CHAPTER II

REV. Kly Or LITERATURE

REVIEW OF LITERATURE
Mammary gland is a unique tissue which through its prodigious synthetic activity synthesizes milk constituents. Proteins have been detected in milk as a major constituent of approximately 150 species studied so far. The importance of milk proteins to the young ones resides in its nutritional qualities (casein) and immunological properties (immuno-proteins). The recent demonstration that one of them (α-lactalbumin) plays a key role in the regulation of processes on which the secretion of milk as a whole depends, further adds to the significance of the study of milk protein synthesis. Mechanism and cellular regulation of the protein synthesis in general, is one of the most fascinating research topics of the day. In spite of the ubiquitous nature of milk proteins as food entity, there is considerable variation between the species in their relative concentrations and their concentrations at different stages of lactation.

Proteins in the milk can be classified into two major groups.

Group I: Caseins, comprising α₂, β- and κ-caseins each of which comprises number of molecular species.

Group II: Non-casein or whey proteins which remain in the solution when casein is precipitated. The principal proteins under this group are α-lactalbumin and β-lactoglobulin.

Also, some serum proteins are present in the milk, their concentration, especially certain immunoglobulins, is high in the colostrum. Very recently, Mepham (1977) has reviewed the current
ideas on the mechanism of milk protein synthesis and their secretion in milk.

Origin of milk proteins

The site of milk protein synthesis or their origin had been a debatable question for many years. Tolley (1949) by his arterio-venous difference measurement techniques concluded that milk proteins are synthesised largely from plasma proteins. Introduction of radiotracer techniques have clearly shown that in the elaboration of milk proteins, amino acids, the precursors, pass from the blood plasma to the lumina of mammary alveoli (Barry, 1961 and Larson, 1965). These studies have also revealed that both essential and non-essential amino acids required for milk protein synthesis are transported from blood to the mammary gland (Black et al., 1957 and Popham and Linzell, 1966).

Quantitative estimation of substrata uptake in relation to milk protein output by the gland has been investigated by arterio-venous difference method in different species (Verbeke and Peeters, 1965). Popham and Linzell (1966) and Spencer et al.(1969) studied the uptake of free plasma amino acids by the lactating cow's udder and amino acid composition of udder lymph.

Experiments involving injection of $^{14}$-labeled amino acids into intact animals with subsequent analysis of labeled proteins suggested that casein is synthesised *de novo* from free amino acids of plasma (Barry, 1961 and Campbell and Work, 1951). The use of isotopes, employing isolated perfused gland preparations has, however, largely confirmed the earlier conclusions (Davis and Popham, 1976).
On the basis of labeling pattern after the injection of $^{14}$-labeled acetate, milk proteins can be divided into two categories: (a) proteins synthesised in the mammary gland from amino acid pool of blood including caseins (except $\gamma$-caseins), $\beta$-lactoglobulin and $\alpha$-lactalbumin; (b) proteins synthesised elsewhere and transferred from blood to the milk like serum albumin and immunoglobulins (Larson and Gillespic, 1957). A significant part of non-essential amino acids of casein is synthesised in mammary gland itself (Mepham and Linzell, 1965, 1966).

**In vivo studies**

Studies carried out by Mepham and Linzell (1965) on the uptake of amino acids by the goat mammary showed that there was no significant arterio-venous difference in the dry goat, but a significant difference was found in the uptake of essential amino acids in the goat colostrum.

Verbeke and Peeters (1965) demonstrated a positive linear relationship between the concentration of arginine, isoleucine, leucine, lysine, valine, histidine and threonine in milk proteins and uptake of these amino acids by the udder.

In goat total amino acid uptake by the udder was significant to account for the protein output, though the individual amino acids showed varying balances (Mepham and Linzell, 1966). Although ornithine does not occur in milk proteins, even then a considerable amount of it was taken up by the mammary gland. Arginine uptake from blood was found to be much higher than its appearance in the milk and it was found that arginine is cleaved to urea and ornithine and latter was used for the formation of proline in the milk.
proteins (Mepham and Linzell, 1967). Hanson and Barry (1958) using $^{14}$C- and $^{14}$N-labelled arginine showed that lactating goat mammary gland takes up free arginine from blood but not aspartic acid residue showing very little synthesis of asparagine from glucose in mammary gland.

