REVIEW OF LITERATURE

A. HISTORICAL NOTE

The first written description of rickets appeared in the year 100 A.D. (Soranus 1894), but the disorder was named for the first time in 1650 (Glisson, 1650) after the widespread appearance of rickets in northern Europe due to industrialisation. However, Loomis (1967) has proposed the possibility of even a pre-historic role of vitamin D in the development of the races. According to his thesis, the requirement of ultraviolet (U.V.) light for vitamin D production was the prime factor which caused natural selection to favour loss of hair and loss of skin pigment in populations as they migrated from the tropics to temperate climate.

The incidence of rickets reached serious proportions with the development of urbanised industrial population. Smoky skies coupled with relatively indoor life necessitated by this environment drastically reduced the solar exposure of the people thereby curtailing the chief source of vitamin D. Cod-liver oil was recognised as a therapeutic measure for curing rickets in 1811 (Schuette, 1824). Sun bathing was recommended as a treatment of rickets in 1890 (Palm, 1890). An excess of osteoid tissue was established as a histological criterion for rickets as early as 1885 (Pommer, 1885). By the beginning of 20th century, the relation between dietary deficiency and many diseases like beriberi and scurvy was demonstrated and the term vitamin was introduced by Funk in 1912. Funk (1914) suggested that rickets also
may be a deficiency disease. In a series of publications Mellanby and Mellanby (1918a, 1918b, 1919, 1921) demonstrated that rickets is a deficiency disease and it could be cured by cod-liver oil or butter fat but attributed the cure to "fat soluble A". However Huldscginsky (1920) provided the experimental proof of the curative effects of U.V. radiation on rickets. In 1925, McCollum had the honour of naming the fourth discovered vitamin as vitamin D (McCollum et al., 1925). In 1924, Steenbock as well as Hess and Weinstock independently demonstrated that U.V. radiation induced vitamin D activity in food. It was soon shown that phytosterol and ergosterol became rich in vitamin D after U.V. radiation (Steenbock and Black, 1925; Rosenheim and Webster, 1925). Subsequently several groups of workers crystallized vitamin D from irradiated ergosterol and the compound was named calciferol (Askew et al., 1930; Windaus et al., 1932). Windaus and Thiele (1935), also determined the chemical structure of calciferol. In 1935, 7-dehydrocholesterol was also synthesized and shown to be a provitamin D (Windaus et al. 1935). Since then, except for official adoption of the name vitamin D$_2$ for ergocalciferol (formerly calciferol) and vitamin D$_3$ for Cholecalciferol (Patterson, 1952), the research activity on vitamin D almost came to stand still, till late sixties, when the role of the liver and the kidney in vitamin D metabolism was elucidated. Subsequently, there has been a spurt of research activity on vitamin D and every year hundreds of papers appear in literature on various aspects of vitamin D metabolism.
B. **VITAMIN D METABOLISM**

Diet is a very poor source of vitamin D. Vitamin D does not occur in the vegetable kingdom. In non-vegetarian diet, egg and fish liver oil are the only important sources of vitamin D. Cow's milk is a poor source. In vegetarian Indian population, hydrogenated vegetable oil fortified with vitamin D (2 IU/g oil) is the chief dietary source of the vitamin.

(i) **CUTANEOUS PRODUCTION OF VITAMIN D**

Most of the vitamin D synthesis occurs in the actively growing layers of the epidermis (strata spongiosum and basale) by exposure to sunlight (Holick et al., 1980). Radiation energies between 290 and 320 nm are most effective (Maclaughlin et al., 1982). 7-dehydrocholesterol present in the epidermis acts as a provitamin D₃. Ultraviolet radiation produces a cleavage of the ring thereby forming previtamin D₃ (9,10-secosteroid). Previtamin D₃ undergoes a temperature-dependent isomerization to form vitamin D₃, taking 2-3 days for completion of the process (Figure 1). The unique thermally regulated synthesis of vitamin D₃ ensures a gradual release of vitamin D₃ from the epidermis into circulation. This concept is confirmed by the observation that subjects exposed to whole body U.V. radiation have a significant increase in the circulating concentrations of vitamin D₃ about 6-9 hours after the exposure that reaches a peak 24-48 hours after the exposure before gradually returning to baseline by 7 days (Adam et al., 1982). Once vitamin D₃ is formed, vitamin D-binding protein in the dermal capillary circulation helps to translocate
the vitamin from blood-less epidermal tissue into circulation.

Melanin pigment present in the epidermis interferes with the synthesis of vitamin D by absorbing U.V. radiation. Loomis (1967) presented the hypothesis that skin pigmentation is evolved for the control of vitamin D synthesis in the skin. Excessive cutaneous melanin seen in populations exposed to greater U.V. radiation seems to support this theory. Moreover when surgically excised skins from blacks and caucasians were exposed to solar radiation, greater vitamin D was produced in the latter (Holick et al., 1981). However now it is clear that melanin is only one of the many factors regulating photosynthesis of vitamin D in the skin. Cutaneous production of vitamin D seems to be under an autoregulatory mechanism. Excessive exposure of even caucasian skin to sunlight does not cause vitamin D intoxication. Continuous exposure to U.V. radiation depletes the cutaneous provitamin D but does not increase the production of previtamin D. Holick et al. (1981) have reported the effect of exposure of skin for different durations to sunlight. During the first 10-15 minutes of exposure, approximately 15% of provitamin D changed to previtamin D. After one hour of exposure, approximately 40% of provitamin D was depleted, yet only 15% of the provitamin was present as previtamin D₃. The other 25% of photolyzed provitamin D₃ was accounted for by the presence of biologically inactive isomers, lumisterol and tachysterol (Fig.1). Further exposure depleted the stores of provitamin D in the epidermis but the concentration of previtamin D₃ or vitamin D₃ did not increase. Vitamin D₃, being sensitive
to sunlight, can also be photodegraded to 5,6-trans-vitamin D₃ and suprasterol (Webb et al., 1986).

Because of the complex mechanism of vitamin D₃ production in the epidermis, the amount of solar exposure required for providing vitamin D adequate for the body shall vary in different individuals and under different conditions. The photosynthesis of vitamin D₃ depends upon (i) the surface area of the skin exposed to sunlight, (ii) the amount of melanin pigment present in the epidermis, (iii) the time of the day (U.V. radiation is most intense between 11 A.M. and 2 P.M.), (iv) latitude (U.V. radiation is most intense at equator) and (v) season (in winter, less U.V. radiation reaches the surface of the earth). However prolonged exposure to sunlight does not necessarily mean greater production of vitamin D since solar radiation can isomerise pre-vitamin D₃ to lumisterol and tachysterol as well as can produce photodegradation of vitamin D₃ (Holick, 1986).

