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Glucocorticosteroid effects on the intestinal absorption of nutrients

by

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A thesis to be submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Glucocorticosteroid Effects on the Intestinal Absorption of Nutrients** in partial fulfilment of the requirements for the degree of Master of Science in Experimental Medicine.

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DEDICATION

Carinhosamente dedico o fruto do meu esforco aos meus pais. Aducio Leonel Thiesen e Lucia Maria de Souza Thiesen.

ABSTRACT

Orally administered systemically active glucocorticosteroids ("steroids") enhance the digestive and sugar absorptive functions of the intestine of weanling and adult rats, but their effect on lipid uptake is unknown. The effect of the locally acting steroid budesonide on intestinal absorptive function also is unknown. Modifications in dietary lipids alter the nutrient transport properties of the intestine. but it is unknown if there is an interaction between dietary lipids and of steroids on the intestinal uptake of sugars and lipids. Accordingly, this study was undertaken to assess the influence of four weeks treatment of weanling male rats with a daily oral gavage of budesonide (BUD; 0.25 mg/kg per day), prednisone (PRED; 0.75 mg/kg per day), or control vehicle (CON) on the *in vitro* jejunal and ileal uptake of sugars and lipids in weanling male rats fed Chow or either a semisynthetic isocaloric saturated fatty acid diet (SFA) or a polyunsaturated fatty acid diet (PUFA). Giving budesonide or prednisone in doses equivalent to those used in clinical practice up-regulates GLUT5 but not SGLT1, and enhances the uptake of some lipids. Feeding a polyunsaturated diet prevents the PRED- or BUD-associated enhanced uptake of fructose in rats fed a saturated diet, whereas feeding this diet increases the effect of these steroids on the absorption of lipids. This adaptive effect of locally and systemically acting steroids on intestinal transport is influenced by dietary lipids by a mechanism other than differences in food intake. body weight gain or villus height. These locally and systemically active steroids, when given in doses which are effective clinically, change the intestinal absorption of fructose and lipids by a process which is modified by the dietary content of lipids.

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LIST OF ABBREVIATIONS

5-ASA	**5-aminosalicylic acid**
ACTH	**adrenocorticotrophic hormone**
ADH	**antidiuretic hormone**
BUD	**budesonide**
BBM	**brush border membrane**
BLM	**basolateral membrane**
BrdU	**bomodeoxyuridine**
CBG	**corticosteroid-binding-globulin**
CDAI	**Crohn's disease activity index**
CIR	**controlled ileal release**
DEAE	**diethylaminoethyl**
FFA	**free fatty acids**
GTP	**guanosine 5'-triphosphate**
Hc	**hydrocortisone**
IGF	**insulin-like growth factor**
IGF BP	**insulin-like growth factor binding proteins**
IL-6	**interleukin-6**
Km	**apparent affinity constant**
LDL	**low density lipoprotein**
MIF	**migration inhibition factor**
MPred	**methyl prednisolone**

MTV-CAT	mouse mammary tumor
NADH	nicotinamide adenine dinucleotide
ODC	ornithine decarboxylase
Pd	passive permeability coefficient
PRED	prednisolone
PGP	P-glycoprotein
PL	placebo
SC	subcutaneous
SI	sucrase-isomaltase
TRF	thyrotropin-releasing factor
TSH	thyroid stimulating hormone
Vmax	maximal transport rate

A) INTRODUCTION

Glucocorticosteroids ("steroids") are widely used to treat a variety of gastrointestinal and hepatic conditions, such as inflammatory bowel diseases and chronic active hepatitis [Bratssand, 1990; Brignola et al., 1992; Brignola et al., 1994; Danielson and Prytz, 1994; Lofberg et al., 1993; Nyman-Pantelidis et al., 1994; Tarpila et al., 1994; Thiesen and Thomson, 1996]. However, systemic steroids may be associated with numerous and potentially serious adverse effects [Bratssand, 1990; Elliot et al., 1992; Girdwood and Petrie, 1987; Haynes and Murad, 1985] . Even topical treatment with steroids using retention enemas or foams depresses plasma cortisol concentrations [Cann and Holdsworth, 1987; Reshef et al., 1992]. For this reason, non-systemic steroids have been developed. The non-systemic steroid budesonide has high topical activity, low systemic bioavailability, and rapid first pass metabolism [Bratssand, 1990]. Budesonide is of proven clinical efficacy when given topically or orally to patients with inflammatory bowel disease [Danielson et al., 1992; Greenberg, 1994; Rutgeerts et al., 1994].

Intestinal adaptation is a process that occurs in response to physiological or pathological processes such as intestinal resection, aging, diabetes, abdominal radiation, chronic alcohol intake, and feeding diets of varying lipid, protein or carbohydrate composition [Thomson et al., 1990; Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b]. Thus, there are different morphological and physiological characteristics of the intestine according to the specific nutrient composition of the diet [Diamond and Karasov, 1984; Thomson and Rajotte, 1983a; Thomson and Rajotte 1983b; Thomson, 1982; Thomson and Wild, 1997a; Thomson and Wild, 1997b]. In adult

rats, the intestinal uptake of sugars and lipids is enhanced by feeding an isoenergetic semisynthetic diet enriched with saturated as compared with polyunsaturated fatty acids [Thomson et al., 1986].

Steroids induce precocious development of the intestinal brush border membrane (BBM) enzymes, and facilitate the induction of several BBM enzymes by dietary carbohydrate [Dere et al., 1967; Lebenthal et al., 1972; Murphy, 1982]. Steroids given by mouth enhance glucose absorption by adult animals [Batt and Peters, 1976; Batt and Peters, 1975; Batt and Scott, 1982], but dexamethasone given subcutaneously blunts the expected morphological adaptive response following intestinal resection: mucosal growth and mucosal hyperplasia [Park et al. 1994]. The adaptive effect of locally or systemically acting steroids on intestinal nutrient transport associated with changes in dietary lipids has not been described.

Budesonide enhances the immunosuppressive effects of cyclosporin A and prolongs small bowel allograft survival without affecting normal adrenocorticotrophic hormone (ACTH) release [Ozcay et al., 1997]. Therefore, budesonide may be used as a immunosuppressive agent in intestinal transplantations.

Accordingly, this study was undertaken to assess the influence of budesonide and prednisone, in doses equivalent to those used in clinical practice, on the intestinal uptake of sugars and lipids in young growing male rats, as well as the influence of feeding isocaloric semisynthetic saturated or polyunsaturated diets on the intestinal uptake of sugars and lipids in young rats receiving four weeks oral gavage of either the systemical

active glucocorticosteroid prednisone (PRED), or the topically active steroid budesonide (BUD).

B) LITERATURE REVIEW

1) Normal Mechanisms

The mechanisms of nutrient uptake are remarkable efficient allowing only 5 % of ingested carbohydrate. fat and protein to be excreted in the stools of adults. Specific mechanisms and transporters have been related to the uptake of sugars, lipids and proteins.

1.1) Sugar Uptake

Since glucose plays a important role in the metabolism of most cells. the transport of D-glucose across plasma membrane is a crucial cellular event. It has been determined by Crane that the Na^+-gradient across the intestinal epithelial cell provides the driving force responsible for the glucose transport through the brush border membrane [Riklis and Quastel. 1958]. The Na^+-gradient is maintained by the action of the Na^+K^+-ATPase which is restricted to the basolateral membrane [Hirayama et al.. 1992]. The Na^+-dependent glucose transporter (SGLT1) mediates the brush border membrane (BBM) Na^+/glucose cotransport [Vehyl et al.. 1993: Vehyl et al.. 1992: Weber et al.. 1991].

The transporter responsible for the exit of glucose through the basolateral membrane is the Na^+-independent sugar transporter (GLUT2). This passage across the basolateral membrane (BLM) occurs by facilitative Na^+-independent diffusion [Caspary and Crane. 1968].

Three kinetic parameters: the maximal transport rate (Vmax). the Michaelis constant (Km) and the passive permeability coefficient (Pd) have been used to characterize the properties of the intestinal glucose transport. Correction for the effective

resistance of the intestinal unstirred water layer (UWL) has been demonstrated to be necessary when estimating the kinetics parameters of sugar uptake. For instance, without correction for UWL, the value of Km is overestimated whereas Pd is underestimated. Although the Vmax is not altered by the UWL resistance, the uptake of higher concentrations of hexoses involves both an active and a passive component [Stevens et al., 1990; Takata et al., 1992; Thomson and Dietschy, 1977; Thomson and Dietschy, 1980; Thomson and Wild, 1997a; Thomson and Wild, 1997b].

1.1.a) SGLT1

The Na^+-dependent glucose transporter was first cloned by Wright and his colleagues [Hediger et al., 1987]. In man, the SGLT1 gene has been mapped to the distal arm of chromosome 22 [Hediger et al., 1989], and encodes polypeptides of 664 amino acids. The functional SGLT1 transporter is about 290 kb, and is thought to be a homotetramer [Smith et al., 1992].

Two potential sites for glycosylation at positions 248 and 306 have been identified. The post-translational glycosylation adds about 15 kd to the apparent mass of SGLT1 [Hediger et al., 1987]. SGLT1 does not have the Arg-X-G-ly-Arg-Arg sequence characteristic of all other sugar transporters [Hediger et al., 1987; Loo et al., 1992]. No evidence has shown regulation of the expression of SGLT1 by phosphorylation. However, a potential site for protein kinase A has been recognized in SGLT1 and the binding sites of SGLT1 to glucose were increased by phosphorylation of the sites of SGLT1 in BBM. These findings indicate that the regulation of phosphorylation of

SGLT1 leads to an alteration of its function and results in the control of glucose transport in the rat small intestine [Ishikawa et al., 1997].

SGLT1 binds two Na^+ for each glucose molecule. The binding of Na^+/Na^+ markedly reduces the Km for glucose from about 100 mM to 0.1 mM. In the presence of a Na^+-gradient, the rate-limiting step in glucose uptake is the return of SGLT1 from the cytoplasmic to the luminal side of the BBM. When the glucose concentration in the cytoplasmic gets too high, glucose stops coming from the SGLT1 and the Vmax falls.

An interaction between the activities of SGLT1 and GLUT2 must occur to result in an increase of cytoplasmic glucose concentration [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Hirayama et al., 1997].

Structure analysis of SGLT1 predicts that this transporter spans the BBM 14 times [Turk et al., 1996]. An aspartic acid is conserved at the interface between the first transmembrane segment and the NH2-terminal. Mutations such as substitution from Asp28 to Asn28 impair sugar uptake resulting in the rare child with glucose-galactose malabsorption [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Martin et al., 1997; Martin et al., 1996; Desjeux and Wright, 1993; Turk et al., 1991].

Since the glucose transport by SGLT1 is only observed from the luminal to the intracellular side of the BBM, and phlorizin inhibits SGLT1 only from the luminal side, we can conclude that this transporter is an asymmetric protein. Cyclosporin binding sites are present on the extracellular side of SGLT1 [Karasov and Diamond, 1983; Koepsell et al., 1988; Lazaridis et al., 1997; Sarker et al., 1997; Suzuki et al., 1996].

The use of monoclonal antibodies against the renal Na^+-dependent glucose transporter that react with intestinal SGLT1 allowed the detection of higher antigenic sites in the jejunum, intermediate in ileum and lower in the duodenum. However, no differences along the length of the villus were realized [Gould and Holman, 1993; Koepsell et al., 1988; Freeman et al., 1993; Hopfer, 1987; Burant et al., 1994]. Using polyclonal antibodies against rabbit intestinal BBM SGLT1, crypt cells show no staining and the onset of SGLT1 appears to start at the crypt-villus junction [Hwang et al., 1991]. The amount of mRNA for SGLT1 also increases towards the mid-portion of the villus, then falls slightly [Freeman et al., 1993].

The functional SGLT1 consists of two subunits: a catalytic one and a regulatory one, both presenting molecular weight of about 70 kd [Vehyl et al., 1992; Poppe et al., 1997]. It is speculated that dietary and hormonal manipulations would affect the expression of SGLT1, modulating the transcription and post-transcription processing of the regulatory subunit of SGLT1 [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b; Dong et al., 1997]. Protein kinase A and protein kinase C regulation of SGLT1 has also been proposed [Wright et al., 1997; Ishikawa et al., 1997].

1.1.b) SGLT2

Low stringency screening with high affinity to SGLT1 has been used to isolate a 2271-nucleotide base pair cDNA from human kidney [Mackenzie et al., 1996]. This clone encodes SGLT2 which presents 59% homologous at the amino acid level to SGLT1 and is present in trivial levels in the intestine [Barfuss and Sachfter, 1981].

1.1.c) GLUT2

The sugar transporter restricted to the BLM which facilitates the transport of glucose, galactose, mannose and fructose is named GLUT2 [Thorens et al., 1988; Thorens et al., 1990; Burant and Bell, 1992; Cheeseman, 1993; Maenz and Cheeseman, 1987]. It has 55 % homology with the aminoacid sequence of GLUT1, a fact that allowed the cloning of GLUT2 using GLUT1 cDNA probe for screening rat and human liver cDNA libraries under conditions of low stringency [Thorens et al., 1988]. GLUT2 has an apparent molecular weight of 61 kd and it is expressed along the villus, being absent from the crypts.