Further studies by Linzell et al. (1968) showed that a considerable proportion of carbon of serine, alanine and also that of glutamate and aspartate and their amines is provided by the glucose. When lactating rats were injected with $^{3}$H-leucine, autoradiographic determination showed that radioactive leucine enters the cells from perivascular space in mammary tissue (Haid, 1970). After the injection of $^{14}$C-labelled glutamic acid, valine and arginine, higher amount of $^{14}$C radio-activity due to glutamate appeared in lactose than in milk proteins whereas most of $^{14}$C from valine and arginine was recovered in milk proteins (Cgan et al., 1970).

Lactating rabbits and guinea pigs when injected with $^{14}$C-amino acids, radioactivity was seen in the milk proteins. The incorporation rate was found to be higher in case of rabbit compared to that in guinea pig (Majumder and Ganguli, 1971a). Also the incorporation of radioactivity was 5-22 folds more in mammary as well as in liver tissue of lactating rats as compared to virgin rats when they were injected with $^{14}$C-valine (Majumder and Ganguli, 1971b).

In vitro studies

Studies have been carried since long back in in vitro mammary system to investigate the mechanism of milk protein synthesis. Mammary gland cell cultures were found to synthesize $\beta$-lactoglobulin. The culture retained such activity up to two weeks though they lost the
ability to synthesize lactose (Larson and Twarog, 1961).

Dispersed cell cultures rapidly take up amino acids. Studies utilizing radioactive amino acid tracers with bovine and rat mammary cultures have shown a significant synthesis of milk proteins including β-lactoglobulin, α-casein and β-casein (Larson, 1965; Groves and Larson, 1965; Schingoethe et al., 1967 and Jorgensen and Larson, 1968).

The uptake of essential amino acids was found to be reasonably in good balance with their output in the milk proteins. Also mammary gland has a capacity to synthesize certain amino acids in accordance with its need for milk protein synthesis (Nepham and Linzell, 1965).

Using rat mammary tissue, in vitro labeling of rat milk proteins was accomplished similar to those obtained for bovine mammary. The labeling patterns of the β-lactoglobulin and β-casein isolated were similar to those isolated from bovine cultures (Hageman and Larson, 1966). Turkington and Topper (1966a) using mouse mammary explants concluded that a pool of unphosphorylated or incompletely phosphorylated casein exists in mammary gland and phosphorylation of peptide chains is a major mechanism for phosphorus incorporation into casein. Milk protein synthesis was augmented considerably by including total non-essential amino acids along with the essential amino acids (Schingoethe et al., 1967 and Larson, 1972). When the ewe's udder was perfused with a substrate mixture containing (1-14C) glycine, about 90 percent of 14C incorporated into casein was in glycine residues, but about 9 percent appeared in the serine residues (Verbeke et al., 1967).

Experiments carried out by Larson (1972) showed that in the
medium containing dispersed cells the ability to synthesise \( \beta \)-lacto-
globulin and \( \beta \)-casein is increased 2-fold if the concentration of
L-methionine is increased 4-6 folds.

Cays et al. (1973a) reviewed that the protein synthesis is
stimulated during lactogenesis and number of polysomes per cell is
also increased. In the bovine mammary cell cultures, \( \beta \)-lactoglobulin
was synthesised at a relatively constant rate for first five days
while \( \alpha \)-lactalbumin synthesis declined much more rapidly. The rate
of synthesis of casein declined at a rate between the two proteins
(Jorgensen, 1973).

Perfusion studies carried out by Davie and Mepham (1976)
demonstrated that the extent of incorporation of labeled amino acids
in casein residue is consistent with their being derived from free
amino acids of perfusate plasma.

Studies on sub-cellular fractions

The initiation of lactation in the mammary gland of rat
resulted in a marked increase in the nucleic acid content of cell
resulting primarily from an increase in the amount of RNA (Waldwin
and Milligan, 1966, and Kuhn and Lowenstein, 1967) coinciding with an
increase in soluble protein indicating a relationship between RNA and
protein synthesis. Nucleus, therefore, must be a regulatory organelle
in cell metabolism through the control of genetic information.