Vitamin D-binding protein has no affinity for lumisterol and tachysterol and hence translocation of these isomers into circulation does not occur. These products are sloughed off during natural turnover of the skin. However these products are in quasiphotoequilibrium with previtamin D₃ (Fig.1). Hence as soon as previtamin D₃ stores are depleted by thermal isomerization to D₃, exposure of lumisterol and tachysterol to U.V. radiation leads to their isomerization to previtamin D₃ (Holick et al., 1980).
Patients with uraemia seem to be unable to produce vitamin D in the skin. It is believed that one or more substances in the epidermis of patients with chronic renal failure act like melanin and absorb the U.V. radiation (Holick, 1986).

(II) HEPATIC METABOLISM OF VITAMIN D:

Vitamin D₃, synthesized in the skin, enters the circulation bound to vitamin D-binding protein. Dietary vitamin D₂ or D₃ enters the circulation through lymphatic system. Subsequently both vitamins D₂ and D₃ are metabolised similarly.

In the liver, vitamin D is metabolised by vitamin D-25-hydroxylase to form 25-hydroxyvitamin D \( [25(\text{OH})\text{D}] \). The enzyme is located in the mitochondrial and microsomal fractions of the hepatic cells (Ponchon and De Luca, 1969; De Luca, 1984). Although there are a few reports of the presence of extrahepatic vitamin D-25-hydroxylase in the chick and the rat (Tucker et al., 1973; Olson et al., 1976), the liver seems to be the only site of 25(OH)D synthesis in the humans. The reserve capacity of vitamin D-25-hydroxylase in the liver is substantial. Severe parenchymal damage is required to lower the level of plasma 25(OH)D (Long et al., 1976). The enzyme vitamin D-25-hydroxylase does not seem to be tightly regulated since the circulating levels of 25(OH)D vary with the amount of dietary intake of vitamin D or the degree of solar exposure (Holick et al., 1986). Decreased plasma 25(OH)D levels are observed in patients with nephrotic syndrome, having proteinuria greater than 4g/day, due to renal
loss of vitamin D tagged to vitamin D-binding protein (Pietrek & Kokot, 1977).

(III) RENAL METABOLISM OF VITAMIN D:

As early as 1833 A.D., Lucas recognised the association between chronic renal disease and bony lesions resembling rickets. Observations of similarity in bony lesions in patients of nutritional rickets and those with chronic renal failure led Liu and Chu (1943) to propose that uraemia interferes with the action of vitamin D. However it was only in 1970 that Fraser & Kodicek demonstrated the intimate relation between the kidney and vitamin D metabolism. These workers demonstrated that homogenates of chicken kidney could metabolise 25(OH)D to a biologically active metabolite. Soon it became apparent that at physiological concentrations 25(OH)D could not stimulate intestinal calcium transport in anephric rat (Boyle et al., 1972). Hollick et al. (1971) as well as Fraser and Kodicek (1970) isolated and identified the active metabolite as 1,25-dihydroxycholecalciferol, \([1,25(OH)_{2}D_{3}]\).

The renal 25(OH)D-1 alpha-hydroxylase is located in the proximal convoluted tubule in the rat (Suda and Kurokowa, 1983) and possibly in humans also. It is now accepted that 1,25-dihydroxy metabolites of vitamin D2 or D3 are the biologically active forms of vitamin D2 and D3 respectively. This metabolite is 10 times more active than vitamin D3 in healing rickets and stimulating intestinal calcium absorption (De Luca, 1984). The
activity of renal 25-OH-D-1 hydroxylase appears to be tightly controlled since plasma 1,25(OH)$_2$D concentration remains normal over a wide range of substrate concentrations of 25(OH)D. Parathormone (PTH) seems to play a crucial role in the synthesis of 1,25(OH)$_2$D since it was found that hypocalcemic vitamin D deficient rats could more effectively metabolise 25(OH)D to 1,25(OH)$_2$D than normocalcemic vitamin D replete rats (Boyle et al., 1971), but when vitamin D deficient hypocalcemic rats were thyroparathyroidectomised, the renal production of 1,25(OH)$_2$D was markedly reduced (Garabedian et al., 1972). Experiments involving manipulation of dietary calcium and phosphorus have shed further light on the regulation of 1,25(OH)$_2$D synthesis. Animals maintained on a low calcium and low phosphorus diet could effectively metabolise 25(OH)D to 1,25(OH)$_2$D despite the absence of PTH (De Luca, 1984; Portale et al., 1986). It is therefore believed that a small decrease in ionic calcium increases PTH secretion by the parathyroid glands. PTH increases renal tubular reabsorption of calcium and increases renal excretion of phosphate. The resultant decrease in intracellular phosphate concentration triggers increased renal mitochondrial production of 1,25(OH)$_2$D. However according to Holick et al. (1986), PTH does not seem to be absolutely essential for 1,25(OH)$_2$D synthesis since patients with hypoparathyroidism often have low-normal concentrations of circulating 1,25(OH)$_2$D. Under certain physiological conditions, factors other than PTH, calcium and phosphate may regulate 1,25(OH)$_2$D synthesis. In pregnancy and
lactation, growth hormone, estrogens and prolactin directly or indirectly enhance the renal production of 1,25(OH)$_2$D (Baksi & Kenny, 1977; Boass et al., 1977).

(IV) ALTERNATE RENAL METABOLIC PATHWAY FOR 25(OH)D:

When vitamin D nutrition and circulating plasma concentrations of calcium and phosphate are normal, 25(OH)D is metabolized into a variety of products (Fig. 2) by hydroxylation at C 24 & C 26 to form 24,25(OH)$_2$D and 25,26(OH)$_2$D (Holick et al., 1986). The concentration of each of these metabolites is 50-100 times more than that of 1,25(OH)$_2$D and is normally directly proportional to the concentration of 25(OH)D. These metabolites seem to have no biological activity at physiological concentrations in anephric animals (De Luca, 1984). Renal 25(OH)D-1-hydroxylase converts 24,25(OH)$_2$D and 25,26(OH)$_2$D into 1,24,25(OH)$_3$D (1,24,25 trihydroxyvitamin D) and 1,25,26(OH)$_3$D (1,25,26 trihydroxyvitamin D) respectively, which again have no biological activity (Holick et al., 1986).