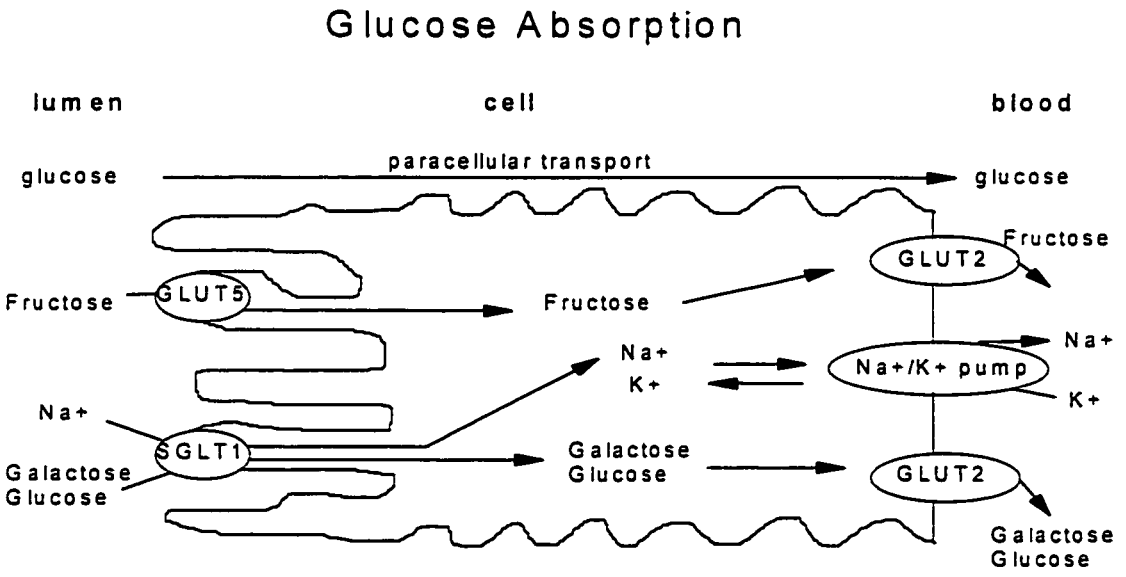
1.1.d) GLUT5

Fructose is absorbed by a facilitated diffusion, a process that is mediated by a member of facilitative sugar carriers named GLUT5 or Na^+-independent fructose transporter [Burant et al, 1992a, Burant et al., 1992b; Crouzlon and Korieh, 1991; Davidson et al., 1992; Rand et al., 1993a; Rand et al., 1993b; Shu et al., 1997; Wasserman et al., 1996]. It presents a Km for fructose uptake of 6mM and it has an apparent molecular weight of about 46 kd. It is confined to the BBM and is maximally expressed at the tip of the villus, contrasting with the maximum level of GLUT5 mRNA in the mid-villus region.

Possible regulation of GLUT5 involving cAMP has been suggested since treatment of Caco-2 cells with forskolin, an activator of cAMP increases the level of GLUT5 mRNA [Brot-larohec et al., 1992]. Analogs of forskolin that do not affect

adenylate cyclase activity do not change the expression of GLUT5 reinforcing the influence of cAMP on GLUT5 regulation.

Figure 1. Model of Sugar Uptake



1.2) Lipid Uptake

Most lipids are absorbed in the proximal intestine. more specifically at the villus tip [Borgstrom et al.. 1957; Haglund et al., 1973; Ladman et al.. 1963; Fingerote et al.. 1964]. In the process of lipid absorption, free fatty acids. monoacylglycerols. lysophosphatidylcholine and free cholesterol must initially diffuse across the UWL and then the BBM. These products of lipid digestion are solubilized in mixed micelles composed of cholesterol. bile acids (mainly taurine or glycine conjugates of cholic and chenodeoxycholic acids) and phospholipids (phosphatidylcholine). Once in the cytoplasm. the lipids are taken up by the cytoplasmic fatty acid binding proteins [Chow

and Hollander. 1979: Stremmel. 1988; Schoeller et al.. 1995a: Schoeller et al.. 1995b: Thomson et al., 1996; Thomson and Wild. 1997a; Thomson and Wild. 1997b].

1.2.a) Passive Lipid Uptake

Classically lipid absorption has been defined as an energy-independent. passive diffusion process down a concentration gradient. Short- and medium-chain fatty acids are not influenced by the UWL. while passive long-chain fatty acid uptake is rate-limited by passage through the UWL [Proulx et al.. 1984a: Proulx et al.. 1984b: Proulx et al.. 1984c: Westegard and Dietschy. 1974].

Three models of passive lipid uptake have been proposed [Thomson and Dietschy. 1981]:

- The entire mixed micelle is absorbed by the BBM. No experimental evidence for this model has been published [Wilson and Dietschy. 1972]
- The micelle collides with the BBM allowing lipids to be taken up directly. The collision model has been proposed for the cholesterol uptake [Proulx et al.. 1984c; Burdick et al. 1994].
- The micelle dissociates in the aqueous compartment of the UWL. releasing the lipids that then are taken up by the BBM. This model has been proposed for fatty acid uptake as well as cholesterol uptake [Westegard and Dietschy. 1976; Chijiiwa and Linscheer. 1987].

Modifications of this last model have been proposed. The dissociation of lipids from the mixed micelle would be under influence of the acidic microclimate adjacent to the BBM [Shiau and Levine. 1980; Shiau. 1990]. The low pH environment increases the

critical micellar concentration of fatty acids and cholesterol, and the fatty acids become protonated, increasing their hydrophobic and lipophilic properties [Small et al., 1984].

Other important aspect influencing the passive uptake of lipids is the membrane fluidity, more specifically the outer third of the BBM bilayer [Meddings, 1988]. The fluidity of the membranes is influenced by the presence of dietary lipids, once the jejunal BBM is exposed to the majority of dietary lipids and presents higher fluidity than the ileal BBM, which is exposed to far less dietary lipids. Other factors influencing the absorption of lipids may involve the translocation of lipids from the outer bilayer to the inner bilayer, which depends on the composition and properties of the two membranes and composition of the lipid to be absorbed [Devaux, 1991; Meddings, 1988; Meddings and Thiessen, 1989].

1.2.b) Protein-mediated lipid uptake

The lipid uptake may also be protein-mediated as it has been evidenced by the following findings: there is a curvilinear relationship between uptake and very low concentrations of fatty acid [Chow and Hollander, 1979; Stremmel, 1988; Schoeller et al., 1995a; Schoeller et al., 1995b]; a plasma membrane fatty acid binding protein has been identified in the BBM of rat intestine [Stremmel et al., 1985; Londraville, 1996]; the fatty acid uptake is Na^+- and pH-dependent [Stremmel, 1988; Ling et al., 1989 and Schoeller et al., 1995); the fatty acid uptake may be inhibited by using a polyclonal antibody to the $FABP_{pm}$ or a monoclonal antibody to the $FABP_{pm}$ [Stremmel, 1988; Schoeller et al., 1995]; and the use of trypsin, chymotrypsin, pronase or heat treatment inhibit fatty acid uptake [Potter et al., 1989, Schoeller et al., 1995; Stremmel, 1988].

The dependence of fatty acid uptake on Na^+- and pH relates the lipid uptake to the NHE_3, the Na^+/H^+-antiporter in the BBM that performs the double exchange of Na^+ and H^+. Experiments performed with oleic acid confirm this dependence. A time-course of oleic acid uptake into rabbit jejunal bush border vesicles was increased markedly in the presence of opposing Na^+ and H^+ gradients [Schoeller et al., 1995a]. Furthermore, antibody to $FABP_{pm}$ inhibits oleic acid uptake in rabbit jejunal BBM vesicles only in the absence of opposing Na^+ and H^+gradients suggesting that oleic acid binds passively to the BBM lipids in a protonated form under acidic conditions maintained by the Na^+/H^+-antiporter, and that the $FABP_{pm}$ is a important component of fatty acid uptake under conditions of low luminal pH or absence of Na^+ [Schoeller et al., 1995a; Schoeller et al., 1995b; Stremmel, 1988; Lucas and Mathan, 1989].

Three isoforms of NHE have been described according to their Na^+ affinity and kinetics, their sensitivity to amiloride and analogues and their regulation by second messengers [Gristein et al., 1989]. The NHE-1 isoform is found in almost all tissues including the BLM of the gut and the NHE-2 isoform is found in the BBM of gut, kidney and adrenal gland. Both isoforms present similar affinity for Na^+, have an internal modifier site, present equal sensitivity to amiloride and are stimulated by serum and phorbol esters. However, different sensitivity to the amiloride analogue ethylisopropylamiloride have been reported [Glesson, 1992]. A third isoform called NHE-3 is found specifically in epithelial cells and it has been isolated in human and rabbit intestine and kidney [Tse et al., 1992].

The intestinal BBM $FABP_{pm}$ is a 40 kd membrane protein found in the BBM of the villus and crypt cells of rat jejunum and ileum [Stremmel et al., 1985]. It is functionally and immunologically similar to $FABP_{pm}$ found in hepatocytes, cardiac myocytes and adipocytes [Potter et al., 1987; Sorrentino et al., 1988], but it is different from the family of cytosolic fatty acid binding proteins [Veerkamp et al., 1991]. This membrane protein was postulated to mediate fatty acid uptake through an active sodium-dependent process, and its kinetics have been described as a Vmax of 2.1 nmoles/min. and a Km of 93 nmoles [Stremmel, 1985; Stremmel, 1988].

More recently, a 88 kd membrane protein named FAT was cloned and associated with the sequestration of fatty acids in adipocytes [Abumrad et al., 1993]. The FAT expression is restricted in the adult rat to the site of long chain fatty acid absorption, i.e. the small intestine, suggesting a complementary role of these proteins in intestinal fatty acid uptake [Poirier et al., 1996]. A polyunsaturated enriched diet has been demonstrated to induce an increase in FAT mRNA [Poirier et al., 1996]. The mechanism of this dietary fat induction remains to be determined.

In the enterocyte cytosol there are other fatty acid binding proteins responsible for transport of the absorbed fatty acids to their sites of metabolism [Ek et al., 1997; Lucke et al., 1996]. The intestinal $FABP_c$ or I-$FABP_c$ is present exclusively in the intestine, has a molecular weight of 14-15 kd [Kaikaus et al., 1990; Jolly et al., 1997], it is observed in mature villus tip cells, it is absent in crypt cells [Iseki and Kondo, 1990; Sweetser et al. 1988], it binds palmitic, oleic and arachidonic acids, it has a higher affinity for saturated fatty acids and has a stoichiometry of moles of fatty acids bound to moles of binding

protein of 1:1 [Cistola et al., 1989; Kaikaus et al., 1990; Peeters et al., 1989]. The liver $FABP_c$ or L-$FABP_c$ is present in both the liver and the intestine; has a molecular weight of 14-15 kd [Kalaus et al., 1990; Jolly et al., 1997], it is confined to the crypt-villus junction [Iseki and Kondo, 1990], it binds saturated and unsaturated fatty acids as well as monoacylglycerols, lisophospholipids and bile salts. it has a stoichiometry of moles of fatty acid bound to moles of binding protein of 1-3:1, and it has a higher affinity for polyunsaturated fatty acids [Cistola et al. 1989; Kaikaus et al., 1990; Peeters et al., 1989].

Bile acid transport in the terminal ileum is a good example of protein-mediated uptake of lipids [Lewis and Root, 1990]. The BBM bile acid transporter is Na^+-dependent and has a higher affinity for unconjugated than for conjugated bile acids. The Na^+-dependent bile acid transporter is a integral membrane glycoprotein that functions in the enterohepatic circulation of bile acids [Gartung et al., 1996; Dawson and Oelkers, 1995; Lester and Ziminiak, 1993; Stieger et al., 1997].

Table 1. Fatty Acid Binding Proteins

Fatty Acid Binding Proteins	Molecular Weight	localization	Fatty Acids Transported
BBM $FABP_{pm}$	40 kd	BBM	saturated and polyunsaturated fatty acids
FAT	88 kd	BBM	unknown
I-$FABP_c$	14-15 kd	cytosol; villus tip cells	preferentially saturated fatty acids
L-$FABP_c$	14-15 kd	cytosol: crypt-villus junction cells	preferentially polyunsaturated fatty acids

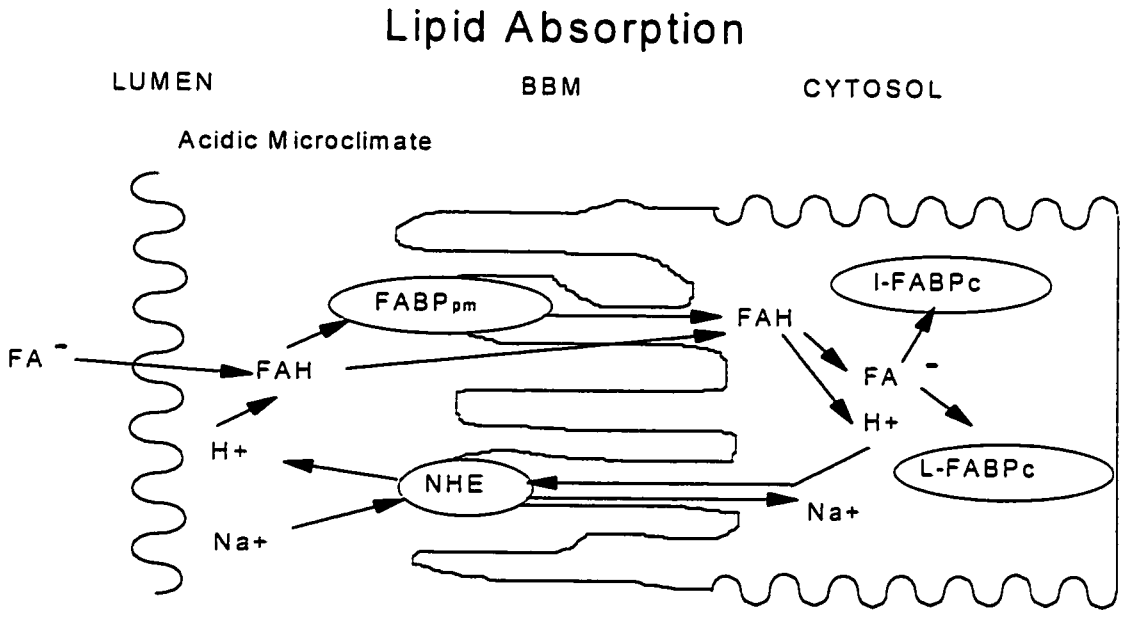
BBM $FABP_{pm}$: Brush border membrane fatty acid-binding protein

FAT: Fatty acid transporter

I-$FABP_c$: Intestinal fatty acid-binding protein

L-$FABP_c$: Liver fatty acid-binding protein

Figure 2. Model of Lipid Uptake



FA: Fatty acids

FAH: Protonated fatty acids

$FABP_{pm}$: Brush border membrane fatty acid-binding protein

$I\text{-}FABP_c$: Intestinal fatty acid-binding protein

$L\text{-}FABP_c$: Liver fatty acid-binding protein FABP

NHE: Na^+/H^+ exchanger

2) Intestinal Adaptation

Intestinal adaptation is one of many biological processes where structural and functional modifications occurs in response to external or internal stimuli. On other words, intestinal adaptation is the ability of the intestine to change functionally in response to differences in environmental conditions. For instance, intestinal adaptation improves the nutritional condition in cases following the loss of a major portion of the small intestine ("short bowel syndrome"), following chronic ingestion of ethanol, and following sublethal doses of abdominal irradiation [Thomson et al., 1989; Tappenden et al., 1996; Tappenden et al., 1997; Gambara et al., 1997; Thomson and Wild, 1997a; Thomson and Wild, 1997b]. Therefore, intestinal adaptation has important implications in survival potential and welfare.