During mammary development the nuclear fraction exhibited a
higher rate of incorporation of amino acids which dropped to normal
when lactation started. The total incorporation of amino acids
increased during pregnancy and subsided at the onset of lactation.
(Lauer and Cole, 1964). Nuclei prepared from lactating cow mammary when incubated with the suitable medium showed protein synthesis in TCA precipitable fraction (Yakovlev, 1966), ATP and to a lower extent GTP enhanced such protein synthesis (Zarowny and Yakovlev, 1967). Intraperitoneal injection of $\omega_1$-valine given to virgin and lactating rabbits, showed a 7-fold increase of incorporation of radioactivity into nuclear proteins over the virgin animal (Majumder and Ganguli, 1970).

Highest level of radioactivity was found in the mitochondrial fraction when the homogenate of lactating guinea pig mammary gland was incubated with radioactive amino acids. The lactating mammary homogenate was found to be several fold active than those of pregnant animals (Majumder and Ganguli, 1970; Yakovlev, 1966; and Drew and Campbell, 1967).

Isolated mitochondria from lactating cow mammary were able to synthesize proteins when they were incubated with amino acids. The addition of ATP and GTP caused stimulation of such synthesis (Ozerova et al., 1967). Mitochondria when combined with microsomal fraction incorporated radioactive amino acids into $\alpha$-lactalbumin (Drew and Campbell, 1967).

Herrington and Hawtrey (1969a) observed that the mitochondria from non-lactating mammary gland are unable to synthesize protein unless they are supplemented with rat liver pH 5 enzyme and not with pH 5 enzyme of bovine mammary gland which may be due to the inability of bovine mammary pH 5 enzyme to activate amino acids.

The mitochondria from lactating gland were found to be
40 fold more active in relation to amino acid incorporation than that of virgin tissue. Furthermore, the mitochondrial fraction was the most active fraction in the lactating tissue (Majumder and Ganguli, 1971b).

Ability of mitochondria to synthesize protein was established by Huang and Keenan (1971). They found both RNA and DNA in the mitochondria of bovine mammary cells and presumably such mitochondria have their own separate pathway for the synthesis of certain mitochondrial proteins. The total mitochondrial protein and mitochondrial marker enzyme activities were found to increase gradually during pregnancy with a rapid 2-3 fold increase occurring during early days of lactation (Jones and Hosano, 1972).

Microsomal fraction obtained by conventional centrifugation consists of a mixture of cell organelles, Golgi apparatus endoplasmic reticulum with its rough and smooth membranes. Milk protein synthesis, as agreed generally, is carried out by the polyribosomes bound to the membranes of endoplasmic reticulum (Maye and Denamur, 1970 and Fairhurst et al., 1971). After parturition microsomal RNA, protein and amino acid incorporation exhibits a marked increase in the mammary gland (Lavrin and Cole, 1964).

Labeled α-lactalbumin was synthesized by the cell-free protein synthesising system when it was incubated with labeled amino acids (Drew and Campbell, 1965, 1967). The radioactive leucine isolated from chymotryptic and tryptic peptides of α-lactalbumin synthesized in the cell-free system was not of uniform radioactivity (Drew and Campbell, 1967). Polypeptides synthesized
by the bound polyribosomes are transported through the endoplasmic
reticulum lumina to golgi region, where polymerization of peptide
chains occur prior to the addition of specific carbohydrates and
phosphorus groups, characteristic of casein complex (Turkington
and Topper, 1966a; Singh et al., 1967; and Schachter et al., 1970).
αS-lactalbumin, synthesized in HLb (Drew and Campbell, 1967), is
transported to the golgi apparatus where it becomes associated with
the galactosyltransferase, an enzyme of lactose synthetase system.
Labeling patterns of proteins indicated that the polypeptide chains
of proteins are assembled from N-terminus and chain initiation
occured in cell-free system. A cell-free system from rabbit mammary
tissue during late pregnancy and lactation requires ATP, GTP and
ATP generating system for its maximum activity to synthesize
protein. Such incorporation is low during late pregnancy and rises
20-fold after parturition. Both ribosomal and supernatant fractions
are involved in increasing the incorporation during early lactation
(Baird and Herriman, 1967).