(V) EXTRARENAL METABOLISM OF 25(OH)D:

Till recently, the kidney was believed to be the only site of 1,25(OH)$_2$D synthesis. Twentyfour hours after injection of $^3$H-25(OH)D, $^3$H-1,25(OH)$_2$D could be detected in the blood and tissues in vitamin D deficient rats, but not in vitamin D deficient rats that had undergone bilateral nephrectomy before receiving $^3$H-25(OH)D (Gray et al., 1971). However bilateral nephrectomy reduced but did not abolish the conversion of
25(OH)D to 1,25(OH)₂D (Weisman et al., 1978b) In vitro studies have confirmed the placenta as the site of 1,25(OH)₂D synthesis in pregnancy (Whitsett et al., 1981). In addition, in vitro, a wide variety of cultured cells from normal human bone, human osteosarcoma, chick embryonic calvaria (Turner et al., 1980; Howard et al., 1981; Howard et al., 1982) have shown capacity to convert 25(OH)D to 1,25(OH)₂D. These studies demonstrate the possibility of extrarenal production of 1,25(OH)₂D. These observations also help to explain why hypercalcemia occurs in some patients of sarcoidosis, tuberculosis, silicosis, Hodgkin's disease and non-Hodgkin lymphoma. Such patients have recently been shown to have elevated levels of 1,25(OH)₂D (Gkonon et al 1984; Breslau et al., 1984; Davies et al., 1985; Rosenthal et al., 1985; Zaloga et al., 1985).

(VI) BIOLOGICAL ACTIONS OF VITAMIN D:

After its synthesis in the kidney, 1,25(OH)₂D is transported in the circulation bound to vitamin D binding protein. In the target cells, the free form of 1,25(OH)₂D is transported to the nucleus where it interacts with nuclear receptors. Next, the transcription of specific genes is activated. In the intestine the result is greater production of not only calcium binding protein, calbindin (Mayer et al., 1984) but also alkaline phosphatase and brush border protein (Wasserman et al., 1984; Bikle and Munson, 1985). The net result is that 1,25(OH)₂D stimulates intestinal absorption
of dietary calcium and phosphate.

Although bone and muscle accumulate about 60% of the injected dose of vitamin D (De Luca, 1977) and gross skeletal abnormalities have been observed in vitamin D deficient animals, no direct effect of 1,25(OH)₂D on the process of ossification has been observed. When serum calcium and phosphate levels were maintained in the normal range in vitamin D deficient rats by dietary manipulation, the skeletal histology was found to be normal (Holtrop et al., 1986). Thus 1,25(OH)₂D does not seem to be essential for ossification of bone. However, in cultured rat osteosarcoma cells, 1,25(OH)₂D stimulates the synthesis of osteocalcin, the bone derived protein in a dose dependent manner (Price, 1984). In patients with postmenopausal osteoporosis, 1,25(OH)₂D administration has been shown to increase circulating osteocalcin level (Zerwekh et al., 1985).

Mobilisation of calcium from the bone is another well known function of vitamin D, specially when administered in pharmacologic doses. At physiologic concentrations, 1,25(OH)₂D acts in concert with parathormone to stimulate osteoclastic activity (Garabedian et al., 1974). At pharmacologic concentrations, 1,25(OH)₂D unlike PTH does not stimulate the activity of mature osteoclasts. In the rat, 1,25(OH)₂D seems to act by inducing the stem cell to become osteoclasts (Haussler et al., 1985). Exposure of human peripheral monocytes that possess receptors for 1,25(OH)₂D results in their differentiation into multinucleated giant cells capable of mobilising calcium from
Fig. 3 Mechanism of action of 1,25-dihydroxy-vitamin D₃ in the regulation cell proliferation and differentiation (Haussler et al., 1985)
bone chips (Gray and Cohen, 1985).

Recently receptor sites for 1,25(OH)₂D have been demonstrated in a wide variety of tissues like gonads, stomach, epidermis, kidney, bone, pituitary gland, pancreas, breast, parathyroid, thymus, cardiac muscle, skeletal muscle, placenta etc. (Stumpf et al. 1979; Reichel et al., 1989). Initially no physiological significance was attached to this finding. Subsequently however, some reports have indicated calcium-binding protein synthesis in many of these tissues including brain (Mayer et al., 1984; Clemens et al., 1985). In vitro 1,25(OH)₂D inhibits proliferation of human fibroblast and keratinocytes; increases TSH synthesis, inhibits PTH synthesis and induces monocytes to differentiate and produce interleukin-1 (Clemens et al., 1983; Smith et al., 1986; Amento et al., 1984). When activated B-lymphocytes are exposed to 1,25(OH)₂D, immunoglobulin synthesis is inhibited (Provvedine et al., 1983). Based upon these observations, vitamin D has been tried in the treatment of hyperproliferative disorders of the epidermis like psoriasis with encouraging results (Morimoto et al., 1986). Haussler (1985) has proposed a role of vitamin D in the regulation of cellular proliferation and differentiation (Fig. 3).

(VII) RELATIVE IMPORTANCE OF ESTIMATION OF VARIOUS VITAMIN D METABOLITES:

Specific and sensitive assays are now available for the estimation of vitamin D₃, 25(OH)D₃, 1,25(OH)₂D₃ and 24,25(OH)₂D₃.
in the plasma. Measurement of plasma vitamin D concentration is of little value since it reflects recent dietary or medicinal intake of the vitamin or recent solar exposure (Holick, 1986). Its normal range may vary from nil to 100 ng/ml. However estimation of plasma vitamin D level 12 hours after administration of a single oral dose of 50,000 IU vitamin D can be used as a test of gastrointestinal absorption of the vitamin (Lo et al., 1985).

25(OH)D level in the plasma is the most important index of vitamin D status of an individual. Its concentration reflects the cumulative effect of vitamin D obtained from dietary, medicinal or cutaneous sources (Holick, 1986). The circulating half life of this metabolite is about 3 weeks. Its normal range is 8-55 ng/ml. Higher levels up to 120 ng/ml, have been reported in sun bathers. Lower levels have been observed in patients with liver disease, intestinal malabsorption or house-bound aged individuals. Values greater than 200 ng/ml indicate vitamin D intoxication.

