The absorption of macromolecules from the intestine of newborns is a well established phenomenon. Also there is a good deal of evidence that intact proteins are absorbed from the intestine in adult animals (Walker and Isselbacher, 1974; Gardner, 1988), although the physiological and pathological significance of such a phenomenon in mature animals is precisely unknown. The absorption of intact proteins from neonatal intestine involves both "selective" and "nonselective" processes. The selective process is mainly confined to the proximal part of the small intestine.

A number of factors are known to influence the absorption of macromolecules from intestine. These include the age of the animal, hormonal and dietary status of the animal. Administration of cortisone and thyroxine during early suckling is known to induce precocious closure of intestinal tissue to macromolecular absorption, however, other factors which bring about and regulate the intestinal closure are not known. Further, how complete is this closure and how it is related to maternal nutrition is not understood. Can the absorption of intact macromolecules from intestine be enhanced in response to dietary factors remains to be investigated. The present studies were undertaken to understand the role of certain factors in the transmission of proteins in developing pups, in particular, the effect of maternal nutrition and hormonal treatments. For this purpose the
absorption of $^{125}$I-labelled BSA, $\gamma$-globulin and $\alpha$-lactalbumin was investigated both \textit{in vivo} and \textit{in vitro} with a view to have an insight into the basic mechanisms involved in the transmission of macromolecules.

The \textit{in vitro} experiments were carried out by incubating the everted tissue segments in presence of labelled proteins. At the end of incubation, the tissues were rinsed with buffer containing the specific non labelled proteins to remove the adhering proteins from the tissue surface. The tissues were homogenized in 10\% TCA and the acid insoluble material was counted for $^{125}$I-radioactivity. For \textit{in vivo} experiments, the animals were gavaged with suitable amounts of $^{125}$I-BSA, $\gamma$-globulin or $\alpha$-lactalbumin and the animals were sacrificed after $1\frac{1}{2}$ hours. Intestine was removed and the blood was directly drained from the heart. After washing the luminal contents with buffer, the intestine was weighed and counted for radioactivity. $^{125}$I-protein was detected in the whole blood, in TCA precipitated blood, and in the serum by immunodiffusion and ELISA.

In agreement to the observations of other investigators (Udall \textit{et al.}, 1981 \textit{b}; Limm and Rowley, 1985), the intestinal absorption of proteins measured by TCA precipitation was always considerably higher than the values determined by immunodiffusion and ELISA methods under various experimental conditions.
In view of the objectives of these studies to investigate the hormonal and nutritional interactions with the macromolecular absorption mechanism, in suckling rats, the experiments were conducted on the effect of: (a) Undernutrition (UN) (b) Feeding Low protein, High protein or High fat diets on the absorption of BSA, γ-globulin and α-lactalbumin in suckling rats. Also the effect of cortisone, thyroxine and insulin administration to control or UN pups was investigated on the macromolecular absorption. The conclusions drawn from these studies are summarized in the followings:

(1) Absorption of $^{125}$I-labelled BSA, γ-globulin and α-lactalbumin in vivo was significantly low (16-86%) in 10-12 day old rats injected with the pharmacological dose of cortisone, thyroxine and insulin daily for 4 days compared to controls. The certain quantitative variations in the absorption rates of proteins in response to hormones treatment were observed.

(2) In vitro experiments using everted intestinal segments from hormone treated pups revealed a similar decrease in the absorption of $^{125}$I-γ-globulin. Kinetic analysis evinced that the observed decrease in γ-globulin uptake in response to hormones was primarily due to an increase (56-92%) in the value of affinity constant ($K_t$) with little change in the maximal velocity ($J_{max}$) under these conditions.
(3) The uptake of BSA, γ-globulin and α-lactalbumin was significantly more at pH 5.5 compared to that at pH 7.0 in suckling rat intestine. Kinetic studies indicated that observed increase in the protein uptake at acidic pH was due to enhanced Jmax (34-130%) with little change in affinity constant (Kᵢ).

(4) There was no change in the uptake of proteins in presence and absence of Na⁺ ions. This suggested that the absorption of macromolecules is insensitive to electrochemical and potential gradient due to Na⁺ ions across the microvillus surface in suckling rat intestine.

(5) The desialylation of intestinal tissue by neuraminidase treatment resulted in 25-28% inhibition of BSA, γ-globulin and α-lactalbumin compared to the control tissue (12-14 old pups). The observed decrease in protein uptake was unrelated to their binding to the microvillus membranes in these animals.

(6) The in vitro uptake of ¹²⁵I-BSA and α-lactalbumin was markedly enhanced in the presence of ATP in the incubation medium. The uptake of these proteins was inhibited by DNP and Sodium arsenite. The uptake of γ-globulin was not effected by ATP, DNP and Sodium arsenite. Presence of HgCl₂ in the incubation medium enhanced the uptake of BSA, γ-globulin and α-lactalbumin. The absorption of α-lactalbumin was inhibited in presence of -SH group reacting reagents, NEM and Iodoacetate.
The presence of Cu\(^{++}\) ions in the incubation medium enhanced the uptake of \(^{125}\)I-labelled BSA and \(\alpha\)-lactalbumin in vitro, but other metal ions (Co\(^{++}\), Ca\(^{++}\), Mg\(^{++}\), Mn\(^{++}\) and Zn\(^{++}\)) essentially had no effect on the protein absorption process in weanling rats. Essentially identical results were obtained at the pH 5.5 and 7.0, under various experimental conditions.