Using cell-free milk protein synthesising system, Seitz
et al. (1969) synthesized β-lactoglobulin, αC-lactalbumin, κ-casein,
αS-casein and β-casein. Synthesis of proteins was found to be
dependent upon the presence of microsomes, an energy source and
partially upon the addition of sRNA and an aminoacyl-sRNA synthetase
preparation (Denisova and Yakovlev, 1972). Microosomal preparation
from lactating mouse mammary gland was found to be 3-times more
active than that from pregnant mammary in relation to protein synthe-
sis, but almost comparable for αS-lactalbumin synthesis (Zarikov
and Rychlik, 1969). Both bound and free ribosomes were found to be
very active in incorporating amino acids into proteins, but \( \beta \)-lactoglobulin appeared to be exclusively synthesized by bound ribosomes (Gaye and Denamur, 1970). In actively synthesizing mammary cells most of the polysomes were membrane bound while in pregnant animal's gland they were in free state. Presumably, bound polysomes were concerned with the synthesis of secretory proteins (casein, \( \beta \)-lactoglobulin and \( \alpha\)-lactalbumin) while free polysomes were concerned with the constitutive proteins (Gaye et al., 1972).

Microsomal fraction of non-lactating mammary gland of rat has the highest specific radioactivity when incubated with amino acids whereas in lactating tissue the mitochondrial fraction was the most active (Majumder and Ganguki, 1970) and the rate of incorporation was very low in non-lactating mammary ribosomes unlike the ribosomes of a functional tissue (Herrington and Hawtrey, 1971; and Fairhurst et al., 1971).

Gaye et al. (1973b) using SDS-extraction and fractionation by centrifugation isolated an active mRNA from mammary gland which was found to direct the synthesis of several proteins in a cell-free system with major milk protein synthesis to be that of \( \alpha\)-s-casein.

RNA from lactating rat mammary was found to be directing the casein synthesis which was identified by radioimmuno precipitation with mouse casein antiserum and electrophoresis of the reaction product (Terry et al., 1975). The major portion of lactating mammary RNA-directed milk protein synthesis in cell-free system appeared to be analogous to \( \alpha\)-s-casein.

Not only the different fractions of the mammary gland were needed for the synthesis of protein, the enzymes present in cytosol
were also found to be essential components of protein synthesising system. Both, amino acid activating enzymes and enzymes responsible for the transfer of tRNA forming AA-tRNA were observed to be located in the soluble fraction of the cell (Brew and Campbell, 1967; Beitz et al., 1969; Fairhurst et al., 1971).

**HORMONAL STUDIES**

Mammary gland begins to differentiate from epithelium of skin early in embryonic life. An underdeveloped mammary gland may be stimulated to a stage of development by proper hormonal treatment.

Over past many decades, relationship both qualitatively and quantitatively, of the mammary gland development and its activity with the hormones has been well documented. Libb (1977) has recently reviewed the hormonal control of mammogenesis and onset of lactation in cows. Hormones of ovary were tried as early as in 1917 by Leeb and Hesselberg. In rats, whose pituitary, gonads and adrenal glands were removed, using replacement therapy Lyons et al. (1955) were able to demonstrate the synergistic effects of hormones from pituitary, ovaries and adrenal glands. Organ culture or in vitro methods have made possible in understanding the qualitative and quantitative relationship between endocrines and the mammary gland.

Experiments with varying doses of estrogens and progesterone on mammary gland showed the best response in terms of mammary growth. 24-96 μg estrogens and 1 mg progesterone/day for 25 days (Lyons and McInty, 1941) or from 15 μg estradiol benzoate and 1 mg progesterone per day for 30 days (Yamamoto and Turner, 1956)

A rapid increase in amino acid incorporation occurred in tissue
slices of mammary gland of rats showing induced lactation by ovariectomy in late pregnancy (Davis et al., 1966). Stilbestrol injection enhanced the uptake of amino acids by mammary gland, but progesterone had no effect. The stimulation caused by former could be increased to a small extent with the simultaneous injection of progesterone (Majumder and Ganguli, 1969a, b). Stilbestrol alone or in combination with progesterone could also increase the mammary RNA (Majumder and Ganguli, 1969c) and mammary protein (Shurovskii et al., 1970). In goats, subcutaneous injection of 17β-estradiol (0.5 - 16 mg) showed that the lower level (0.5 mg) resulted in a slight decrease in protein content, but higher level injections were accompanied by increase in protein content (Chowdhry and Forbes, 1972). Progesterone injected into pregnant rats (6 mg/day) prevented the appearance of casein in mammary gland (Davis et al., 1972). Danamur and Deloixus (1972) observed that injection of 10 mg of progesterone/day to the pregnant rabbit reduces the RNA content of the mammary gland, but has no effect on DNA.