Estimation of plasma 1,25(OH)₂D is not of as much value in assessing the nutritional status of vitamin D as the differential diagnosis of metabolic bone disease. Its normal range is 25-65 pg/ml and its circulating half life is 4-6 hours (Holick et al., 1986). Assay of this metabolite can help to differentiate between types I & II renal rickets since 1,25(OH)₂D level is low in type I renal rickets because of an inborn error in its synthesis. In type II renal rickets, plasma 1,25(OH)₂D level is high because of the end-organ resistance to the hormone. The normal range of plasma 24,25(OH)₂D is 1-4 ng/ml. This metabolite is chiefly
used in research and its estimation has no clinical value as yet.

C. ROLE OF VITAMIN D IN PREGNANCY

The study of vitamin D metabolism in pregnancy and lactation was prompted by the observations of osteomalacia in pregnant and lactating women in India and China (Maxwell and Miles, 1925; Ford et al., 1973). The requirement of the fetus for calcium during gestation and of the neonate during lactation puts considerable demand on the female. Now it is being realised that even caucasian women tend to go into biochemical osteomalacia during pregnancy and lactation and need to be supplemented with vitamin D (Watney and Rudd, 1974).

(I) INTESTINAL CALCIUM ABSORPTION IN PREGNANCY;

Greater calcium demand of the body during pregnancy and lactation can be met with by increasing the calcium intake or increasing calcium absorption from the intestine or decreasing calcium losses in the urine. In the rat as well as humans, greater appetite for food occurs in pregnancy (Cripps and Williams, 1975; Toverud and Boass, 1979). But in the women residing in underdeveloped countries, financial constraints limit the actual increase in food intake. Obviously increased efficiency of intestinal calcium absorption remains the chief mechanism of increasing the availability of calcium to the mother. A marked increase in intestinal calcium absorption in later months of pregnancy has been observed in humans (Heaney and Skillman, 1971), and sheep (Braithwaite et al., 1970). Greater efficiency of
intestinal calcium absorption has been attributed to vitamin D as well as some other factors. Halloran et al. (1980b) estimated intestinal calcium transport ratio (serosal Ca⁺⁺/mucosal Ca⁺⁺) in vitamin D replete and vitamin D deficient rats during pregnancy. In vitamin D replete pregnant rats the ratio was 6 compared to 3 in vitamin D replete control rats. Surprisingly even in vitamin D deficient pregnant rats the ratio was 3.5 compared to 2.0 in vitamin D deficient control rat. In the rat on a fixed dietary intake of vitamin D, 25(OH)D and 24,25(OH)₂D concentrations diminish while the concentrations of 1,25(OH)₂D and PTH increase during the later part of pregnancy (Halloran et al., 1979; Reitz et al., 1977; Pitkin et al., 1979; Bouillon and De Moor, 1973). Such studies have been cited to explain the (PTH mediated) increased synthesis of 1,25(OH)₂D in pregnancy leading to increase in intestinal absorption of calcium. However many other workers have failed to observe any increase in PTH during pregnancy (Gillette et al., 1982; Wieland et al., 1980; Whitehead et al., 1981). It has been suggested that in pregnancy growth hormone, prolactin and placental lactogen stimulate 25(OH)D-1 alpha-hydroxylase activity of the kidney (Tanaka et al., 1976; Spanos et al., 1976; Spanos et al., 1978; Baksi and Kenny, 1977; Baksi et al., 1978; Tanaka et al., 1978).

Prolactin has been shown to increase the intestinal calcium absorption in not only vitamin D replete but also vitamin D deplete animals (Mainoya, 1975a, b; Pahuja and De Luca, 1981). Mechanism by which prolactin increases the intestinal calcium
absorption is not exactly clear. Possibly the effect of prolactin on calcium transport is mediated through intestinal mucosal hypertrophy. During pregnancy, the intestinal mucosa shows substantial hypertrophy (Cripps and Williams, 1975; Burdett and Ruk, 1979). Villus height, absorptive cell number and tissue weight, all increase in pregnancy. Prolactin is a trophic hormone for mammary glands and may be at least partly responsible for the intestinal hypertrophy in pregnancy (Harding and Cairnie, 1975; Mainoya, 1978).

Renal conservation of calcium is another mechanism which may be utilised by the body for improving the supply of calcium to the fetus during pregnancy. However due to increased G.F.R. in later months of human pregnancy, the excretion of many urinary constituents like amino acids, glucose and calcium increases.

(II) BONE METABOLISM IN PREGNANCY:

The effect of pregnancy on bone metabolism is not entirely clear. It has been suggested that mineral accumulates in the bone during pregnancy in anticipation of the calcium requirement during lactation (Heaney and Skillman, 1971; Denzie et al., 1955). In vitamin D deficient rat, there is roughly 25% increase in the femoral bone mineral content by the end of pregnancy (Halloran and De Luca, 1980c). In vitamin D replete rat, however, no change in bone mineral content could be demonstrated. Many studies have suggested that in the rat under normal dietary conditions of vitamin D, calcium and phosphate, bone turnover
and total bone mineral are not changed during pregnancy. In such a condition the calcium requirements of the fetus are met with mainly by increasing the calcium absorption in the intestine (Miller et al., 1982; Naismith, 1966). Under conditions of dietary restriction of calcium, bone mineral is sacrificed to support the fetal demand (Rasmussen, 1977a; 1977b).

(III) PLACENTAL CALCIUM TRANSPORT:

To meet the fetal demand, calcium is actively pumped across the placenta (Twardock and Austin, 1970). This view is supported by the observation of higher serum calcium level in the fetus (12-13mg%) than in the mother (9-11mg%) (Watney and Rudd, 1974). In the later months of pregnancy the increase in the rate of calcium transport across the placenta is accompanied by the appearance of calcium-binding protein in the placenta (Bruns et al., 1978; Delorme et al., 1979). This protein has properties similar to those of the intestinal calcium binding protein associated with vitamin D induced transport of calcium in the intestine. Thus vitamin D may play an important role in fetal calcium metabolism. However the amount of calcium transferred to the fetus during pregnancy in vitamin D deficient female rat is slightly greater than that transferred in vitamin D replete female (Halloran and De Luca, 1981). This suggests that the active transport of calcium across the placenta is not dependent on vitamin D alone.
25-Hydroxyvitamin D: Plasma 25(OH)D level in pregnant women has been determined by numerous workers with conflicting results. Many workers (Rosen et al., 1974; Dent and Gupta, 1975; Weisman et al., 1978; Wieland et al., 1980) have reported similar plasma 25(OH)D level in pregnant and non-pregnant women. On the other hand, Turton et al. (1977), MacLennan et al. (1980); and Cockburn et al. (1980) have reported lower values of 25(OH)D in the plasma of pregnant women in the third trimester than in normal non-pregnant controls. Bedouins in Israel (Biale et al., 1979) and Asian immigrants in the U.K. (Heckmatt et al., 1979) have shown lowest 25(OH)D levels in pregnancy. Pregnant women in Belgium (Bouillon et al., 1977), Switzerland (Paunier et al., 1978) have also shown lower levels of 25(OH)D in pregnancy than their North American counterparts (Steichen et al., 1980). The variations in the reports of 25(OH)D level in pregnancy (and even in non-pregnant state) may be attributed not only to the latitude of the cities from where these reports have originated but also to the variation in the degree of solar exposure and the amount of dietary intake of vitamin D in different populations.