The mechanisms of intestinal adaptation occur at a variety of levels: physiological, cellular and molecular, and most of the signalling process involved in adaptation is still not well understood. Signals of adaptation may relate to hormone levels, ATP levels or changes in luminal solute concentration. The signals, mechanisms and the adaptive process may be different in the jejunum and ileum, explaining the found site-specific alterations.

The adaptive process has been defined in terms of kinetics as changes in the maximal transport rate (Vmax) and in the Michaelis affinity constant of nutrients transported actively (sugars and amino acids), as well as alterations in the passive permeability coefficient of nutrients obviously transported passively (short-, medium-

and long fatty acids. and cholesterol) [Thomson et al.. 1996: Thomson and Wild. 1997a: Thomson and Wild. 1997b].

Dynamic morphologic parameters may also adapt. For instance. the crypt cell production rates or the enterocyte migration rates are known to change in some situations of intestinal adaptation [Thomson et al.. 1994]. Although. both kinetics and dynamic morphologic parameters are altered in the adaptive process. it is much easier to explain the influence of this process on nutrient uptake by kinetic alterations.

Many models of intestinal adaptation have been described. For instance. glucose uptake has been found to be increased during pregnancy [Musacchia and Hartner. 1970]. lactation [Cripps and Willians. 1975]. high carbohydrate intake [Diamond and Karasov. 1984]. hyperglycemia [Csaky and Fisher. 1981: Fischer and Lauterbach. 1984: Maenz and Cheeseman. 1986] and after intestinal resection [Glesson et al.. 1972: Robinson et al.. 1982]. Aging has been associated to an increase in the protein-mediated transport of hexoses and a decrease in the passive uptake of lipids [Bowman and Rosemberg. 1983: Hollander and Morgan. 1979; Tolozoa and Diamond. 1992; Thomson. 1979. 180. 1981]. Diabetes mellitus increases both protein-mediated and passive transport of sugars [Fedorak et al.. 1987: Thomson and Rajotte. 1983]. External abdominal irradiation and chronic ethanol consumption decrease both active and passive components of uptake [Thomson et al.. 1983. 1984].

Phospholipid. cholesterol and fatty acid composition of plasma membranes may be modified in mammalian cells [Spector and Yorek. 1985]. These changes in the membrane lipid composition may affect the physical properties of the membrane. which

can alter and influence the activity of membrane-bound proteins as SGLT1. GLUT5 and other transporters. For example, changes in the BBM fluidity have been demonstrated to influence the passive uptake of lipids as well as the carrier-mediated D-glucose uptake [Brasitus et al., 1989: Meddings, 1988, 1990; Meddings and Thiessen, 1989: Hyson et al., 1997].

Dietary constituents provide a continual signal for intestinal adaptation [Thomson and Keelan, 1986]. Every day enterocytes are exposed to different nutrients which vary according to the intake and availability. For that reason, the intestine must be able to adapt as soon as possible to variations in the dietary load and composition [Diamond, 1991]. The exposure to different diets appear to have extreme importance when occurred at young age, and this concept has led to the theory of "critical period programming" [Karasov et al., 1985: Keelan et al., 1990]. This periods is defined as "a biological mechanism which is turned irreversibly on or off only once during an individual's lifetime in response to conditions prevailing at some critical stage" [Karasov et al., 1985].

Changes in villus height, BBM lipid composition, nutrient transport and BBM enzyme activity in response to alterations in the dietary macronutrient content (carbohydrate, cholesterol, essential fatty acids and protein) have been demonstrated [Thomson and Rajotte, 1983; Keelan et al., 1987, 1990]. For example, animals fed PUFA enriched diets have a decline in glucose uptake as compared to animals fed SFA enriched diet [Thomson et al., 1987]. In relation to the carbohydrates, animals fed a glucose enriched diet have an increased sugar uptake, resulting from up-regulation of both BBM and BLM glucose transporters [Cheeseman and Maenz, 1989; Cheeseman and Harley,

1991, Ferraris et al., 1992; Dyer et al., 1997]. A fructose enriched diet also results in enhancement of fructose uptake and increased expression of GLUT5 [Burant and Saxena, 1994; Shu et al., 1997; Monteiro and Ferraris, 1997]. Therefore, changing the composition of the diet, modifications in the transport of nutrients are expected.

2.1) Patterns and Mechanisms of Adaptation

The alterations in the cell kinetics that result in modification of the nutrition status may be specific or non-specific. The last one involves kinetic alterations that would result in changes in the intestinal mucosal mass and villus surface area, leading to a non-specific alteration in the nutrient uptake. It would affect nutrients absorbed actively, as well as those absorbed passively [Rand et al., 1993]. On the other side, specific mechanisms involve up- or down-regulation of specific transporters responsible for the uptake of particular nutrients, or alterations in the lipid composition of the membrane cellular, changing the passive permeability properties of the brush border membrane [Thomson et al., 1996; Thomson and Wild, 1997a; Thomson and Wild, 1997b].

2.2) Dietary Lipids

Early exposure of weaning rabbits to a high-cholesterol diet for 6 weeks leads to an increase of acyl-CoA-cholesterol acyltransferase (ACAT) activity [Subbish et al., 1989]. The persistence of this enhanced capacity to form cholesterol esters is evidenced when these animals are re-exposed to high-cholesterol diets after being fed a normal diet for nine weeks. Therefore, depending upon early dietary lipid experience, there may be a different amount of cholesterol absorbed in later life. This fact brings up the possibility

that lipid absorption and metabolism may be influenced genetically by a process that is modulated by early childhood events involving nutritional experiences.

Current North American diet contains up to 34 % of fat [NHANES III, 1993]. Several kinds of cancer and cardiovascular diseases are associated with the intake of high dietary fat. More specifically, cholesterol and saturated fats are highly involved in the process of pathogenesis of many oncogenic diseases as well as cardiovascular diseases. On the other side, polyunsaturated fats are thought to be beneficial in decreasing the incidence of these pathogenic processes. Further benefits may be observed with a diet enriched in ω3 polyunsaturated fatty acids as compaired with a diet rich in ω6 polyunsaturated fatty acids [Bang et al., 1980; Carrol, 1986].

BBM total lipid composition is not changed significantly in animals fed diets of different fatty acid composition [Keelan et al., 1990; Thomson et al., 1986. Thomson et al., 1987]. However, in other models of intestinal adaptation such as streptozocin-induced diabetes, chronic ethanol feeding, intestinal resection, aging and following external abdominal irradiation, the BBM total phospholipid content composition changed, and may contribute to the adaptive alterations in these models [Keelan et al., 1985, Keelan et al., 1986]. Therefore, the adaptive alterations in nutrient transport observed with dietary lipids are not fully explained by alterations in the BBM lipid composition.

Feeding diabetes rats a high fat diet enriched in ω6 polyunsaturated fatty acids results in a decrease in the intestinal sugar uptake and an improvement in the clinical control of the diabetes [Rajotte et al., 1988; Thomson et al., 1987; Thomson et al., 1988]. Further negative effect on intestinal sugar uptake is again observed with diets enriched in

ω3 polyunsaturated fatty acids [Thomson et al.. 1988]. Therefore. a diet enriched in polyunsaturated fatty acids may be the importance when handling diabetic patients.

The effects of dietary lipids on another model of intestinal adaptation. 50% ileal resection. have also been published [Keelan et al.. 1996]. Once again negative effects on intestinal uptake were obtained with those animals fed the polyunsaturated diet. while those fed the saturated diet had a jejunal uptake of D-glucose twice as high in those fed the polyunsaturated diet. In this case. it would be beneficial to prescribe a diet rich in saturated fatty acids for those patients which have undergone an intestinal resection.

Although the effects of dietary lipids have been described. the mechanisms by which dietary lipids may mediate changes in membrane structure and function are not well understood.

Dietary fatty acids are modulators of BBM phospholipid fatty acid composition [Keelan et al.. 1990]. A diet enriched with saturated fatty acids is associated with increases in the saturation of BBM phospholipid fatty acids. while a diet enriched with ω6 polyunsaturated fatty acids is associated with an increase in the unsaturation of BBM phospholipid fatty acids [Thomson et al.. 1996: Thomson and Wild. 1997a: Thomson and Wild. 1997b]. The degree of fatty acid unsaturation or saturation is one of the factors that influence the fluidity of the BBM. Changes in the fluidity of the membrane may alter the permeation of molecules and nutrients through this barrier, as well as the conformation of binding sites on proteins as SGLT1, GLUT5.

The diet changes in the membrane lipid composition may be modulated by alterations in enterocyte microsomal membrane desaturase activity [Keelan et al. 1994].

Changing the enzymes involved in lipid synthesis. dietary lipids would subsequently alter the lipid composition of the membrane. Interestingly, there is a lack of changes in BBM cholesterol in response to dietary lipids suggesting that the BBM cholesterol is tightly controlled [Keelan et al.. 1994]. However. the enterocyte microsomal membrane cholesterol level is influenced by diet and endogenous synthesis.

Alterations in enterocyte microsomal membranes lipid composition do not always explain the alterations found in the BBM lipid composition [Keelan et al. 1996]. There may be heterogeneity of the lipids along the villus. and studies done with mixtures of cells collected from the entire villus may obscure alterations occurring at just one portion of the villus. Alternatively. there may be some modification of the phospholipid fatty acids after the lipids leave the microsomes of the enterocyte.

2.3) Enterocyte Microsomal Membranes

Once in the cytosol the absorbed free fatty acids are transported to the endoplasmic reticulum by the fatty acid binding proteins (see section 1.2.b). where they are used for re-synthesis of triacylglycerols. re-synthesis of phospholipids and esterification of cholesterol. These re-synthesized lipids and newly synthesized lipids are then directed to membrane synthesis or lipoprotein assembly [Keelan et al.. 1994].

The first step of fatty acid metabolism involves the acyl-CoA synthetase action that converts these free fatty acids in acyl-CoA. This acyl-CoA may now be used for reesterification of absorbed cholesterol and lysophosphatidylcholine. for synthesis of phosphatidic acid and for synthesis of diacylglycerol. The diacylglycerol may be used for

the synthesis of triacylglycerols and/or phospholipids by the action of choline and ethanolamine phosphotransferases [Brindley and Hubscher, 1966; Ohkubo et al., 1991].

In relation to the phospholipid synthesis, two enzymes are important: one is the CTP:phosphocholine cytidyltransferase, enzyme that converts phosphocholine to CDP-choline, a conversion that is the rate-limiting step in the synthesis of phosphocholine, the largest phospholipid found in many membranes, including intestinal BBM [Pelech et al., 1983a; Pelech et al., 1983b]; and the other is the phosphatidylethanolamine N-methyltransferase that converts phosphatidyethanolamine to phosphocholine [Bremer and Greenberg, 1961].

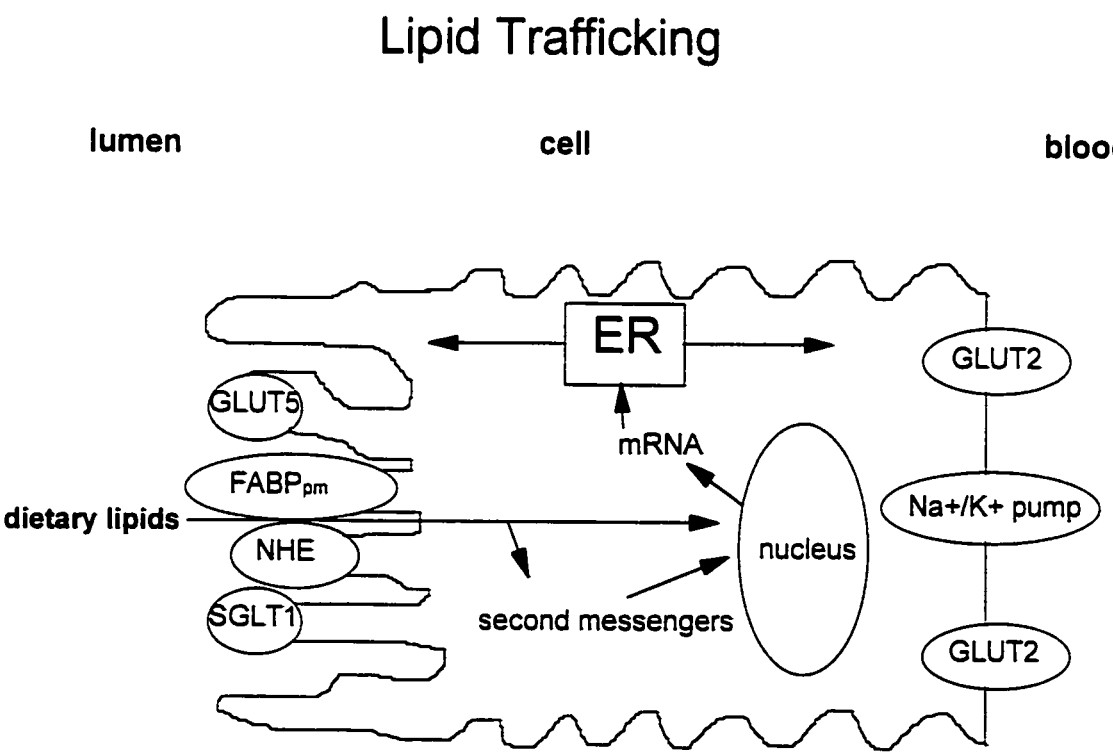
Free fatty acids are also desaturated and elongated in the endoplasmic reticulum to form long-chain polyunsaturated fatty acids [Strittmatter and Rodgers, 1975; Holloway and Holloway, 1975, 1977]. The conversion of linoleic acid (18:2ω6) to γ-linolenic acid (18:3ω6), and α-linoleic acid (18:3ω3) to 18:4ω3, is performed by Δ6-desaturase which is the rate-limiting step in the synthesis of arachidonic acid (20:4ω6) and eicosapentanoic acid (20:5ω3) [Sprecher, 1981]. Desaturases are endoplasmic reticulum intrinsic membrane proteins that remove two hydrogen atoms from acyl-CoA derivated from exogenous or endogenous sources, forming a cis double bond [Jeffcoat, 1979; Rogers and Strimatter; 1973, 1974]. It is suggested that desaturase activity may influence or regulate the differences found in jejunal and ileal EMM (enterocyte microsomal membranes) and BBM in terms of fatty acid composition [Garg et al., 1990; Keelan et al., 1990].