Gardener and Witliff (1973) detected significantly higher levels of specific receptors for 17β-estradiol in the lactating mammary of rat, but much lower levels were observed in the gland of pregnant or virgin animals. Estradiol benzoate and progesterone injection led to udder development and initiated milk secretion in non-pregnant intact ewes (Fulkerson and McDowell, 1974).

Prolactin could maintain 25 percent of the total milk yield in a hypophysectomized lactating rats (Lowie, 1967 and Lowie et al., 1969) whereas human chorionic somatotrophin could substitute for
prolactin in pseudopregnant or pregnant rabbits (Friesen, 1966)
or cultured mammary cells to secrete casein (Turkington and
Topper, 1966b). Turkington (1969) observed that ovine prolactin
or the human placental lactogen can induce the synthesis of
casein, α-lactalbumin and β-lactoglobulin. The metabolic changes,
as measured by increase in RNA content during luteal phase of
oestrous is associated with the changes in pituitary prolactin
concentrations (Sinha and Tucker, 1969). The protein synthesis by
mitochondria from a lactating rabbit was increased when prolactin
was added to the incubation medium (Yakovlev and Lyasenko, 1969).
Mammary gland development was induced by estradiol and progesterone
administration to hypophysectomized rabbits and RNA, and protein
synthesis was obtained by the injection of prolactin (Simpson, 1970).
Overiectomized rabbits treated with progesterone and oestrogens,
were injected with prolactin. The total content of DNA and RNA
was increased after the prolactin injection (Simpson and Schmidt,
1971). Radiocautographic studies carried out by Bourne et al. (1972)
indicated that prolactin stimulates DNA synthesis and cell division
in mammary gland of pseudopregnant rabbit.

About 1.5 fold increase in DNA concentration was observed
when the mammary gland of a pseudopregnant virgin rabbit was
injected with 125 µg prolactin (Bourne et al., 1974).

Prolactin added to nuclei isolated from mammary epithelium
showed a stimulation in RNA synthesis. The effect was tissue
specific and it was suggested that the hormone may perform its
function within the mammary cell (Chomczynski and Topper, 1974).
Bullough and Wallis (1974) showed in a dispersed mammary cell
preparation, that casein synthesis can be stimulated by 5-20 μg/ml of prolactin and mammary gland of pseudopregnant rabbits can incorporate p³2⁵ in casein at a higher rate after the prolactin injection (Journe and Bryant, 1974).

Insulin was shown to be vigorously stimulating the incorporation of L¹⁴⁻leucine in protein of mammary gland slices from pregnant rats, but the effect was much less marked in lactating rats (Wayne and Barry, 1965 and Wayne et al., 1966). Injecting 1, 2 and 3 units of insulin to the ovariectomized and progesterone +oestradiol benzoate treated rats, Kumaresan and Turner (1965) observed 8 percent, 17 percent and 32 percent increase in DNA content/100 gm body weight, respectively. To alloxan treated ovariectomized rats injected with ovarian hormones, when 3 units of insulin was injected about 66 percent increase in DNA/100 gm body weight was observed.

Raskin et al. (1973) showed increase in protein synthesis in mammary explants by insulin and put forth that the stimulation of protein synthesis is not due to increased transport of amino acids, but it appears to act rapidly on processes which subsequently lead to enhanced synthetic activity (Wang and Amor, 1971). Even the protein synthesising activity of parenchymal cells isolated from rat mammary gland was stimulated when they were incubated with insulin (Amger et al., 1976).

Voogt et al. (1969) observed an increase in the level of corticosteroids with the onset of lactation. This increase was about 1-fold over the last few days of pregnancy. Adrenalectomy caused 50 percent reduction in the protein synthesis in pregnant rats and also inhibited casein synthesis in lactating gland.
A selective increase in incorporation of labeled amino acids was observed in lactating rats on the administration of cortisol, showing that protein synthesis in mammary tissue was more dependent on adrenal corticoids during functional stage than the stage of structural differentiation (Banerjee et al., 1971). Chadwick (1971) demonstrated that ACTH injected into pseudopregnant rabbits has a lactogenic effect and this effect was also observed in the rabbits treated with corticosteroids or ACTH. Free polysomes from lactating gland of adrenalectomized mice had 4-fold low rate of incorporation of amino acids into proteins than in a corresponding system from a normal mice presumably due to release of polysomes from rough endoplasmic reticulum, thus suggesting that adrenal corticoid hormone regulates milk protein synthesis by controlling association between polysomes and the membrane (Banerjee, 1972).