Halloran et al. (1979) have studied vitamin D metabolism in pregnant rats. On day 18th and 20th of pregnancy, 25(OH)D and 24,25(OH)_{2}D levels were significantly lower and 1,25(OH)_{2}D level significantly higher than in non-pregnant control rats. Numerous human studies have shown a marked rise of plasma 1,25(OH)_{2}D concentration in pregnant women (Bouillon et al., 1981;
The mechanism of increase in plasma level of 1,25(OH)₂D in pregnancy is not exactly clear. It is said to be related to increased level of plasma PTH, prolactin, and oestrogens (Gray, 1981).

(V) PLACENTAL TRANSFER OF VITAMIN D AND ITS METABOLITES:

The importance of placental transfer of vitamin D and its metabolites was suggested many decades ago by the clinical reports of fetal rickets in the off-spring of Chinese osteomalacic women (Maxwell and Miles, 1925). More recently, neonatal rickets has been observed in vitamin D deficient Asian immigrants in the U.K. (Ford et al., 1973). In animal studies, vitamin D supplements given to pregnant rats produced a delay in the onset of rickets in pups given rachitic diet at weaning (Korenchevsky and Carr, 1923). Now more direct evidence of the transfer of vitamin D and its metabolites across the placenta is available in the sheep (Ross et al., 1979; Hidiroglou and Williams, 1981), rat (Hadded et al., 1971), cow (Barlet et al., 1979), and Rhesus monkey (Schediwie et al., 1980).

In pregnant sheep, ³H₂-vitamin D₃ was administered in physiologic doses and maternal and fetal concentrations of vitamin D₃ and its metabolites were estimated 19 hours later. ³H-vitamin D₃ and ³H-25(OH)D₃ could be detected in the maternal as well as fetal plasma but equilibration between maternal and fetal compartments was not observed (Ross et al., 1979). These
observations demonstrate the permeability of the placenta to vitamin D₃ and 25(OH)D₃. However no conclusion could be drawn regarding the placental permeability to dihydroxylated metabolites because of the possibility of fetal and placental metabolism of the maternally administered vitamin D₃ or 25(OH)D₃. Fetal accumulation of such a metabolite may be the result of maternal metabolism of the substance followed by placental transfer to the fetus or the substance itself could have been transferred to the fetus and metabolised there or the placenta itself may metabolise the substance and transport its metabolites to the fetus. However the studies mentioned above at least demonstrate that vitamin D₃ and its hormone 1,25(OH)₂D₃ are available to the fetus.

The possible transfer of 1,25(OH)₂D₃ across the placenta has been indirectly demonstrated in the cow. Oral administration of dried powdered leaves of Solanum Glaucophyllum containing a glycoside of 1,25(OH)₂D₃ for 6 days to pregnant cows resulted in both maternal and fetal hypercalcemia (Barlet et al., 1979). Since maternal hypercalcemia induced by calcium infusion did not increase the fetal calcium level, it was concluded that 1,25(OH)₂D₃ glycoside crossed the placenta and produced fetal hypercalcemia. Rapid placental transfer of 1,25(OH)₂D₃ to the fetus has been demonstrated in Rhesus monkey also (Schediwie et al., 1980). Weisman et al. (1976) suggested that despite the demonstrated ability of fetal tissues to metabolise 25(OH)D in vitro (as shown in the kidney homogenates), the fetus does not metabolise
25(OH)D₃ during intrauterine life. The fetal levels of vitamin D₃ metabolites are dependent on the maternal supply and the placental transfer of such metabolites.

D. **ROLE OF VITAMIN D IN LACTATION**

Lactation presents a calcium challenge to the mother similar to that experienced in pregnancy. In some species e.g. the rat, the stress on the calcium homeostatic mechanism is a far greater in lactation than in pregnancy. In 21 days of lactation, the rat transfers to her litter over 2.5 g of calcium, equal to 60% of calcium content of her skeleton. The daily loss of calcium in the milk in lactating rat usually exceeds 100mg which is 100 times more than the daily urinary calcium excretion. In human female, the calcium secretion in milk (350mg/day) is only marginally greater than the 24 hrs urinary calcium excretion.

1) **INTESTINAL CALCIUM ABSORPTION:**

In the lactating rat, food intake is 3-4 times that of a non-lactating rat. The increase in appetite is attributed to suckling-induced stimuli as well as to the metabolic drain of milk production. The structural changes in the jejunum include increase in villus height, crypt depth and total tissue mass (Cripps and Williams, 1975; Burdett and Ruk., 1979). The mechanism of intestinal adaptive changes appear to include both the presence of an increased amount of food in the intestine and the action of some hormones. Prolactin has been suggested as one of such hormones (Mainoya, 1978).
In vitro preparation (Kostial et al., 1969a; Kostial et al., 1979b; Toverud et al., 1976) have confirmed the increased intestinal calcium absorption in lactating rats. In the studies of Fournier and Susbielle (1952) when the diet contained 100 mg calcium per day, calcium absorption in lactating rats was 50% of the dietary intake as compared to 10% in controls. When the dietary calcium was reduced to 27 mg/day, the intestinal absorption of calcium was almost 100%.