Dietary lipids may produce changes in EMM lipids, BBM lipids and BBM uptake as a result of unknown signals that would affect the activities of EMM lipid enzymes or

would interfere in the gene expression of EMM lipid enzymes and BBM transporters. Changes in enterocyte microsomal enzyme (EMM) activities may be mediated by dietary lipids acting to modify signal transduction by second messengers as cAMP, Ca^{+2} and diacylglycerol, altering RNA expression [Keelan et al., 1994].

The lipids present in the diet may also influence lipid synthesis. During the phospholipid synthesis, ω3 fatty acids compete with ω6 fatty acids for the sn-2 position of phospholipids, resulting in the enrichment of ω3 fatty acids in phospholipids. Long chain fatty acids may also serve as substrates for desaturases and may be desaturated and elongated to form long-chain polyunsaturated fatty acids.

Figure 3. Lipid Trafficking



3) Glucocorticosteroids

3.1) Introduction

Thus far in the literature review, I have considered normal mechanisms and have reviewed the topic of intestinal adaptation. Because glucocorticosteroids have been implicated as possibly being important in the ontogeny of intestinal development, and because glucocorticosteroids affect the intestine, I developed hypotheses related to intestinal adaptation, glucocorticosteroids, dietary lipids and intestinal nutrient transport. In this section of my literature review I provide a summary of the effects of glucocorticosteroids on the intestine [Batt and Peters, 1976a; Batt and Peters, 1976b; Scott et al., 1980; Scott et al. 1981; Batt and Scott, 1982; Murphy, 1982; Park et al., 1994; Spitz et al., 1994; Guo et al., 1995; Marti and Fernandez-Otero, 1994; McDonald and Henning, 1992; Lebenthal et al., 1972; Nsi-Emvo et al., 1996; Sangild et al., 1995; Smeard and Walker, 1988]. For sake of completeness, I will also consider the effect of glucocorticosteroids on other body systems.

There is a large number of bioactive hormones secreted from the adrenal cortex The hormones secreted in significant amount are: hydrocortisone or cortisol, corticosterone and aldosterone. All three have mineralocorticoid activity and cortisol and corticosterone have glucocorticoid activity [Haynes and Murad, 1985; Haynes and Larner, 1975; Crossland J, 1980; Bowman and Rand, 1980]. The most abundant of these is cortisol [Haynes and Murad, 1985, Haynes and Larner, 1975; Crossland, 1980; Baxter, 1979]. Cortisol accounts for approximately 95 % of all glucocorticoid activity in humans.

Glucocorticosteroids are a type of corticosteroids that have potency based on liver glycogen deposition. The major naturally occurring glucocorticosteroid in man is cortisol [Baxter, 1979; Peterson and Pierce, 1960], and in rats and mice the major glucocorticosteroid is corticosterone [Shimizu et al., 1983].

3.2) Historic Perspectives

Cortisone was the first corticosteroid to be discovered in 1935 by Edward C. Kendal of the Mayo Foundation for Medical Education and Research. Cortisone was initially called compound E [Aviado, 1972]. In 1946, Sarret partially synthesized cortisone [Aviado, 1972; Baxter, 1979]. Adrenal extracts containing cortisol had been used in therapy even earlier [Baxter, 1979; Ehrich et al., 1992]. In 1948, the 37 steps in the synthesis of cortisone were elucidated [Aviado, 1972; Laurence and Bennet, 1992; Grollman and Grollman, 1970]. In the same year, compound E was named cortisone [Aviado, 1972].

In 1946 Hennch undertook the first therapeutic use in rheumatoid arthritis, with dramatic results [Crossland, 1980; Aviado, 1972; Laurence and Bennet, 1980]. Shortly afterwards corticosteroids were introduced to treat asthma. Since 1950, the glucocorticosteroids have been one of the mainstays in the therapy of active ulcerative colitis and Crohn's disease [Bratssand, 1990].

3.3) Biosynthesis

Corticosteroids are obligatory obtained from cholesterol. Part of this cholesterol is from the diets [Haynes and Murad, 1985; Haynes and Larner, 1975; Bowman and Rand, 1980]. The another portion of cholesterol is synthesized and stored within the adrenal

glands [Bowman and Rand. 1980]. This biosynthesis is called corticosteroidogenesis. It occurs at rest or following the administration of adrenocorticotrophic hormone (ACTH) at the inner mitochondrial membrane [Stocco and Clark. 1996; Simpson et al.. 1979]. In fact the rate-limiting step of biosynthesis of glucocorticosteroids is the delivery o the substrate. cholesterol. to the inner mitochondrial membrane. Many proteins such as sterol carrier protein 2 (SCP2). steroidogenesis activator protein polypeptide (SAP). peripheral benzodiazepine receptor (PBR) and steroidogenic acute regulatory protein (StAR) have been described to regulate the transport of cholesterol to the inner mitochondrial membrane [Stocco and Clark. 1996].

When administration of ACTH does not result in an increase in cortisol (major human glucocorticosteroid) or corticosterone (major rat glucocorticosteroid) blood concentrations. there is inhibition of the hypothalamic-pituitary-adrenocortical axis. The use of synthetic glucocorticosteroids frequently blocks the ACTH response. A stimulatory action of free fatty acids (such as oleic and linoleic acids) on corticosteroidogenesis has been proposed. This modulation of corticosteroidogenesis by these naturally occurring lipids may be an important component of the control mechanisms within the hypothalamic-pituitary-adrenocortical axis in disorders of lipid homeostasis. such as obesity. starvation or diabetes [Sarel and Windmaier. 1995].

Adrenocortical cells have large numbers of receptors that mediate the uptake of low density lipoprotein (LDL). the main source of cholesterol [Haynes and Murad. 1985: Haynes and Larner. 1975; Grollman and Grollman. 1970; Myles and Daly. 1974: Simpson and Mason. 1979; Cope. 1972; Rainey et al.. 1992]. The reactions catalyzed by

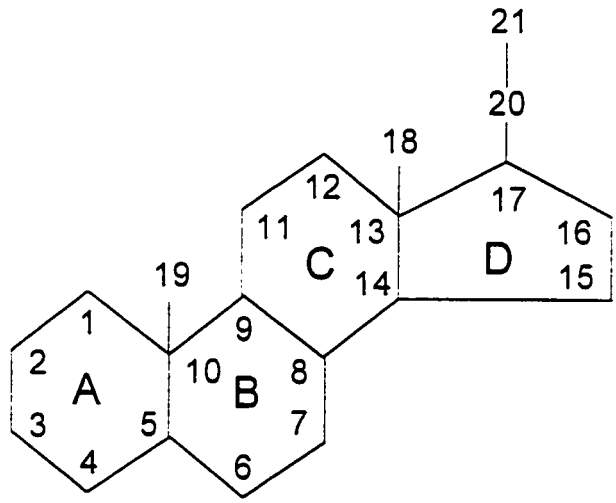
mixed-function oxidases that contain cytochrome P-450. and require NADPH and molecular oxygen [Haynes and Murad. 1985: Haynes and Larner. 1975; Simpson and Mason. 1979: Bowman and Rand. 1980: Nonaka Y. et al.. 1995]. and convert cholesterol to 21-carbon corticosteroids. Many reactions involved in steroid metabolism are hydroxylation reactions. in which one atom of oxygen is inserted into the hydroxyl group of the product. and the other is reduced to water. These reactions are called mixed function oxidase [Simpson and Mason. 1979], and consist of 17α-hydroxylation to form Δ17α-hydroxyprogesterone. 5-3β-hydroxyl configuration to Δ4-3 keto configuration to form progesterone, 21-hydroxylation to form 11-desoxycortisol. and 11-β-hydroxylation to form cortisol [Barnes. 1992; Bowman and Rand. 1980]. The forms of rat CYP11B genes CYP11B1 and CYP11B2 encode steroid 11 beta-hydroxylase and aldosterone synthase. respectively [Mukai et al.. 1993]. The products of corticosteroidogenesis are not stored in adrenal tissue. since the rate of biosynthesis is equal to the rate of secretion [Haynes and Murad. 1985: Haynes and Larner. 1975: Simpson and Mason. 1979; Bowman and Rand, 1980].

3.4) Chemical Structure

Steroids are classified as lipids, because of their greater solubility in organic than in aqueous solvents. As the number of hydroxyl or carbonyl groups on the steroid nucleus increases. so does its solubility in water [Myles and Daly. 1974]. For example. budesonide is 100-fold more water soluble than another steroids. beclomethasone dipropionate. and this facilitates its dissolution and transport into the bowel wall [Bratssand. 1990].

Glucocorticosteroids are adrenocortical steroids with 21 carbon atoms. because all bear two carbon atoms. disposed in four rings: A. B. C and D. Its carbon skeleton is named the cyclophenantrene nucleus (i.e.. has 17 carbon atoms) [Haynes and Murad. 1985: Haynes and Larner, 1975: Crossland. 1980: Grollman and Grollman. 1970: Cope. 1972: Myles and Daly. 1974; Phillips, 1974; Dluhy et al.. 1975; Applezweig. 1962].

Figure 4. Carbon Skeleton

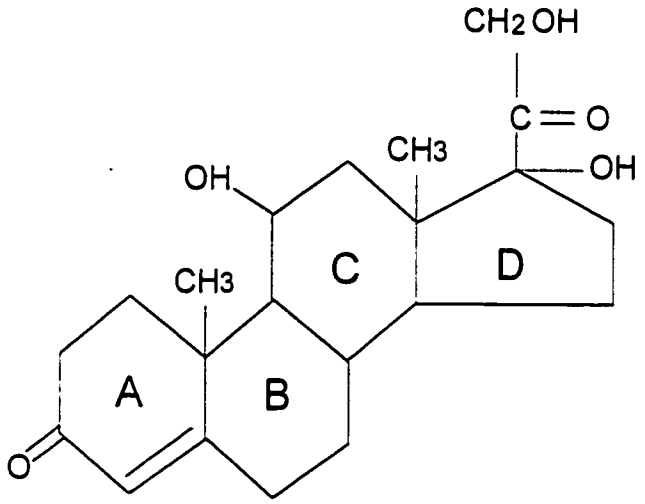


CARBON SKELETON

The other basic feature of glucocorticosteroids is the ketol grouping at position 20. oxygen atom on carbon atom 3, a double bond between carbon atom 4 and carbon atom 5. the methyl groups at carbon atom 18 and 19 and the hydroxyl group at carbon atom 11 [Haynes and Murad, 1985; Haynes and Larner, 1975; Crossland, 1980; Grollman and Grollman. 1970: Cope. 1972: Myles and Daly, 1974; Phillips. 1974; Dluhy et al.. 1975: Applezweig, 1962; Sutherland,, 1970]. The two-carbon ketol side chain at carbon

atom 17 is designated β, and the hydroxyl group at the same carbon atom is designated α [Haynes and Murad. 1985: Haynes and Larner, 1975; Crossland. 1980: Grollman and Grollman. 1970: Phillips. 1974; Bowman and Rand. 1980; Benet et al.. 1984].

Figure 5. Hydrocortisone



CORTISOL (HYDROCORTISONE)

Cortisol's three-dimensional configuration resembles a chair. due the double between carbon atom 4 and carbon atom 5 [Haynes and Murad. 1985; Haynes and Larner. 1975]. These chemical characteristics determine the biological activity of corticosteroids.

According to their biological activity, the corticosteroids are classified as glucocorticosteroids and mineralocorticosteroids. If a corticosteroid has potency based on liver glycogen deposition. it is called a glucocorticosteroid. If one has potency based on sodium retention. it is called a mineralocorticosteroid. However. a corticosteroid may have both potencies. This biological activity is measured by effects of

glucocorticosteroids on the metabolism of carbohydrates and on the kidney reabsorption of sodium [Haynes and Murad. 1985; Barnes. 1992; Haynes and Larner. 1975; Crossland. 1980; Laurence and Bennet. 1980; Grollman and Grollman. 1970; Cope. 1972; Myles and Daly, 1974; Phillips. 1974; Dluhy et al.. 1975; Applezweig. 1962]. Topical anti-inflammatory and systemic glucocorticoid potencies are determined by intracutaneous vasoconstriction and depression of plasma cortisol levels respectively [Johanson et al.. 1982]. Examples of the importance of chemical structure can be demonstrated: the high anti-inflammatory activity requires an α-hydroxyl group at C7 and a β-hydroxyl group at C11. Hormonal activity requires a double bond linking carbon atoms 4 and 5, and an oxygen atom on C3. Halogenation always increases anti-inflammatory and mineralcorticoid activity, but when associated to hydroxylation or methylation at C16, it retains anti-inflammatory activity and reduces mineralocorticoid activity [Crossland. 1980]. Esterification in the 17α position increases glucocorticosteroid potency [Bratssand. 1990].