Casein synthesizing ability of the lactating cell polysome was reduced after the adrenal ablation, with 85 percent reduction in casein mRNA activity (Terry et al., 1976). Cortisol treatment of adrenalectomized mice maintained high level of casein synthesizing activity, thus providing the evidence that role of corticosteroids in lactogenesis involved in the modulation of casein.

Protein synthesis in mammary gland was increased 30 percent when thyroxine was injected in cows. This was also accompanied by an increase in milk yield (Perahin et al., 1969) whereas reserpine could reverse the effect of thyroxine (Riva et al., 1968).

In vitro addition of insulin + hydrocortisone to the mammary epithelial cells of pregnant mice caused a marked increase in ribosomes and organisation in polysomes (Turkington and Riddle, 1970).
Raskin et al. (1973) reported that insulin together with cortisol increased milk yield but did not affect DNA in vitro.

Decrease in RNA polymerase activity in hypophysectomized lactating rat mammary can be restored to normal values by the injection of cortisol (1 mg) + prolactin (60 I.U.) per day (Baldwin et al., 1969). Similar studies carried out by Baldwin and Martin (1968) showed that decrease in rate of casein and cytoplasmic protein synthesis can be partially maintained by cortisol, but apparently can be maintained by administering cortisol (1.0 mg) + prolactin (40 I.U. per day).

Turkington (1970a) observed a two-fold increase in nuclear RNA synthesis in mammary epithelial cells isolated from pregnant mice on incubation with insulin and prolactin. Prolactin caused the proliferation of epithelium of virgin mice mammary, but insulin was mitogenic in its effect. It was observed that insensitive epithelium can be sensitized to insulin by prolactin injection in vivo (Oka and Topper, 1972). Addition of insulin and prolactin (5 μg/ml) each to the epithelium from human mammary in organ culture caused an increase in number of cells synthesising DNA (Flaxman and Lasfargues, 1973). Effect of insulin was much faster in stimulating incorporation of H3-leucine in protein compared to that of prolactin in mammary explants.

Human placental lactogen, combined with insulin and cortisol produced histological development distinguishable from that produced by prolactin + insulin + hydrocortisone (Turkington and Topper, 1966b). Experiments conducted by Turkington et al. (1965),
Lockwood et al. (1966) and Voytovich et al. (1969) confirmed that by incubating mammary gland from mid pregnant mice with insulin + hydrocortisone + prolactin medium, an increase in all the major casein components is obtained. While incubation with insulin + cortisol led to a decrease in the rate of synthesis of such components. The effect was selective for casein components only (Juergens et al., 1965). Mammary gland from a three week old mice was induced to synthesize casein in vitro in the absence of lobulo-alveolar development by incubating in the presence of insulin, prolactin and hydrocortisone (Voytovich and Topper, 1967).