Increased intestinal calcium absorption may be attributed to the increased level of plasma 1,25(OH)_{2}D in view of the well known action of the hormone on calcium-binding protein synthesis. However, the intestinal calcium absorption remains high even in vitamin D deprived lactating rat (Toverud et al., 1978). Halloran and De Luca (1980b) studied the intestinal calcium absorption by everted gut sac technique. Duodenum sac of the lactating rat which had been deprived of vitamin D for long time (and had undetectable circulating levels of 25(OH)D and 1,25(OH)_{2}D) showed significantly higher calcium absorption than the duodenum sac of a non-lactating rat. It may be added that the duodenum sac of a vitamin D replete lactating rat showed still higher calcium absorption. From these experiments it has been concluded that while vitamin D is important for the increased active transfer of calcium in the intestine during lactation, there is also a vitamin D independent component of active transfer associated with pregnancy and lactation.
While scanning the literature on intestinal calcium absorption in lactating women it may be pertinent to note the species difference in calcium metabolism. In the rat the calcium requirement of the fetus is almost negligible as compared to the calcium requirement during lactation, while the daily fetal calcium requirement in the last two months of human pregnancy usually exceeds the amount of calcium secreted in the milk (Spray, 1950). This fact may explain why firm evidence for enhanced calcium absorption from the intestine in lactating women is not available. Some studies have revealed enhanced calcium absorption in the later months of pregnancy but no further increase during lactation (Heaney and Skillman, 1971). Many studies have, although revealed the improvement in intestinal calcium absorption in a lactating woman after vitamin D supplementation (Toverud and Toverud, 1931; Liu et al., 1937).

(II) URINARY CALCIUM EXCRETION:

In the lactating rat, urinary calcium excretion appears to be lower than that of pregnant or non-lactating rat. In a study reported by Fournier and Susbielle (1952), daily urinary calcium excretion decreased from 2 mg during pregnancy to a negligible amount during the second and third week of lactation. The decrease in urinary calcium excretion in lactation may be attributed to a decrease in serum calcium level (lowered filtered load) and to higher plasma PTH level. However the contribution of a reduced urinary calcium excretion to the
calcium economy of a lactating rat is negligible.

In women almost 200 mg calcium is lost daily in the urine and a similar amount in the milk (Toverud and Toverud, 1931). Hence small changes in urinary calcium excretion may make an important contribution to the calcium balance in a lactating woman. Retallack et al. (1977) have reported a marked decrease in urinary calcium excretion during the later months of pregnancy and in lactation.

(III) BONE METABOLISM:

In rats on a diet containing 0.44% calcium, 0.30% phosphorus and 25IU of vitamin D$_3$/day, 27% loss of femur calcium has been observed during lactation. While a vitamin D replete rat lost about 40mg of calcium from its femur during lactation, vitamin D deficient rat lost 46mg. Histological examination revealed a loss of both cortical and trabecular bone (Halloran and De Luca, 1980c). In vitamin D deficient rat, at day 14 of lactation, the amount of bone loss was almost similar to that in vitamin D replete rat. On the basis of these experiments it has been concluded that although vitamin D is necessary to ensure normal calcium homeostasis during lactation, some other hormonal mechanism promotes bone mineral release. A likely candidate for such a factor is prolactin. Prolactin has been shown to stimulate the release of calcium from the bone in vitamin D deficient rat (Pahuja and De Luca, 1981).
(IV) VITAMIN D METABOLISM:

A non-pregnant, non-lactating rat has to be deprived of vitamin D for several weeks before hypocalcemia develops. On the other hand if vitamin D is withheld even after the first week of pregnancy hypocalcemia may be observed by the middle of lactation period (Halloran et al., 1979; Boass et al., 1981a). Increased plasma level of \(1,25(\text{OH})_2\text{D}\) in lactating rat was demonstrated for the first time by Boass et al. in 1977. Consuming similar diet, vitamin D replete lactating rats had significantly greater serum \(1,25(\text{OH})_2\text{D}\) level and lower serum calcium level than the non-lactating rats. Halloran et al. (1979) not only confirmed the elevated circulating levels of \(1,25(\text{OH})_2\text{D}\) in pregnant and lactating rats but also showed a reciprocal relationship with circulating levels of \(24,25(\text{OH})_2\text{D}\). At midlactation, the ratio of \(1,25(\text{OH})_2\text{D}\) to \(24,25(\text{OH})_2\text{D}\) was 6:1 compared to the ratio of 1:6 in non-pregnant, non-lactating rats. Kumar et al. (1979) and Lund and Selnes (1979), also observed elevated levels of \(1,25(\text{OH})_2\text{D}\) in pregnant and lactating women.

Hypocalcemia, hypophosphatemia and increased circulating PTH are the known stimulators of renal 1-alpha-hydroxylase synthesis. Of these hypophosphatemia may be eliminated as a stimulus for the increased \(1,25(\text{OH})_2\text{D}\) synthesis in lactating rats since serum phosphorus level is actually higher than in non-lactating rats (Hughes et al., 1975). Hypocalcemia and increased PTH level have been demonstrated in lactating rats.
(Pike et al., 1979). Parathyroidectomy in the rat at mid-lactation led to a marked decline in serum calcium as well as $1,25(OH)_2D$ level. However, some factors other than PTH may also be involved in the increased synthesis of $1,25(OH)_2D$ in lactation since even after parathyroidectomy, the serum level of $1,25(OH)_2D$ was twice that of non-lactating controls (Pike et al., 1979). Recently, evidence for increased plasma PTH level in lactating goats (Hove, 1981), and humans (Retallack et al., 1977) has been reported.

(V) TRANSFER OF VITAMIN D AND ITS METABOLITES IN MILK:

Vitamin D and its metabolites circulate in the blood bound to a specific transfer protein called vitamin D-binding protein (DBP). DBP expresses binding preference for $25(OH)D_3$, $24,25(OH)_2D_3$ and $25,26(OH)_2D_3$ when compared to the parent vitamin and $1,25(OH)_2D_3$ (Haddad and Walgate, 1976; Imawari et al., 1976; Belsey et al., 1974). In man, DBP appears to be an alpha globulin with a molecular weight of 60,000. In human plasma DBP concentration is 525 ug/ml in normal individuals but reaches up to 1,254 ug/ml in pregnant women. Since the total amount of vitamin D sterols in normal individuals is approximately 35ng/ml (Lambert et al., 1981; Shepard et al., 1979), it has been calculated that under normal circumstances 98% of the plasma DBP circulates with its binding sites unoccupied by any vitamin D metabolite.

Human milk contains two types of DBPs, one which appears to be identical to the plasma DBP while the other
resembles DBP previously isolated from a number of different tissues (Vanbaelen et al., 1977). Actual level of DBP in human milk seems to be rather low. In early lactation it is 18 ug/ml but 3 weeks after initiation of lactation, it is about 3 ug/ml i.e. about 1.2% of the plasma DBP level of a normal woman (Haddad and Walgate, 1976).

