been shown to affect the lipid metabolism (Cayen and Dvornik, 1978, 1979; Cayen et al., 1979). It has been shown to be a potent inhibitor of cholesterol absorption and lowers the serum cholesterol level in the rat. The decreased absorption of cholesterol in diosgenin treated rats is shown to result in increased hepatic cholesterol synthesis.
Fig. 4. Structure of diosgenin.
Diosgenin markedly increases neutral sterol excretion without enhancing the elimination of bile acids (Cayen et al., 1979; Uchida et al., 1984). Cayen et al. (1979) have further observed that diosgenin is poorly absorbed and the amount which is absorbed undergoes extensive biotransformation.

Diosgenin has been shown to be distributed in many organs namely, gastrointestinal tract, liver, kidney, cortex spleen, heart, brown fat, salivary glands, skeletal muscles and adrenals. The site of action of diosgenin is gastrointestinal tract and the level remains high in it even after 24 hours of treatment (Cayen et al., 1979).

Keelar et al. (1976) did not observe any teratogenic effect of diosgenin in rats.

Asparagus

Asparagus roots have been used to prepare a drug Shatawar which is considered to be antidiarrhoeic, antispasmodic, nerve tonic and galactogogue (Medicinal Plants of India, 1976). The powdered dried roots of Asparagus racemosus have been shown to possess sarsapogenin and sitosterol. The pharmacologically active saponins have been shown to be anti oxytocic (Gaitonde and Jetmalani, 1969). The crude alcoholic extract of the roots has been shown to increase the weight of mammary glands in post-partum and estrogen-primed rats and the uterine weight in estrogen-primed group. The increase in the weight of adrenals coupled with the depletion of
ascorbic acid suggests the release of pituitary adrenocorticotropic hormone (ACTH). Estrogen-primed rats receiving the extract show well developed lobulolveolar tissue with milk secretion. The mechanism of action of the extract may be through a direct action on the mammary gland or through the pituitary or pituitary-adrenal axis due to the secretion of prolactin and ACTH (Sabnis et al., 1968). In a veterinary study, the roots of Asparagus racemosus have been found to have galactogogue action in buffaloes in which the milk yield was found to be significantly increased after the use of the drug (Patel and Kanitkar, 1969).

The alcoholic extract of the aerial parts of Asparagus racemosus showed anti-cancer activity in human epidermal carcinoma of the nasopharynx in tissue culture (Medicinal Plants of India, 1976).

Rao (1981) has demonstrated that when animals are put on Asparagus-root extract diet before their exposure to DMBA, there is a reduction of mammary tumor incidence. When animals primed with estradiol are put on Asparagus-root extract diet before their exposure to DMBA, there is a further reduction in the incidence of mammary tumors. It has been proposed that Asparagus root extract may exert its mammotrophic and/or lactogenic influence on normal as well as on estrogen primed animals and thereby render the mammary epithelium refractory to the carcinogen.