Synthesis of both the proteins A and B of lactose synthetase was increased in an organ culture supplemented by insulin, prolactin and hydrocortisone (Turkington et al., 1968 and Palmiter, 1969). The activity of glucose-6-phosphate dehydrogenase was increased by insulin in mammary culture of late pregnant mice, but further addition of prolactin and cortisol had no effect (Leader and Barry, 1969). Studies carried out with mammary explants showed that the secretory epithelial cells, which contain hydrocortisone induced rough endoplasmic reticulum, can be converted into secretory cells by treatment with prolactin and insulin (Green and Topper, 1970). Turkington (1970a) observed that prolactin can cause a rapid increase in the rate of RNA synthesis in the mammary explants which had been previously incubated with insulin and hydrocortisone. About 53 percent increase in RNA synthesis could be achieved in the mammary cell culture when it was incubated with insulin, prolactin and cortisol (5 µg/ml, 5 µg/ml, 1 µg/ml, respectively) (El-Darwish and Rivera, 1971), and incorporation of
lysine was increased in lysine/histones (Mohmann and Cole, 1971) or
the $^{14}$C-amino acid incorporation into TCA precipitated protein
was stimulated in non-lactating mammary tissue (Mohrenwiess, 1971).
Prolactin, insulin, hydrocortisone (5 μg/ml) each caused the cassein
synthesis by preneoplastic and neoplastic mouse mammary tissue
in vitro, which was accompanied by the incorporation of lysine
into individual lysine rich histone protein (Hohmann et al., 1972).
Insulin was found to stimulate phosphorylation of proteins in epi-
thelial cells of mammary explants while prolactin synergised with
it to further stimulate protein phosphorylation in explants or in
cells cultured with insulin and hydrocortisone (Majumdar and
Turkington, 1972). In pregnant and virgin mice mammary glands, in
a medium containing insulin (5 μg/ml) and corticosterone (1 μg/ml),
the $^{32}$P incorporation was maximum when 1 μg/ml of prolactin was
also used (Wang et al., 1971 and Hollows et al., 1973). Epithelial
cells from mature virgin mice synthesized minute amounts of cassein
and exhibited low level of lactose synthetase A protein, whereas B
protein had a barely detectable activity. When the cells were
cultured in the presence of prolactin, insulin and hydrocortisone
the capacity to synthesize all these proteins developed in the
cells (Vandernaar et al., 1973).

Rate of protein synthesis and RNA synthesis in mammary tissue
were increased when pseudopregnancy was induced with estradiol and
progesterone. It decreased when artificial lactation was induced
with hydrocortisone and growth hormone in the same animal. Additional
stimulation of lactation by testosterone had no effect on rate of
protein synthesis (Dzirakov and Hychlik, 1968). Hypophysectomy
depressed the activity of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, citrate cleavage enzyme, malic enzyme in lactating rat mammary gland which could be restored to normal levels by administering prolactin 26 I.U./day and 0.5 mg hydrocortisone/day (Korstad and Baldwin, 1969).

Denisova and Yakovlev (1971) demonstrated that 0.5 μM adrenocorticotrophin (ACTH) + 0.25 μM gonadotrophin per ml of medium with mammary ribosomes increase protein biosynthesis. Addition of insulin 0.125–0.5 units, prolactin 0.5–5.0 units, diethyl-stilbestrol 25 μg and 6.5 x 10^{-6} M thyroxine per ml further stimulated such synthesis. Experiments by Dilley (1971) showed that insulin + prolactin + oestradiol + progesterone and aldosterone stimulate maximum mitotic activity and alveolar development in a cultured mammary gland from 3–4 week old female rats. Progesterone blocked or reduced the RNA in pregnant rabbit mammary, but DNA was not affected. Prolactin injections, 12.5 I.U., increased mammary DNA and RNA, and reversed the effect of progesterone (Uenamur and Delouis, 1972). Mammary glands of 4 week old virgin mice could be stimulated to full lobuloalveolar development by culture in presence of insulin + prolactin + aldosterone or oestradiol + progesterone which was accompanied by sequential rise in RNA, protein and DNA synthesis (Mehta and Banerjee, 1975). RNA was isolated from membrane bound polysomes of lactating rabbits which directed the synthesis of casein. But such mRNA was not detected in non-secretory tissue of pseudopregnant rabbits. On the injection of prolactin into the animal, mRNA appeared accompanied by protein synthesis, but progesterone inhibited the appearance of casein synthesis directing mRNA (Houdebine and Gaye, 1975).
toudemire et al. (1975) observed that prolactin and progesterone increased \(^3\)H-thymidine in DNA in an in vitro system, but prolactin alone or in combination with estrogens did not.

**Cyclic AMP**

The chemical reactions those proceed in a living cell are catalyzed by large molecules called enzymes. If all the enzymes work at top speed the result will be a chaos. Many mechanisms have been evolved that control the speed at which these enzymes function.

A small molecule that plays a key role in regulating the speed of chemical processes in organisms is cyclic 3'-5' adenosine-monophosphate called 'Cyclic AMP'. Among many other functions performed by cAMP it acts as a chemical messenger and has also been shown to control the activity of genes.

Cyclic AMP has also been found to regulate the metabolism of the mammary gland. Many reports have appeared in the last decade on the cAMP regulation of mammary metabolism.