Maternal blood levels of various vitamin D metabolites determine the amount of these metabolites in the milk. Milk from a mother who is vitamin D-deficient would be devoid of vitamin D and its metabolites. On the other hand, vitamin D content of milk can be increased by administration of a large amount of vitamin D to the mother (Polskin et al., 1945; Hibb and Ponden, 1955). Like plasma the concentration of 25(OH)D seems to be the stable in the milk, whereas concentration of the parent vitamin is variable depending upon short term intake and solar exposure of the mother. Dihydroxylated metabolites of vitamin D constitute an insignificant component of antirachitic properties of milk (Hollis et al., 1981).

Since DBP occurring in milk has its origin in the plasma and vitamin D and all the metabolites bind to the protein, DBP entering into milk provides an important route for the transfer of vitamin D and its antirachitic metabolites. Colostrum is extraordinarily rich in plasma proteins (Larson, 1974). Hence colostrum is particularly rich in antirachitic sterols (Oh and Horst, 1981).
The relative importance of placental transfer and milk transfer of vitamin D has not been addressed to by many workers. However the observation of Mendelsohn and Haddad (1975) that serum 25(OH)D levels are lower during suckling period than at birth suggest that the milk transfer of this metabolite of vitamin D or its precursor is limited.

E. EXPERIMENTAL RICKETS

The long bones develop embryologically on a cartilaginous model and hence are called cartilaginous bones although most of the diaphysis is ultimately replaced by periosteal (membranous) bone. Most of the increase in length of the long bone is brought about by interstitial growth. The increase in diameter of both the shaft and its marrow is due to periosteal deposition of new bone and endosteal resorption of older bone. In the epiphyseal cartilage and the adjoining region of diaphysis (metaphysis), 5 zones can be normally identified. 1. Resting cells, some of which by division lead to (2) the columns of proliferating cells, (3) each cell of which develops into a cylinder thereby compressing and elongating the surrounding matrix. It is in zone 2 and 3 that the increase in length of the bone occurs. (4) The matrix, primarily the longitudinal trabeculae, then calcifies with hypertrophy, vacuolization and death of the cells. 5. The uncalcified, or less well calcified transverse cartilaginous trabeculae are destroyed by marrow elements.
Osteoblasts which develop from the connective tissue cells of the marrow penetrate between the calcified longitudinal trabeculae and deposit the bone matrix on the exposed surface. The bone matrix is promptly calcified producing a firm union between the cartilaginous and osseous portions of the bone.

The characteristic features of rachitic metaphyseal region are (i) a near normal rate of growth and proliferation of zone 2 and 3, (ii) lack of mineralization and maturation of the cartilagenous matrix and cells (zone 4) and (iii) reduced erosion and uneven penetration by elements from the marrow (zone 5) and renewed but atypical removal of the cartilage leading to a mixed mass of cartilage and osteoid.

Even short term vitamin D deprivation in pregnant rats (6th day of pregnancy onward) leads to the characteristic changes in the long bone of the pups. Boass et al. (1981a) observed the changes described above in 19 days old pups.

Rickets and Osteomalacia can be produced in experimental animals. Initially rickets was determined by gross examination of the width of epiphyseal plate in the long bones and the increased width was easier to distinguish when the diet was low in vitamin D as well as phosphorus (Mc Collum et al., 1925). This led to the belief that low serum phosphorus is necessary in the rat to cause rickets and low serum calcium due to vitamin D deficiency alone does not cause rickets.
However such rats did not gain weight; their growth was stunted and the rats soon died presumably as the result of malnutrition. Therefore commonly used vitamin D deficient diet contained only 0.44% calcium and 0.3% phosphorus so as to produce hypocalcemia as well as hypophosphatemia. Rasmussen (1969) fed rats on vitamin D deficient diet containing 1.1% calcium and 0.8% phosphorus for eight weeks. Such animals showed widening of epiphyseal plate in the tibia indicating that rickets can be induced to develop in vitamin D deficient rats despite normal serum phosphorus.

Rickets is not necessarily associated with vitamin D deficiency alone. Rats fed on low phosphorus diet adequate in vitamin D also develop rickets (Carttar et al., 1950). Rickets has also been reported in the rat on low calcium diet (Shohl, 1936). However calcium deficiency also increases parathormone secretion resulting in bone resorption. Osteoporosis without osteoid seams was observed in bone when vitamin D in the diet was adequate but osteomalacia with wide osteoid seams developed when the diet was deficient in vitamin D as well as calcium (Harrison and Fraser, 1960). Such studies emphasize the importance of serum calcium, serum phosphorus as well as vitamin D in the mineralization of cartilage and bone. On the other hand normal serum calcium and phosphorus can prevent rickets in young growing rats on vitamin D deficient diet. Holtrop fed 1-week pregnant rats and subsequently their pups on diets deficient in vitamin D and containing varying concentrations of calcium and phosphorus (Holtrop et al., 1982). The pup at 5 weeks of
age, in the stage of active growth, had 25(OH)D and 1,25(OH)\textsubscript{2}D levels undetectable or very low. Tibial epiphyseal plate was normal in the rat with normal serum calcium and slightly low serum phosphorus but widened in the rat with low serum calcium as well as serum phosphorus.

Numerous studies on weaning rats have shown that rickets, evidenced by widened epiphyseal cartilage and hypophosphatemia, can be produced only by depriving the animals of vitamin D as well as phosphorus for several weeks (Boass et al., 1981). However, typical rickets may be observed in pups by 20 days of age when the mothers are deprived of only vitamin D from 6th day of pregnancy. Boass et al. (1981) have shown that with such a procedure pups at 8th day of age had hypocalcemia and hypophosphatemia. By 15th day of age serum 25(OH)D was undetectable, serum calcium and phosphorus were low and body weight was reduced by 26%. In 19 days old pups, the ratio of bone weight to body weight was not reduced but ash weight as a percent of bone weight was 33.8% as compared to 36.2% in control pups. Histological examination of the tibia revealed the characteristic feature of rickets including irregularity and widening of the hypertrophic cartilage cell layer, uneven line of ossification and widened and irregularly arranged bone trabeculae. Thus rat pups suckling vitamin D deprived mothers can develop biochemical and histological evidence of vitamin D deficiency similar to that of human vitamin D-deficiency rickets.
F. EFFECTS OF HYPO-AND HYPER-VITAMINOSIS D DURING PREGNANCY ON REPRODUCTIVE FUNCTION

(i) HYPOVITAMINOSIS D:

Liu and Chu (1943) described in detail the effects of vitamin D deficiency in pregnancy. The clinical, biochemical, radiological and histopathological features of osteomalacia seen in pregnant and lactating women (Fourman and Royer, 1968) are too well known to warrant repetition. In babies born to such mothers neonatal hypocalcemia (Cockburn et al., 1980) or even congenital rickets have been reported (Maxwell et al., 1939; Liu et al., 1940; Snapper, 1956). The possible role of vitamin D in reproductive function has attracted attention only recently. Sunde et al. (1978) observed abnormal embryonic development of chicks when hens were put on vitamin D deficient diet. Henry and Norman (1978) studied the effect of administration of vitamin D₃, 1,25(OH)₂D₃ and 24,25(OH)₂D₃ in hens. Eggs from hens receiving 1,25(OH)₂D₃ or 24,25(OH)₂D₃ alone were virtually incapable of hatching. Normal egg hatch-ability was seen only in hens receiving either the parent vitamin D₃ or both of its dihydroxylated metabolites.