The possible corticosteroids isomers are derived from reduction of the ketone at C3: 3β-hydroxyl and 3α-hydroxyl: two are derived from saturation of the 4,5 double bond: 5α and 5β: and two derived from reduction of the ketone at C20. This forms an asymmetrical carbon in this point: α and β [Haynes and Murad. 1985; Haynes and Larner. 1975].

Most of the synthetic compounds are glucocorticoids with little or no mineralcorticoid activity. The following describes the changes in the chemical structure and the associated products:

- Introduction of a 1,2 double bound in the ring A: prednisone and prednisolone [Haynes and Murad, 1985; Barnes, 1992; Haynes and Larner, 1975; Crossland, 1980; Aviado, 1972; Grollman and Grollman, 1970; Myles and Daly, 1974; Phillips, 1974; Dluhy et al., 1975; Applezweig, 1962; Cope, 1972; Sutherland, 1970; Benet et al., 1984; Bratssand, 1990];
- A methylation or fluoridation at position C6: methyl prednisolone and fluprednisolone [Barnes, 1992; Crossland, 1980; Aviado, 1972; Grollman and Grollman, 1970; Phillips, 1974; Cope, 1972; Sutherland, 1970; Bratssand, 1990];
- Fluoridation at C9 associated to hydroxyl group: triamcinolone [Haynes and Murad, 1985; Barnes, 1992; Haynes and Larner, 1975; Crossland, 1980; Aviado, 1972; Grollman and Grollman, 1970; Myles and Daly, 1974; Phillips, 1974; Sutherland, 1970; Bratssand, 1990];
- Fluoridation at C9 associated to methylation at C16: dexamethasone and betamethasone (is isomeric with dexamethasone, but its methyl group takes the β instead of the α configuration) [Haynes and Murad, 1985; Barnes, 1992; Haynes, and Larner, 1975; Crossland, 1980; Aviado, 1972; Grollman and Grollman, 1970; Myles and Daly, 1974; Phillips, 1974; Dluhy et al., 1975; Sutherland, 1970; Bratssand, 1990];
- Fluoridation at C6 associated with methylation at C16: paramethasone [Barnes, 1992; Crossland, 1980; Aviado, 1972; Grollman and Grollman, 1970; Myles, and Daly, 1974; Sutherland, 1970];

- Fluoridation at C9 and C6 associated with an hydroxyl group: fluocinolone (has mineral and glucocorticoid activity) [Crossland. 1980: Sutherland. 1970]:
- Chlorination at C11 and C9, without substitution on C16: dichlorisone (has mineral and glucocorticoid activity)[Crossland. 1980; Sutherland. 1970];
- Substitution at the 17α ester position: beclomethasone dipropionate or budesonide [Barnes. 1992: Greenberg. 1994; Bratssand. 1990: Greenberg et al.. 1994: Johanson et al.. 1982: Clissold et al.. 1984].

3.5) Absorption

Glucocorticosteroid molecules enter the cells by diffusion across the plasma membrane. This is supported. for example. by studies in tissue culture where the steroid concentrations required for binding to the glucocorticosteroid receptor were similar in either intact cells or in cell free homogenates [Haynes and Larner. 1975]. It is possible that steroids may be actively transported out of some cells. The extrusion process is temperature-dependent. saturable. glucose-dependent. and operates for only a few selected steroids [Gross et al.. 1970].

Within the cytoplasm of the cell there is a specific glucocorticoid-binding protein which is the receptor for these steroids. These receptors exist in many tissues. since the glucocorticosteroids have wide-spread effects throughout the organism [Haynes and Larner. 1975; Bamberg et al., 1996]. There are cytoplasmic receptors for steroids in most tissues of the juvenile rat and rabbit [Ballard et al.. 1974]. The glucocorticosteroid receptor of the rat liver has a molecular weight estimated to be 66 kd. However. the

fibroblast receptor has a molecular weight of about 600 kd. This discrepancy, added with the demonstration that phospholipase decreases the apparent size of the receptor, suggests that the receptor may be a lipoprotein [Haynes and Larner, 1975; Hackney and Pratt, 1971].

Other proteins have been described in liver, kidney and other tissues such as the brain which bind some glucocorticosteroids. In general, the proteins are found in the cytosol in higher concentration than the specific receptors, and they do not have a high affinity for the synthetic glucocorticosteroid. These cytosolic proteins can be distinguished from transcortin on the basis of their elution on DEAE columns, and by other types of protein fractionation techniques [Haynes and Larner, 1975].

The receptor-hormone complex undergoes a transformation that results in a change in its sedimentation coefficient. When this structural change (the exact nature is unknown) takes place, the receptor-steroid complex becomes capable of diffusing into the nucleus of the cell. Within the nucleus the receptor-steroid complex is bound to chromatin [Haynes and Larner, 1975].

The glucocorticoid receptors recognize active glucocorticoid, proportional to their concentration and activity. The levels of plasma corticoid may be evaluated by quantifying their ability to compete with a radioactive glucocorticoid for binding to the specific receptor. The major advantage of this assay is that it can quantify levels of any of the synthetic glucocorticoids. There are occasional major differences in absorption and metabolism of the synthetic steroids [Haynes and Larner, 1975]. Cortisol and its synthetic analogous are effective when they are given orally [Haynes and Murad, 1985; Barnes,

1982: Sutherland. 1970; Girdwood and Petrie. 1987]. Cortisol is readily and rapidly absorbed from gastrointestinal tract [Aviado. 1972]. Enteric coatings that are designed to reduce the incidence of dyspepsia delay absorption but do not reduce the total amount of cortisol which is absorbed [Barnes. 1982]. In general, the corticosteroids are absorbed in the upper jejunum. and peak concentrations in plasma occur in 30 min to 2h after administration [Ehrich et al.. 1992]. However, the desoxycorticosterone is ineffective when it is given orally [Haynes and Murad. 1985].

The water-soluble esters of cortisol and their synthetic congeners are given by the intravenous routes. in order to achieve high concentrations in body fluids rapidly [Haynes and Murad. 1985]. Intramuscular suspensions of cortisol have prolonged effects [Haynes and Murad F. 1985]. The glucocorticoids are absorbed from sites of local application: synovial spaces. conjuntival sac. skin. intestinal mucosa. The administration of topical glucocorticosteroids for long periods or for use in large areas can cause systemic effects [Haynes and. 1985; Sutherland . 1970: Girdwood and Petrie. 1987].

3.6) Transport

More than 90% of cortisol. is reversibly bound to plasma proteins [Haynes and Murad. 1985; Myles and Daly. 1974: Baxter, 1979; Bowman and Rand. 1980]. The responsible proteins are globulin. a glycoprotein, and albumin. The globulin is called transcortin or corticosteroid-binding-globulin (CBG) [Rocci et al., 1982]. A physiological role of the multidrug transporter P-glycoprotein (PGP) in the transport of cortisol is suggested [Van Kalken et al.. 1993]. CBG physiological levels are 3 to 5 mg/dl [Myles and Daly. 1974]. CBG increases in pregnancy. and decreases in patients with hepatic

disease [Haynes and Murad. 1985; Myles and Daly. 1974; Bowman and Rand. 1980; Sutherland. 1970; Benet et al.. 1984]. The molecular weight of CBG is about 52 kd: it can bind one cortisol per molecule of protein. CBG binding capacity is 20 μg of cortisol per 100 ml of plasma. Its half life in the plasma is 5 days: and in electrophoresis CBG migrates as an α1-globulin [Myles and Daly. 1974; Ballard. 1979].

The Δ4–3-ketone is an essential chemical characteristic for binding to CBG. Many of the synthetic corticosteroids have substituent groups with the α configuration. particularly at C9 and C6. which will markedly interfere with binding to CBG. This feature of synthetic corticosteroids results in an increase in the free corticosteroid. and consequently an increase in its biological activity [Myles and Daly. 1974].

The CBG has high affinity for cortisol but low total binding capacity whereas albumin has low affinity and high total binding capacity for cortisol [Haynes and Murad. 1985: Myles and Daly. 1974; Baxter. 1979: Bowman and Rand. 1980: Girdwood and Petrie. 1987: Benet et al.. 1984]. In situations of low or normal cortisol concentration. most cortisol is bound to globulin. Otherwise. in situations of high cortisol concentrations. the amount of cortisol bound to globulin increases. the free cortisol increases. and the amount of cortisol bound to albumin changes little [Haynes and Murad. 1985; Myles and Daly. 1974; Baxter. 1979; Bowman and Rand. 1980].

The physiological concentration of albumin is about 4.0 g/dl of plasma [Myles and Daly. 1974]. The free cortisol or unbound plasma cortisol in physiological conditions is 0.1 to 1.8 μg/dl of plasma. and it accounts for the biological activity of the plasma

[Barnes, 1992: Myles and Daly. 1974; Baxter, 1979; Bowman and Rand. 1980; Benet et al., 1984).

3.7) Metabolism

The liver is responsible for 70% of corticosteroid metabolism [Haynes and Murad. 1985; Barnes. 1992: Cope. 1972: Sutherland. 1970; Girdwood and Petrie. 1987; Hunter and Chasseaud. 1976; Johanson. 1982]. The glucocorticoid metabolism can occur by:

- Reduction of the 4.5 double bond in the liver and in extrahepatic sites. This reaction results in an inactive substance, called dihydrocortisol. In the second step dihydrocortisol is converted to tetrahydrocortisol [Haynes and Murad. 1985; Cope , 1972; Peterson and Pierce. 1960; Bowman and Rand. 1980];
- Reduction in the liver of the 3 ketone substituent to a 3-hydroxyl, to form tetrahydrocortisol. Tetrahydrocortisol is then converted to tetrahydrocortisone [Haynes and Murad 1985; Aviado. 1972; Cope. 1972: Peterson and Pierce. 1960: Bowman and Rand. 1980: Hunter and Chasseaud. 1976];
- Reversible oxidation of the 11-hydroxyl group occurs slowly in extrahepatic tissues, and rapidly in the liver [Haynes and Murad. 1985; Cope, 1972]. The corticosteroids with 11-ketone substitution require reduction to 11-hydroxyl group compounds for their biological activity [Haynes and Murad. 1985. Bowman and Rand. 1980; Sutherland. 1970; Benet et al., 1984];

- Reduction of the 20 ketone to a 20-hydroxyl which has little. if any. biological activity. This reduction forms cortol (α and β) and cortolone (α and β) [Haynes and Murad. 1985; Cope. 1972; Peterson and Pierce. 1960; Cope. 1972; Bowman and Rand. 1980; Sutherland. 1970]. The tetrahydrocortisol and tetrahydrocortisone represent 35% of the corticosteroid metabolites excreted. while the cortol and cortolone represent 30% of the same metabolites [Peterson and Pierce. 1960].
- Oxidation of the 17-hydroxyl group to form 17-ketosteroids and a two-carbon fragment. The 17-ketosteroid lacks in corticosteroid activity, but has weak androgenic properties [Haynes and Murad. 1985. Bowman and Rand. 1980; Sutherland. 1970):

There are liver enzymes capable of carrying out the reduction the ketonic group: one in the soluble fractions (5β-isomer) and another in microsomes (5α-isomer) [Aviado. 1972; Cope . 1972]. These enzymes require NADPH as a hydrogen donor [Cope. 1972]. There are other enzymes responsible for reduction of Δ1 unsaturation of prednisone. 2-hydroxylation. and 20-hydroxylation [Cope.. 1972]. Most of these enzymes are part of the cytochrome p-450 system. that can easily inactive glucocorticosteroids with lipophilic groups such as 17α-valerate and 17α.21-dipropionate [Bratssand. 1990]. Regioselective and stereospecific hydroxylation of these molecules in positions 2.6.7,15,16. and 21 has been studied in detail and shown to be catalysed by several forms of cytochrome P450 from families 1. 2, and 3 in rats and from families 3 and 4 in humans [Pichard et al.. 1991]. Dexamethasone and prednisone are inducers of cytochrome P450 3A. increasing

the accumulation of mRNA and protein [Pichard et al.. 1991]. The locally-acting steroid budesonide is metabolized by cytochrome P450 3A enzymes in human liver [Jonsson G. et al.. 1995].

Most of the ring-A-reduced metabolites are coupled through 3-hydroxyl in the liver or kidney with sulphate or glucuronic acid. to form water soluble sulphate esters or glucuronides. These compounds can be excreted in the urine [Haynes and Murad. 1985: Cope. 1972: Bowman and Rand. 1980; Ehrich et al.. 1992: Greenberg. 1994]. Excretion is largely dependent on prior conjugation of the steroids with glucuronic acid [Peterson and Pierce. 1960]. Glucuronyl transferase catalyses glucuronidation of various endogenous substances [Sutherland. 1970].

Corticosteroid use is considered relatively safe during pregnancy due to a placenta enzyme: 11β-OH-dehydrogenase. This enzyme inactivates corticosteroids and leads to low concentration of active drug in the fetus [Ehrich et al.. 1992].

In humans. the intestinal sites of metabolism of cortisol and cortisone are saturated before the hepatic sites [Barr et al.. 1984].

Glucocorticosteroids with 16α, 17α acetyl groups such as budesonide. have topical anti-inflammatory activity. stability in extra-hepatic tissues (airways and intestinal walls). and about 90% first pass hepatic metabolism to products which posses minimal or no biological activity [Greenberg. 1994; Bratssand. 1990].