(7) The observed increase in the uptake of proteins at pH 5.5 was associated with enhanced binding of \(^{125}\)I-proteins to microvillus membranes as a function of protein concentrations at the acidic pH. The binding of proteins at all different protein concentrations showed a significant decrease in the binding of BSA and \(\gamma\)-globulin at pH 7.0 compared to that at pH 5.5.

(8) The imposition of UN by increasing the litter size from 6-8 pups in the control to 16-18 pups per mother in the experimental group resulted in reduction of body weight and intestinal weight. At day 21 after birth, a decrease in sucrase activity and an increase in lactase activity in UN pups was observed compared to the controls. This type of the developmental pattern of the disaccharidase indicated "delayed maturation" of intestinal tissue in UN animals.

(9) The absorption of \(^{125}\)I-labelled BSA, \(\gamma\)-globulin and \(\alpha\)-lactalbumin in vivo was significantly \((p < 0.001)\) enhanced in UN pups compared to the controls. The observed increase in protein absorp-
tion was associated with enhanced binding of the proteins to MVM from malnourished pups. The administration of cortisone, thyroxine and insulin to UN pups produced a marked decline in the absorption of macromolecules in vivo compared to saline treated UN animals. Again there were quantitative differences in the uptake rates of these proteins in response to the hormones in the UN pups. The binding of \(^{125}\)I-labelled proteins to microvillus membrane from UN and hormone injected UN pups was unaffected.

(10) There were no interactions between BSA, \(\gamma\)-globulin and \(\alpha\)-lactalbumin for their binding to brush borders in UN animals. This suggested the involvement of different sites for their binding on microvillus membranes.

(11) The uptake of BSA, \(\gamma\)-globulin and \(\alpha\)-lactalbumin in UN pups was significantly more at pH 5.5 compared to that at pH 7.0. Kinetic studies revealed that observed increase in the protein uptake at acidic pH was due to enhanced \(J_{\text{max}}\) (13-50%) with no change in affinity constant \((K_{1})\).

(12) The effect of various compounds on the uptake of proteins in UN pups in vitro were essentially identical to those observed with the control tissue as explained above.

(13) Feeding low protein (8%) diet to lactating rats considerably enhanced the absorption of \(^{125}\)I-proteins in vivo in pups compared to those from the control group. The observed increase in protein
absorption was associated with enhanced binding of the proteins with microvillus membranes under these conditions.

(14) Analysis of various brush border enzymes revealed a significant elevation (25%) in the activities of lactase, sucrase and alkaline phosphatase in low protein group. The body weight, intestinal weight and intestinal length were significantly low in pups from low protein group compared to the controls. This indicated that feeding low protein diets to lactating dams results in malnutrition in the developing pups. Thus "delayed maturational" development of the intestinal tissue may be responsible for the observed enhancement of macromolecules in these pups.

(15) Feeding high protein (30%) diet to lactating rats also produced a significant (180%) increase in the absorption of labelled proteins compared to controls in vivo. This increase was associated with enhanced binding of the proteins to microvillus membrane under these conditions.

(16) Pups nursed on lactating rats given high protein diet showed a significant increase in body weight, intestinal weight and intestinal length. The activities of sucrase and lactase were reduced in experimental group compared to the controls. The observed changes in enzyme activities may be attributed to the normal developmental process of the tissue. However, the enhanced protein uptake in these animals may be due to the
adaptation of intestinal tissue to high protein load in the diet (Forsum and Lonnerdal, 1980). This also suggested that the mechanism(s) responsible for increased capacity of intestine to transport proteins in pups from low protein and high protein fed groups might be quite different.

(17) The feeding of fat rich diet (26%) to lactating rats essentially had no effect on the body weight and intestinal weight of the developing pups. There was however a significant increase in sucrase and lactase activities and a decrease in alkaline phosphatase activity in pups from high lipid fed groups compared to the controls.

(18) The absorption of proteins in vivo was significantly reduced in pups nursed on lactating dams given high fat diet. The observed decrease in protein absorption was associated with a corresponding decrease in the binding of these proteins to microvillus membrane.

Thus, it is evident from the findings presented herein, that the imposition of UN during early suckling period and feeding diets of different composition to lactating dams resulted in considerable changes in the absorption of macromolecules measured by a variety of techniques in suckling rats. UN and feeding of low or high protein diets to lactating rats augmented the transmission of proteins from intestine. However, pups nursed on
mother given high fat diet exhibited a marked decline in the absorption of proteins. The observed changes in the absorption of proteins was associated with similar changes in their binding to microvillus surface under the various experimental conditions. This indicated that the binding characteristics of these proteins are similarly effected in malnourished pups, and in pups nursed on lactating rats fed various experimental diets. Studies with UN animals revealed a significant decrease in the luminal proteolytic activity. This may be responsible for the observed increase in protein absorption in these animals.

A number of factors are known to influence the capacity of intestinal tissue to transmit macromolecules. These include (a) Age of the animal, (2) Hormonal status, (3) Pinocytotic activity, (4) Luminal proteolytic activity, (5) Exocytosis on the basolateral side of enterocytes, (6) Activation of the local immune system in the gut, (7) Alterations in the binding of macromolecules to microvillus surface, (8) Changes in the integrity of intestinal epithelial tissue and (9) Changes in the chemical composition of microvillus surface. Since the absorption of macromolecules has been shown to occur through both the paracellular and transcellular pathways in intestine, any change in these pathways may also contribute to the net absorption of proteins.

From the results of present studies it is not possible to surmise the underlying mechanism responsible for the observed
changes in the absorption of macromolecules under various experimental conditions but any one of these or all may be related to the observed changes in the absorption of macromolecules in suckling rat intestine. These findings may be of considerable significance since they relate to the effect of maternal nutrition on the absorption of macromolecules in developing intestine.