Specific activities of phosphokinases 1 and 11 bound to a cAMP binding protein in the cytosol fraction of mammary epithelial cells of mice increased 7-9 folds during pregnancy. Insulin acted synergistically with prolactin for induction of two phosphokinases. Neither insulin nor prolactin stimulated adenyl cyclase activity of isolated mammary cell membranes and cAMP in the medium could not substitute for prolactin, insulin or hydrocortisone for \(^{32}\)P incorporation in casein (Majumder and Turkington, 1971).

Studies of Hagar and Greenbaum (1973) showed that adenyl cyclase reaches its maximum activity late in pregnancy and
fell continuously through lactation. Cyclic GMP has prolactin-like effect on uridine transport and its incorporation in RNA in mid-pregnant mice mammary explants and hence casein synthesis takes place. These effects can be suppressed by dibutaryl cAMP. The activity of adenylyl cyclase and level of cyclic nucleotides in mammary gland rose to the peak at the end of pregnancy, but the levels fell sharply after parturition. Fluoride ion at 1.0 - 10 mM concentration inhibited the adenylyl cyclase activity of pregnant rat mammary, but had no effect on lactating mammary adenylyl cyclase. Low concentrations of Ca\(^{2+}\) (1 \(\mu\)M) activated the adenylyl cyclase of mammary gland. \(\beta\)-estradiol and progesterone caused stimulation of adenylyl cyclase activity of pregnant rat mammary, but hydrocortisone, insulin and prolactin did not. But none of these hormones had effect on the enzyme from lactating mammary thus suggesting that tissue content of cAMP is needed for the development and growth of mammary gland while initiation and scale of lactation is related to cAMP removal from the gland (Spag Hagar and Greenbaum, 1974a and Ribbens, 1976a). The level of cGMP had reciprocal changes to that of cAMP during pregnancy and lactation (Spag Hagar and Greenbaum, 1974b). Adrenalectomy decreased cAMP by increasing the activity of phosphodiesterase, but hydrocortisone therapy returned these to normal. Also during insulin insufficiency the cAMP concentration increased showing that insulin causes decrease while glucocorticoids increase the cAMP level in rat mammary gland (Louis and Baldwin, 1975a).

Prostaglandin \(F_{2\alpha}\) increases the activity of adenylyl cyclase by 270 percent. In rat mammary there was fall in cAMP level at the
onset of lactation, but remained steady during lactation (Louis and Baldwin, 1975b). Georg (1976) showed that the cAMP level decreased whereas cGMP, DNA and RNA levels increased from pregnancy to lactation.

OTHER FACTORS AFFECTING MAMMARY PROTEIN SYNTHESIS:

In the recent past many reports have appeared relating to the control of mammary protein synthesis by certain extraneous substances in vitro. Herrington and Hawtre (1969b) observed that the protein synthesis by rat liver polysomes is inhibited by the pH 5 enzyme fraction of non-lactating mammary gland of rat even when the pH 5 enzyme of liver was also present. Both, the formation of amino acyl-tRNA and the transfer of amino acids from amino acyl-ttRNA to protein was affected. Crude nucleotide-incorporating enzyme from pH 5 enzyme fraction of non-lactating bovine mammary gland inhibited the adenosine-5'-monophosphate incorporation activity of rat liver nucleotide-incorporating enzyme due to high nuclease activity. The tRNA from non-lactating mammary gland was unable to accept amino acids due to the absence of -p-p-p terminal sequence, possibly due to nuclease activity of pH 5 enzyme (Herrington and Hawtre, 1970a,b).

Milk as well as whey proteins of goat milk inhibited the incorporation of laucine in the in vitro preparations of mammary gland from normal as well as lactating animal. The boiled extract from lactating goat's liver stimulated the amino acid incorporation in the mammary gland proteins irrespective of physiological status of the animal, but the extract from the liver of nonlactating goat
had an inhibitory effect (Singh et al., 1971 and Singh and Ganguli, 1972).

Harrington and Hawtrey (1971) found that ribosomes from lactating bovine tissue synthesized protein whereas those from the non-lactating tissue did not. Neither polyuridylic acid used as synthetic mRNA nor activated amino acyl-tRNA complex was able to bind to ribosomal proteins. However, both were found to associate with ribosomes isolated from rat liver and ribosomes from lactating mammary gland thus indicating the absence of certain ribosomal proteins in the non-lactating mammary gland.