Halloran and De Luca (1980a) maintained weanling female rats on vitamin D-deficient diet. Such animals showed poor growth and hypocalcemia as compared to vitamin D replete rats. It was found that the likelihood of vitamin D deficient rats becoming pregnant was roughly one half as great as in the vitamin D replete females. Moreover even when the rats became pregnant, only 40%
of the vitamin D deficient females reached full term and gave birth to normal litter as compared to 80% incidence in vitamin D replete female rats. Moreover mean litter size was 7.8 in vitamin D deficient females as compared to 11.2 in vitamin D replete rats. Mean birth weight of each pup was however similar in the two groups. Histological examination of the fetal tissues did not reveal any abnormality in the liver, kidney, brain, or spleen. Examination of the fetal bones, however, revealed a slight yet significant increase in the amount of osteoid in trabecular bone surfaces (Miller et al., 1982).

Vitamin D deficiency during pregnancy seems to cause adverse effects in the offspring that become more evident during the postnatal life. Brommage and Neuman (1981) have observed growth retardation in the pups at the age of 12-14 days when the mother was not given vitamin D during pregnancy. Hypocalcemia, hypophosphatemia and impaired bone calcification was observed by the age of 23 days (Halloran et al., 1979; Halloran and De Luca, 1980c). Boass et al. (1981) undertook a systemic study of the effect of short term vitamin D deprivation of the mother from 6th day of pregnancy on the suckling and weaned pups. By 15th day, serum 25(OH)D was undetectable and body weight was reduced by 26%. Serum calcium and phosphate levels were also reduced. In 19 day old pups the ratio of bone weight to body weight was not reduced but the ash weight as a percentage of bone weight was significantly reduced to 33% as compared to 36% in pups from vitamin D replete mothers. The histologic examination of bone revealed irregularity and widening of epiphyseal cartilage of
long bone, a characteristic feature of rickets.

Brommage and De Luca, (1984a) tried to determine whether the failure of vitamin D deficient pups to grow properly was due to a maternal or neonatal defect. When vitamin D₃ was provided to the pups there was no improvement in their growth, but administration of vitamin D₃ to vitamin D deficient mothers produced a threefold increase in growth rate of the pups. The position was further clarified when vitamin D deficient mothers were given only two pups each to nourish whereas eight pups each were nourished by vitamin D replete mothers. The growth rate of pups was now similar in the two groups. With isotope studies vitamin D deplete rats were found to produce about 20% of the milk produced by vitamin D replete rats. On the basis of these experiments, it was concluded that vitamin D deficient rats produce a reduced quantity of nutritionally adequate milk. (Brommage & De Luca, 1984b).

Another recent study has shown that vitamin D is necessary for reproductive function even in the male rat. When vitamin D deficient male rats were allowed to mate with vitamin D replete females, the fertility was 73% less than controls (Kwiecinski et al., 1989).

In human pregnancy, even mild deficiency of vitamin D seems to reduce the fetal growth. In Asian immigrants in the U.K., due to low dietary intake of vitamin D and reduced solar exposure, low serum 25(OH)D level as well as low serum calcium, and phosphate levels have been observed in the mothers as well
as the new borns (Heckmatt et al., 1979; Cockburn et al., 1980). Administration of vitamin D to such pregnant mothers resulted in improvement in serum calcium status of the mother as well as the fetus (Brooke et al., 1980; Heckmatt et al., 1979). More interestingly it reduced the incidence of low birth weight babies (Brooke et al., 1980; Maxwell et al., 1981). In women of Haryana who do not show any evidence of vitamin D deficiency during non-pregnant state (Marya et al., 1981b), administration of vitamin D supplements in pregnancy produced a dose related increase in birth weight of the babies (Marya et al., 1981c). Many anthropometric measurements of the newborn like length, head circumference and skinfold thickness were also found to increase after vitamin D supplementation in pregnancy (Marya et al., 1988).

(II) HYPERVITAMINOSIS D:

Excess of vitamin D in the diet of pregnant rats leads to a persistent defect in bone formation in the pups. Administration of 20,000 or 40,000 IU of vitamin D per day to pregnant rat from 10th day to 21st day of gestation induced severe growth retardation of suckling pups. Osteogenesis of long bones was impaired as evidenced by retarded epiphyseal ossification and persistence of endochondral bone trabeculae within the diaphysis (Ornoy et al., 1968). There was increased number of bony trabeculae but these often had wide borders of osteoid. Thus many of these changes resemble those seen in rickets but there is absence of the characteristic epiphyseal changes (Follis, 1955; Yendt et al., 1955). Similar results have been obtained in the rat after administration
of 1,25(OH)$_2$D$_3$ (Zusman et al., 1981). However a pregnant rat subjected to hypervitaminosis D is less susceptible to the toxic effects of the vitamin than a non-pregnant animal. Hypervitaminosis D does not seem to produce hypercalcemia in pregnant rat whereas similar doses do so in nonpregnant rats (Ornoy et al., 1971).

In rabbits the teratogenic effects of hypervitaminosis D resemble those of a disorder in infants called supravalvular aortic stenosis syndrome. In both conditions, there are aortic lesions and abnormalities of the cranial, facial and dental development (Chan et al., 1979). In rabbits, administration of high doses of vitamin D in pregnancy resulted in low birth weight of the newborn and hypoplasia of the lower jaw and of tooth enamel, a congenital absence of teeth and malocclusion. Supravalvular aortic stenosis syndrome (Idiopathic infantile hypercalcemia) observed in some newborn children is associated with hypercalcemia, osteosclerosis, mental retardation, dwarfism and impaired kidney function. Although such children have shown high vitamin D levels in the blood, the disorder seems to be due to an abnormal sensitivity of the fetus to vitamin D rather than the excessive intake of the vitamin by the mother (Committe on nutrition, 1967).