The majority of corticosteroids are excreted in the urine. [Haynes and Murad. 1985; Aviado. 1972; Fukushima et al, 1960; Cope. 1972]. Corticosteroids are detectable

in small amounts in fecal, biliary and CO_2 excretion [Haynes and Murad, 1985; Haynes and Larner, 1975; Sutherland, 1970].

The cortisol plasma half-life is about 1.5 h, and the prednisolone half-life is 1 to 2 h. However, the prednisolone half life of its biological effect is in the range of approximately 24h [Haynes and Murad, 1985; Cope, 1972; Sutherland, 1970]. The corticosteroid metabolism is greatly slowed by a 1,2 double bond, and by fluoridation. These chemical features prolong its half-life [Haynes and Murad, 1985; Cope, 1972].

The physiological human plasma cortisol level in the morning is 5-20 μg/ml, and in the afternoon is 2.5-10 μg/ml [Greenspan and Baxter, 1994; Griffin and Wilson, 1992; Jonetz-Menztel and Wiedemann, 1993]. In rats and mice, the plasma corticosterone levels vary greatly according to the technique and sample collection methods. Generally a baseline range of 50 to 300 ng/ml and 50 to 400 ng/ml can be expected for mice and rats respectively depending on the time of day the sample is taken [Shimizu et al., 1983]. In both mice and rats the highest concentrations of plasma corticosterone are observes between 4:00 pm and 10:00 pm [Coffigny et al., 1978].

3.8) Mechanism of Action

Glucocorticosteroid molecules enter cells by diffusion across their plasma membrane [Haynes and Larner, 1975]. The action of steroids does not require cytosolic kinases [Alberts et al., 1994]. Within the cytoplasm there is a specific glucocorticoid-binding protein which is considered to be the receptor for these steroids. These receptors exist in many tissues, and they constitute the intracellular receptor superfamily or steroid-hormone receptor superfamily, as well as those receptors related to

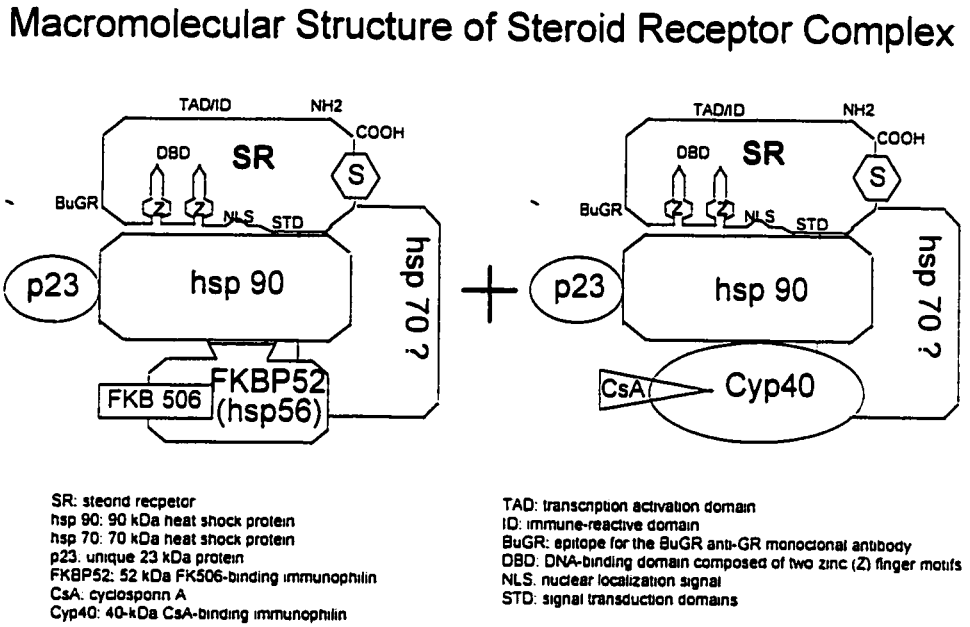
thyroid hormones, retinoids and to vitamin D [Haynes and Larner, 1975; Alberts et al., 1994; Schulman et al., 1994]. The receptor-hormone complex undergoes a transformation that results in its activation. The activation of this receptor consists of binding of ligand to the receptor, which dissociates an inhibitory protein complex. This results in the exposition of the DNA-binding site of the receptor [Alberts et al., 1994]. When this structural change takes place, the receptor-steroid complex becomes capable of diffusing into the nucleus of the cell through the nuclear pores. Within the nucleus, the complex is bound to the nuclear chromatin [Haynes and Larner, 1975; Bamberger et al., 1996]. This reaction influences the synthesis of specific mRNA and consequently protein synthesis, which is ultimately responsible for the glucocorticoid response [Baxter, 1979; Bamberger et al., 1996]. The cell response to glucocorticosteroids involves two steps: 1) an early primary response, which consists of activation of primary-response genes by steroid hormone-receptor complexes, and consequently induction of synthesis of primary-response proteins; and 2) a delayed secondary response which involves activation of secondary-response genes by the primary-response proteins. These secondary-response proteins also shut off the primary-response genes [Alberts et al., 1994].

A model of the macromolecular structure of inactivated or untransformed steroid receptor (SR) complex is presented in figure 6. The SR complex has one molecule of hormone binding receptor [Denis and Gustafsson, 1987] with the indicated functional domains: transcription activation domain (TAD), immune-reactive domain (ID), DNA-binding domain (DBD), nuclear localization signal (NLS) and signal transduction domain

(STD). In addition, the complex also has two molecules of hsp 90, and unknown numbers of FKBP52, cyclophilin-40 and p23 molecules [Denis and Gustafsson, 1987; Tai et al., 1986; Owens-Grillo et al., 1986; Smith et al., 1990; Bresnick et al., 1990]. Another heat shock protein, hsp 70 is also included in the model [Srivinasan et al., 1994]. The binding of hsp 90 to the steroid receptor as indicated occurs in the domain named STD [Housley et al. 1990]. The binding of p23 to the complex occurs through hsp 90, as well as the binding of FKBP52 and Cyp40 to the steroid receptor [Jhonson and Toft, 1995; Lebeau et al., 1992; Renoir et al., 1992]. Two distinct SR complexes may exist, each distinguishable by the presence of FKBP52 or Cyp40 subunits as is the case of the mouse glucocorticosteroid receptor. These two distinct receptors may be functionally different as well as one may be the precursor of the other [Owens-Grillo et al., 1986]. These two different SR isoforms are generated by alternative splicing of the human SR pre-mRNA, and are termed SRα and Srβ [Bamberger et al., 1996]. The distinction between SRα and Srβ may be more relevant than previously thought. In fact overexpression of SRβ antagonizes the effects of hormone activated SRα on a glucocorticoid-responsive gene [Bamberger et al., 1995]. This finding would explain why we observe positive effects of glucocorticosteroids on gene expression in certain conditions, and negative effects in other conditions.

Figure 6. Steroid Receptor

(From webpage: http://www.mco.edu/depts/pharm/ssrc.html)



Most tissues are direct targets for glucocorticosteroids. and all tissues may be influenced indirectly by this steroid. The physiological responses are diverse [Baxter. 1979]. The glucocorticosteroid response depends on the nature of the particular steroid. and on the target tissue. The various types of steroids are separated according to their activity in four classes [Samuels and Tomkins, 1970]:

- Optimally active steroids: when the steroids are present in a concentration sufficient to saturate the receptors. and elicit the maximal glucocorticosteroid response;

- Suboptimal steroids: when the steroids are not present in a concentration sufficient to saturate the receptors. and elicit the intermediate glucocorticosteroid response:
- Anti-inducers steroids: when the steroids do not elicit a glucocorticosteroid response:
- Inactive steroids: when the steroids neither bind to receptor nor influence glucocorticosteroid response.

If one compares the biological activities of the steroids with suboptimal and antiglucocorticoid activity in different tissues studies, considerable variation is seen. One reason for this is that metabolism may convert an inactive glucocorticoid to an active one [Samuels and Tomkins. 1970] .

After exposure of cultured hepatoma cells to dexamethasone. about 70% of the total cytoplasmic receptor localizes in the nucleus [Rousseau. 1973], suggesting that the function of the glucocorticoid is to influence the nuclear localization of the glucocorticoid-receptor complexes. In the hepatoma cells. there is a rapid disappearance of the glucocorticosteroid response upon removal of the inducer steroid. Consequently. there is a rapid dissociation of steroid from the receptors. which is associated with a disappearance of receptors from the nucleus. and their reappearance in the cytosol. These findings suggest that in the induced state, the cytoplasmic receptor remains in the nucleus stoichiometrically combined with the corticosteroid [Samuels and Tomkins. 1970; Rousseau. 1973]. The level of glucocorticoid receptor mRNA in a number of target

tissues and in many different cells is regulated by the presence of glucocorticosteroids. and it is different according to their half-life [Shimojo et al., 1995].

The activation of the SR complex is favored by heating. dilution. salt treatment or by divalent cations. "Activation" is the change that must occur in the SR complex following binding of steroid to receptor and prior to nuclear binding. This activation is a temperature-dependent step. Cytoplasmic fractions were exposed to salt. then chilled. and the salt was removed: this resulted in SR complexes binding to the nuclei. The nature of this change is not understood. In some systems divalent cations may influence the activation of the SR complex [Baxter. 1979].

As cytosolic binding increases. nuclear binding also increases. and does not appear to level off [Willian and Gorski. 1972]. Thus. the nuclear acceptor capacity appears to exceed the cytosol receptor capacity. Nuclear sites are highly specific in relation to the type of cytoplasmic receptor. and to the tissue of origin. The nuclear acceptors are localized on the chromatin. which binds the glucocorticoid-receptor complexes [Baxter. 1979]. SR complexs bind to glucocorticosteroid responsive elements as a dimer. which is thought to be the active regulatory form [Chalepaski et al.. 1990: Drouin et al.. 1992].

In order to activate a gene. an intracellular receptor needs the presence of the right combination of other gene regulatory proteins. Thus, each steroid has a specific response. and each cell with receptors has a different combination of other gene regulatory proteins [Johnson, 1987]. Since chromatin is a complex of DNA and associated proteins. the acceptors are in one and/or both these components. Two lines of evidence suggest a role

for DNA in acceptor activity. First, the glucocorticosteroid-receptor complexes bind to purified DNA, and the capacity of DNA for binding to the complexes is even larger than that of chromatin. Second, the loss of nuclear binding capacity parallels the DNA loss caused by treatment with deoxyribonuclease (DNAase). DNA binding is much more sensitive to ionic strength and to divalent cations than is nuclear binding [Baxter, 1979; Tomkins et al., 1972].

In general, the glucocorticosteroid response requires RNA synthesis, and subsequently protein synthesis. The factor that accumulates in response to the glucocorticoids is the mRNA for the enzyme in question. This occurs for example with trehalase mRNA and trehalase, a glycoprotein of the brush border membrane of the enterocyte and kidney proximal tubule epithelial cells [Galand et al., 1995]. Furthermore, the glucocorticoid effect is blocked by inhibitors of RNA synthesis, and the number of polysomes involved in enzyme synthesis are increased in response to corticosteroid. However, in some situations the response induced by glucocorticosteroid may be indirect. Initially, this response may involve only protein synthesis and consequently other processes in relation to this first system [Baxter, 1979].

There is a repressor of tyrosine aminotransferase mRNA which inhibits the translation of the mRNA. This repressor is labile, and when RNA synthesis is inhibited the repressor disappears. It is postulated that the glucocorticoid-receptor complex inhibits the synthesis of this "repressor", resulting in an accumulation of mRNA. However, the interaction of the glucocorticosteroids with its nuclear receptor results in the induction of either positive events (transactivation), or negative events (transrepression) by repression

of gene transcription and/or alteration of mRNA half-lives [Krane . 1993: Sakai et al.. 1988].

Glucocorticoid receptors function as a necessary and sufficient factor for the transcriptional activation of the MTV-CAT (mouse mammary tumor virus as measured by chloranphenicol acetyl transferase assays) fusion gene [Giguere et al.. 1986]. The magnitude of this induction reveals that glucocorticoid receptors may act as a transcriptional "switch". which may involve a silent promoter containing a glucocorticoid response element to an active state. This stimulation is totally dependent upon the presence of glucocorticoid hormones. The mechanism involved in the activation of glucocorticoid-receptor is poorly understood. but it is likely to involve allosteric transitions within the protein [Giguere et al.. 1986].

Godowsaki and colleagues (1987) proposed the derepression mechanism. by which glucocorticoid hormone binding might yield the functional enhancer-activating protein. Ligand-stimulated structural transition may unmask DNA-binding or enhancer-activation domains already folded in their functional configuration. This derepression mechanism predicts that constitutive enhancer activating proteins. functional even in the absence of hormone. might appear upon deletion of a region that masks or represses otherwise functional or enhancer-activation domains [Godowsaki. 1987].

There are special situations where unresponsiveness or hyper-responsiveness occurs to glucocorticoids. In the course of the treatment of childhood lymphoblastic leukemia. unresponsiveness to glucocorticosteroid. may occur [Klumper et al.. 1995]. possibly due to a decrease in the amount of cytoplasmic receptors. This response is not

influenced by the hormone, and is increased by mutagens [Sibley and Tomkins, 1974]. Thus, a mutational event may be involved in unresponsiveness to glucocorticoid. Although the receptor defect appears to be the most common, other steps in glucocorticoid action are also blocked because a mutational event interferes with steps in corticosteroid action. However, defects in cellular entry and steroid metabolism have not been found [Sibley and Tomkins, 1974]. In contrast, defects in the receptor itself, in the ability of the receptor-glucocorticosteroid complex to bind to the nucleus (whereby the steroid receptor complex binds more tightly to the nucleus) have been found [Sibley and Tomkins, 1974; Yamamoto et al., 1974; Stratakis et al., 1994]. In other cases of unresponsiveness to glucocorticosteroids, normal steroid binding with the receptor and nuclear binding have been demonstrated. It was suggested that the mechanism of unresponsiveness is localized in some site distal to the initial steroid-receptor interaction [Lippman and Thompson, 1974].

Glucocorticoid resistance can emerge through defective nuclear binding and/or mutation in the receptor as indicated by a slower sedimentation in density gradients. Clones of steroid-resistant lymphoma cells have been found which have cytosolic receptors which bind steroids normally, but the receptor-glucocorticoid complexes bind less to the nucleus. In some cases, the glucocorticoid resistance may emerge through blocks in mechanisms distal to nuclear binding of the complex, because some cell lines have apparently normal cytosol receptors, normal nuclear binding, and yet have glucocorticoid resistance. Several steroid resistant lines appear to be a mutation in the

receptor as indicated by a slower sedimentation in density gradients [Sibley and Tomkins. 1974: Yamamoto et al.. 1974].

Some patients with colitis do not respond to high doses of corticosteroids. and these persons have a low glucocorticoid receptor content in their mononuclear cells [Madrestma et al., 1995]. Interestingly, antibodies to lipocortin-1 do not have a role in the development of steroid-resistance in children with inflammatory bowel disease [Beattie et al. 1995]. In contrast. hyper-responsiveness occurs in patients with open-angle glaucoma. and in persons homozygous for the postulated gene defect which causes this disease [Baxter. 1979: Tripathi et al., 1992].

The glucocorticoid tendency to produce hyperglycemia and carbohydrate intolerance follows from the inhibition of glucose uptake. tissue catabolism. and conversion of substrate to glucose. Other hormones can also influence these reactions [Baxter. 1979: Sutherland , 1970: Girdwood and Petrie. 1987: Wise et al.. 1973: Weber et al.. 1968; Pagano et al.. 1983]. The increase in the tissue catabolism results in the release of fatty acids. glycerol and nucleic acids. These substrates are used for gluconeogenesis. The mechanism of glucocorticoid-induced gluconeogenesis has been postulated to be explained by the increased precursor amino acids and/or the induction of gluconeogenic pathways in the liver. The steroid stimulation of gluconeogenesis in the liver may be blocked by inhibitors of protein or RNA synthesis [Exton. 1972: Weber et al..1965]. The free fatty acids (FFA) from lipolysis may spare glucose and amino acids from being utilized for energy production. This can save glucose and amino acids. the most important substrates for gluconeogenesis and for enzyme production). FFA provide an energy

source and influence gluconeogenesis through production of NADH. However, these possibilities are controversial: while FFA can increase gluconeogenesis from a variety of substrates in liver slices and in the perfused liver, there is no stimulation of gluconeogenesis when FFA are added in a physiologic manner. Thus, further studies *in vivo*, specially in man, are required to establish whether or not FFA are important in gluconeogenesis [Exton, 1972].

The major site of gluconeogenesis is the liver, but the renal gluconeogenesis can be important, especially in the fasting state [Exton, 1972]. The mechanisms involved in the increase of gluconeogenesis are the increase in the hepatic level of glycogen synthetase, and secondary hyperinsulinism (insulin is a potent activator of the glycogen synthetase) [Baxter, 1979; Haynes . and Murad, 1985; Haynes and Larner, 1975; Girdwood and Petrie, 1987].

If the actions of other hormones are not observed unless there has been exposure to glucocorticosteroid, this action is termed "permissive". This occurs in the sensitivity of the fat cell to epinephrine-induced lipolysis. Many of these permissive actions are associated with influences on hormones that stimulate adenyl cyclase [Baxter, 1979; Haynes and Murad, 1985; Haynes and Larner, 1975]. The glucocorticoid actions on fat cells result in the development of the Cushingoid appearance (redistribution of fat, with an accumulation in the face and trunk and wasting in the extremities). Steroids increase lipolysis and decrease glucose uptake and metabolism in fat cells. It is likely that fat tissues have varied hormonal sensitivity, such that glucocorticosteroid effects are

dominant in the extremities, and insulin actions are more effective in the trunk [Baxter. 1979; Sutherland. 1970].

Glucocorticosteroid administration increases the FFA in plasma. There are two mechanisms involved in this: direct action of glucocorticosteroids on increasing fatty acid release. This apparently results 1) from inhibition of glucose metabolism. with concomitant decrease in glycerol production; and 2) from potentiation of the actions of a wide variety of lipolytic hormones including cathecolamines, thyroid hormones. growth hormone and ACTH [Baxter. 1979].

Glucocorticosteroids have also been reported to have an specific effect on plasma lipoproteins. Corticotropin administration resulted in rapid decrease in apolipoprotein B. low- density lipoprotein (LDL). cholesterol and plasma triglyceride concentration. In contrast. dexamethasone treatment did not change any of the apo B-containing lipoproteins. High-density lipoproteins (HDL) increased with both treatments and apo A-I increased only with dexamethasone. Up-regulation of low-density lipoprotein receptor activity *in vitro* is equally observed after incubation with corticotropin. Therefore. glucocorticosteroids have a direct effect on the lipoprotein metabolism. primarily on apo B-containing lipoproteins [Berg and Nilsson-Ehle. 1994]. Knowing that the lipoproteins are responsible for the transport of the re-synthesized lipids to the blood or lymphatic system, if we had a negative effect on the synthesis of lipoproteins as observed with glucocorticosteroids we would expect alterations in the lipid uptake and\or accumulation of lipids at the enterocyte level.

The glucocorticoid catabolic actions are mainly seen in lymphoid cells, muscle, skin, fat cells and fibroblastic tissues. The steroid catabolic effect is not generally seen in liver, gut, kidney, brain, heart or blood cells. It is likely that this catabolic or anti-anabolic effect is due to the induction of proteins which are ultimately toxic for the cell [Baxter, 1979].

3.9) Liver

The liver is one of the few targets for glucocorticosteroids in which there is anabolism. However, some functions are inhibited; for example, DNA synthesis [Loeb et al., 1973]. This glucocorticoid action in the liver involves gluconeogenesis.

3.10) Muscle

Muscle is the target tissue where the glucocorticoid actions are catabolic, and is responsible for the negative nitrogen balance observed in patients with Cushing's syndrome. This steroid myopathy is associated with elevations in the concentration of muscle enzymes in the plasma [Ehrich et al., 1992; Baxter, 1979; Kusunoki et al., 1992].

3.11) Immunology and Inflammatory Responses

Glucocorticoids are the most potent drugs for treating a number of diseases in which abnormal immunological reactions are thought to be a major cause of the pathology. The catabolic actions of the corticosteroid on mast cells, lymphoid cells, macrophages, other blood elements, endothelial cells and fibroblastic tissues form the major basis for immunological and inflammatory suppressive responses [Ehrich et al., 1992; Baxter, 1979; Sutherland, 1970; Klebl et al., 1994]. Glucocorticosteroids affect different stages of the immunological response: antigen processing and penetration of

basement membranes by antigen-antibody complexes; actions on macrophage function: delayed hypersensitivity reaction; T-cell mediated and spontaneous cytoxicity; immunoglobulin levels without interference in specific antibody production; reticuloendothelial clearance of antibody-coated cells; cellular helper cell functions; inflammatory reactions; and vascular reactions [Ehrich et al., 1992; Baxter, 1979; Cupps and Facui, 1982; Gillis et al., 1979; Arya, 1984; Wu et al., 1991; Bowen and Fauci, 1984; Goulding and Guyre, 1993].

The inflammatory reactions involved with glucocorticoid response are : increase circulating neutrophils; decrease circulating lymphocytes and monocytes; inhibit accumulation of neutrophils and macrophages at inflammatory sites (may be the most important factor); reduce edema formation; and lessen the increase in vascular permeability. Some of these effects are mediated by interfering with the synthesis or release of prostaglandins, leukotriens and bradykinins; complement activation and action; margination and sticking of leukocytes to vascular endothelium; membrane permeability; and the action of migration inhibition factor (MIF) which causes macrophages to adhere to the endothelium [Ehrich et al., 1992; Baxter, 1979; Haynes and Murad F, 1985; Haynes and Larner, 1975; Skubitz et al., 1981; Coates et al., 1983; Blackwood et al., 1982].

Inflammatory cytokines have selective and specific inhibitory effects on the expression of the brush border hydrolase sucrase-isomaltase in vitro and in vivo, providing evidence for a previously unrecognized mechanism for disaccharidase deficiency in intestinal inflammation. The most well characterized cytokines involved in

this process include IL-6, IL-1α ,IL-1β, TNF-α and IFN-γ [Ziambaras et al., 1995]. This finding brings up the question whether glucocorticosteroids inhibiting the production, expression and release of cytokines, enhance the expression and activity of other brush border membrane hydrolases. For example, budesonide has been described to have a negative effect on the expression of IL-3 and IL-5 [Lantero et al. 1996].

3.12) Healing and Scar Tissue

Glucocorticoids exert a negative influence on wound healing when given systemically or topically, due to a decrease in collagen formation and in the fibroblast response [Baxter, 1979; Sutherland. 1970].

3.13) Blood Cells

Glucocorticoids increase the plasma hemoglobin concentration by about 15%. The marrow response to glucocorticoid is normoblastic, and it can sometimes mask a megaloblastic anemia. The blood eosinophils and basophils are depressed in Cushing's disease or after glucocorticoid therapy. The acute response to giving steroids is an increase in thrombocytes, but after chronic administration there is thrombocytopenia. The polymorphonuclear cells increase and mononuclear cells decrease (70% in circulating lymphocytes, and 90% in monocytes). Although the neutrophil number increases, their phagocytic ability actually decreases [Baxter, 1979; Sutherland, 1970; Girdwood and Petrie, 1987; Haynes and Murad, 1985; Haynes and Larner, 1975]. This increase in neutrophil number is due to the glucocorticoid's protective effect on human neutrophil survival by delaying apoptosis [Liles et al., 1995].

3.14) Lung

Glucocorticosteroid improve severe bronchial asthma by enhancing the β adrenergic responses (permissive action) that result in bronchodilatation [Baxter. 1979]. Glucocorticosteroids induce the production of surfactant in the lung of the fetus and accelerate pulmonary maturation preventing the respiratory distress syndrome. [Avery. 1995; Gonzales et al.. 1994; Boggaram et al., 1991; Delemos. 1970; Motoyama. 1971].

3.15) Cardiovascular System

The heart contains glucocorticoid receptors. and some persons given steroids have an increase in cardiac output. with a decrease in peripheral resistance [Sambhi. 1965; Haynes and Murad. 1985; Haynes and Larner. 1975; Ballard et al.. 1974].

Patients with spontaneous Cushing's syndrome and hypertension have been found to have elevations of plasma renin substrate. the protein which acts to release angiotensin. Furthermore. increased vascular reactivity to infused pressor substances has been observed in patient with Cushing's syndrome. and after high doses of glucocorticosteroid given to normal subjects [Krakoff et al.. 1975; Sutherland. 1970; Kusunoki et al.. 1992]. This steroid-induced hypertension is not associated with steroid-induced hyperinsulinemia [Whitworth et al.. 1994].

Electrocardiographic changes associated to hypokalemia have been reported [David et al.. 1970]. due to the glucocorticoid action influencing in serum electrolytes such as potassium and calcium. Other changes such as cardiac conduction alterations and even arrhythmia were described after glucocorticoid administration. These can be explained by hypokalemia and/or enhancement of β adrenergic stimuli [Shimidt. 1972].

3.16) Endocrine System

The glucocorticosteroids have some actions on the metabolism of the thyroid hormones. decreasing uptake, clearance rate. and turnover of ^{131}I. These effects are reversible by the administration of thyroid stimulating hormone (TSH). The mechanism that causes this effect is probably at the pituitary level. decreasing the thyrotropin-releasing factor (TRF) from the hypothalamus. However. with continued glucocorticoid administration there occurs almost always an escape from the suppression of serum TSH concentrations. This results in a normal thyroxine concentration in plasma. Otherwise. thyroid hormone accelerates the metabolism of corticosteroids [Baxter. 1979; David et al.. 1970].

The glucocorticosteroid actions appear to be generally antagonistic to growth hormone. This is likely due to the fact that a major mechanism of growth hormone action is to influence the production of somatomedin. which may have insulin-like actions. Otherwise. growth hormone decreases the uptake of glucose in muscle and enhances lipolysis in adipose tissue. These latter actions of growth hormone are parallel to those of the glucocorticoids [Baxter. 1979; David et al.. 1970].

In general, glucocorticoid tends to depress the serum calcium. whereas parathyroid hormone tends to elevate it [Baxter. 1979].

3.17) Fluid and Electrolyte Balance

The glucocorticoids influence salt and water balance by their mineralocorticoid actions [Sabatini et al.. 1993]. For example. dexamethasone and prednisolone have very weak sodium-retaining properties. The corticosteroids also influence fluid balance by

increasing the glomerular filtration rate and renal plasma flow. and antagonizing the release of antidiuretic hormone (ADH) and/or exerting an anti-ADH-like action. These latter effects are more noticeable in the Addisonian patient who is treated with only aldosterone or desoxycorticosterone [Sutherland. 1970: Baxter. 1979].

3.18) Bone and Calcium Metabolism

The action of glucocorticoids on bone and calcium metabolism is the major limitation to long-term therapy. The glucocorticosteroid-induced osteoporosis tends to be severe and debilitating. with damaging changes in bone persisting even after cessation of glucocorticoid therapy. It is likely that glucocorticoid-induced catabolism in the matrix of bone is responsible for this bone damage [Ehrich. 1992: Sutherland. 1970: Baxter. 1979: Madsen and Andersen. 1994; Kasperk et al.. 1995].

Corticosteroids promote hypocalcemia due to a steroidal alteration in the distribution of calcium between the extracellular and intracellular compartments. and due to a decrease in the intestinal absorption and renal tubular reabsorption of calcium [Baxter. 1979: Krane. 1993].

The regular measurement of bone density in persons taking steroids may be helpful in identifying persons at risk for the development of osteoporosis [Zelissen et al.. 1994].

3.19) Growth

Corticosteroids have catabolic and anti-anabolic effects on connective tissue and muscle, suppress growth hormone secretion. and thereby limit the use of corticosteroids in children. because of their suppression on growth [Baxter, 1979; Kusunoki et al.. 1992].

In rats, administration of corticosteroids as cortisone acetate, classically impairs the growth, a finding that is reflected on the weight gain. Furthermore, this classical growth-impairing effect is more pronounced in the older animals than in the younger animals [Henning and Leeper, 1982].

3.20) Central Nervous System

Psychiatric disturbances are associated with the use of glucocorticosteroids. Depression is the most common manifestation in Cushing disease, whereas elevation of mood or frank euphoria is common in the iatrogenic disease caused by the intake of steroids. The moods changes are reversible with reduction in dose or discontinuation of therapy [Ehrichet al., 1992; Sutherland, 1970; Kusunoki et al., 1992]. The perception of taste, smell and sound stimuli are elevated in hypercorticism [Haynes and Murad, 1985; Haynes and Larner, 1975]. Benign intracranial hypertension is a rare condition with unknown etiology, although steroid use has been implicated [Newton and Cooper, 1994].

3.21) Gastrointestinal Tract

Cushing's syndrome has been implicated as causing an increased incidence of peptic ulceration. It is suggested that the glucocorticoids increase acid secretion [Nicoloff, 1969; Okabe et al., 1971; Cooke, 1973]. It is likely that the corticosteroids have catabolic effects in the stomach, and this could explain the mechanism for ulceration in animals [Ehrich et al., 1992; Baxter, 1979, Kusunoki et al., 1992]. Steroid effect on the level of prostaglandins in the gastric mucosa is another factor that explains the corticosteroid induced ulceration [Orlicz-Sczesna et al., 1993; Avunduk et al., 1992). Ulceration risk doubles with steroid therapy [Messer et al., 1983].Other gastrointestinal

effect described in humans is the increase of appetite [Drug Evaluations Annual. 1991]. In contrast, in rats no significant differences in food intake have been described in the literature [Henning and Leeper, 1982].

The following sections will be give more details about glucocorticoid action on the gastrointestinal tract.

3.22) Effects of Glucocorticosteroids on Intestine

In rats, the most used animal model for the description of effects of steroids on the intestine, corticosteroids classically have a negative effect on the body weight gain of these animals, being this finding more prominent in older animals. No alterations in the food intake have been described [Henning and Leeper. 1982].

3.22.a) Effects of Glucocorticosteroids on Intestinal Uptake

The administration of prednisolone for seven days has little effect on the intestinal mucosal structure or cell kinetics, but does enhance the absorptive capacities of the jejunum and ileum for galactose [Batt and Peters. 1976a: Batt and Peters. 1976b]. This is due to an increase in the value of the maximal transport rate V_{max} of the transporter, without alterations in the value of the apparent affinity constant (K_m) [Scott et al.. 1980: Batt and Scott. 1982]. There is also steroid-enhanced activity of the intestinal brush border membrane enzyme activities, such as α-glucosidase, naphthylamidase and β-glucosidase [Scott et al., 1980]. Steroids increase the rate of synthesis of brush border membrane proteins, associated with an increased glycoprotein content of this membrane [Scott et al., 1981], and an increase in the membrane-bound ribosomal RNA (mRNA) content of the enterocytes. This leads to enhanced synthesis of membrane proteins

associated with a likely proliferation of the rough endoplasmic reticulum [Batt and Scott . 1982]. Short-term prednisolone dosing enhances the ileal epithelial cell migration rate. and decreases cell turnover time [Batt and Scott, 1982]. Also. the basolateral membrane enzyme activities and mitochondrial enzymes are enhanced with steroids; however. the lysosomal or peroxisomal enzyme activities do not change [Scott et al.. 1980]. Administration of prednisolone for longer intervals (28 days) sustains these short-term stimulatory effects on intestinal absorptive and digestive functions. but long-term prednisolone is associated eventually with a small decrease in the height of the villus [Murphy. 1982].

In contrast. the administration of betamethasone-17-valerate (a locally rather than a systematically active glucocorticosteroid) decreases intestinal crypt cell turnover. decreases villous height and crypt depth. decreases the epithelial cell migration rate. and increases the transit time of enterocytes along the villus [Murphy. 1982]. Absorption per enterocyte is increased. but betamethasone reduces the total number of enterocytes. and hence there is no net change in the absorption per centimeter length of intestine. The predominant activity of this steroid may be the stimulation of enterocyte function or the reduction of the enterocyte population.

Following intestinal resection in rats the short-term administration of prednisolone by mouth increases the adaptive hyperplasia in the remaining ileum. with an increase in villus height and in crypt depth, an enhancement of the epithelial cell migration rate. and of the villus transit time. As well, prednisolone increases enzyme activity per centimeter length of intestine. without changes in the enzyme activity in

individual enterocytes. Higher doses of prednisolone result in only slight villus atrophy. In contrast, more prominent morphological changes may result from the administration of long-acting glucocorticoids, as they have a more marked effect on DNA metabolism and decreased crypt cell proliferation [Batt and Scott, 1982].

Dexamethasone [128 µg/ kg / day given subcutaneously (SC) for 7 days], dropped the weight, protein and DNA content of the duodenojejunal and ileal mucosa of sham operated rats, as well as in rats which underwent an 80% jejunoileal resection. Dexamethasone did not elevate the BBM enzyme activity, and inhibited the normal mucosal growth and mucosal hyperplasia in resected rats [Park et al., 1994]. Insulin-like growth factors and insulin are considered to be important regulators of somatic growth [Daughaday and Rotwein, 1989]. IGF-I and IGF-II have been reported to stimulate the proliferation of intestinal epithelial cells as well as to enhance the mucosal hyperplasia that occurs after massive small bowel resection [Park et al., 1992; Lemmey et al., 1991; Vanderhoof et al., 1992; Wheeler and Challacombe, 1997]. The serum insulin-like growth factor (IGF) levels were diminished in both groups (dexamethasone-infused resected and sham-operated rats) as well as the serum level of IGF binding proteins (IGF BP), the IGF BP-2 and IGF BP-3. However, the IGF BP-1 was increased and the IGF BP-4 did not change. This suggests that the growth inhibiting effects of dexamethasone in small intestinal mucosa may be partially mediated by decreasing serum IGF levels, or by alterations in IGF activity associated with changes in serum levels of IGF BP. Other findings confirm this hypothesis: glucocorticosteroids decreased the number of mitoses in both the gastric and duodenal mucosa, and reduced the weight of the stomach, small

intestine and colon in growing rats. This process is reversed by IGF administration which induces proliferative events, such as the mitogenesis of intestinal cells [Stteb et al., 1995]. Dexamethasone enhanced the activity of disaccharidases in jejunum and ileum of sham-operated rats, and enhanced the sucrase and lactase activity in the duodenojejunum but not in the ileum of resected rats. Thus, glucocorticosteroids may control the site along the gastrointestinal tract responsible for nutrient digestion and absorption during intestinal adaptation via differential responsiveness of the proximal compared with the distal bowel. The mechanisms involved in this process are unknown [Park et al., 1994].

Dexamethasone (0.2 μg/ g / day, given by infusion into the fetal amniotic cavity for seven days in a rabbit fetal gastroschisis model) increases the fetal small intestinal lactase activity by 70% [Guo et al., 1995]. However, the level of this enzyme is lower than in normal fetuses. The rate of glucose uptake is increased by 100% by dexamethasone, but again it is still lower than in normal fetuses.

Administration of dexamethasone (0.8 mg/150 g/ day SC for 2 days) was associated to a significant increase in bacterial adherence to the mucosa, accompanied by alterations in intestinal permeability. Thus, the bacterial-mucosa cell interactions may be responsible for the changes in the intestinal permeability after dexamethasone administration [Spitz et al., 1994].

3.22.b) Glucocorticosteroids and Intestinal Ontogeny

The human fetal serum hydrocortisone concentration doubles during the last 4 weeks of gestation [Murphy, 1982]. Release of corticosterone, a hormone similar to cortisol but less potent, has been observed to be one of the main factors in small intestinal

maturation [Marti and Fernandez-Otero, 1994; McDonald and Henning, 1992]. This may represent a possible specific modulatory influence of glucocorticosteroid hormones on human fetal intestine. Several lines of evidence suggest this possibility: in immature animals, glucocorticosteroids induce precocious development of adult BBM activities, and facilitate the induction of specific enzymes by dietary carbohydrate. [Lebenthal et al., 1972; Nsi-Emvo et al., 1996; Sangild et al., 1995; Smeard and Walker, 1988]. The BBM activities of sucrase and isomaltase are not detectable in rats before 15 or 16 postnatal days. Glucocorticosteroids precociously induce the activity of α-glucosidases. Sucrase and isomaltase are not detectable, nor are they induced by sucrose administration in adrenalectomyzed rats. And yet, the administration of glucocorticosteroids caused the appearance of these hydrolytic enzymes. After adrenalectomy, a decrease in the expected developmental rise of sucrase activity on the 17th postnatal day is observed, but on days 18, 21 and 28 no effects are seen [Henning and Sims, 1979]. After the administration of hydrocortisone (50 mg/g) on the 15th or 16th postnatal day, an increase in sucrase activity is observed. However, the hydrocortisone given on the 17th, 18th or 28th postnatal days does not affect sucrase activity. Neither adrenalectomy nor the administration of glucocorticosteroid have an effect on the activities of disaccharidases in the adult animal [Deren et al., 1967]. The rise in the sucrase-isomaltase mRNA after glucocorticosteroid administration indicates that these glucocorticoid effects on intestinal development are mediated by the crypt epithelial cells, and are due to the synthesis of new proteins, rather than to post-transcriptional enzyme activation [Nanthakumar and Henning, 1993]. The dosing of corticosteroid together with sucrose has a synergistic effect. The adult values of

BBM sucrase and isomaltase occur by the 30th postnatal day. At this time, the intestine is altered, so that it displays a more mature histological appearance [Lebenthal et al., 1972]. There is a parallel increase of corticosterone levels with that of ornithine decarboxylase activity (ODC) and ODC mRNA in preweaned intestine. Following these increases, the mucosal polyamine content also increases. It is speculated that polyamines mediate the premature expression of sucrase-isomaltase mRNA and sucrase activity [Nsi-Emvo et al., 1996; Rosewicz and Longsdon, 1991].

Different enzymes and functions mature after glucocorticosteroid administration such as salivary amylase, pepsinogen, gastrin receptors, pancreatic amylase, ileal lysosomal hydrolases, jejunal and ileal pinocytosis, ileal bile salt transport, intestinal absorption of immunoglobulins, intestinal pyruvate kinase, hepatic tryptophan oxygenase, hepatic ornithine aminotransferase, glutamine synthetase and hepatic production of α-protein [Henning, 1981; Sarantos et al., 1994]. Thus, these effects show the glucocorticosteroid capacity of maturation in different functions and systems.

How is the sucrase-isomaltase (SI) in rat small intestine modulated by exogenous and endogenous glucocorticosteroids during 2nd and 3rd weeks? Although sucrase and isomaltase are distinct enzymes, they are synthesised as a single polypeptide from a 6-kb sucrase-isomaltase mRNA. Adrenalectomyzed rats on 9th postnatal day had retarded appearance of sucrase, and the SI mRNA paralleled the enzyme activity. Administration of dexamethasone daily starting on 10th postnatal day had a precocious appearance of SI and SI mRNA. Starting dexamethasone on the 16th day produce an accelerated rise in SI and SI mRNA; starting on 18th day did not have effect. These results suggest that there

are three distinct phases of glucocorticoid action: 1) an early phase, where activation of the gene occurs (probably around 10th postnatal day): 2) a late phase, where changes in cell kinetics occurs (probably around 16th postnatal day): and 3) a final phase, where there is a loss of responsiveness. It is likely that glucocorticosteroids regulate sucrase activity at a pretranslational level such as transcription and/or mRNA stability [Nanthakumar and Henning, 1993].

The appearance and elevation of sucrase activity are preceded by a marked increase in its mRNA. However, even the mRNA increase is relatively slow (12-24 hours) after glucocorticoid administration. Glucocorticoid induction of sucrase in the epithelium is dependent on the underlying mesenchyme, and suggests that glucocorticosteroid most likely acts indirectly through one or more regulatory genes [Nanthakumar and Henning, 1993].

The thymidine analogue 5-bromo-2-deoxyuridine (BrdU) selectively inhibits differentiation in a number of tissues in suckling and mature animals. The BrdU had no effect on lactase and sucrase activity; thus, BrdU does not inhibit crypt-villus differentiation [Nanthakumar and Henning, 1995]. The administration of dexamethasone plus BrdU on the 10th postnatal day inhibited glucocorticoid-induced elevation of sucrase, trehalase and glucoamylase, but had no effect on lactase activity. This suggests that such maturation involves a different molecular mediator than does normal ontogeny. In contrast, administration of BrdU during normal development accelerated the ontogenic rise of sucrase and trehalase, as well as the ontogenic decline of lactase. This acceleration was also seen in adrenalectomyzed animals, which suggests that a

glucocorticosteroid-independent component of normal intestinal ontogeny is activated by BrdU [Nanthakumar and Henning, 1995].

3.22.c) Effects of Glucocorticosteroids on Ion Transporters

In glucocorticoid deficient rats, Na^+ is not absorbed and Cl^- and HCO_3^- are secreted. In the group treated with methylprednisolone (40 mg per day for two days), more Na^+ was absorbed and more HCO_3^- was secreted. Also, the maximal secretory stimulus by 8-Br-cAMP of Cl^- secretion was similar in all three groups [Sellin and Field, 1981]. This study suggests that the glucocorticoids enhance Na absorption and HCO_3^- secretion, and do not affect the maximal secretory capacity of Cl^-. Corticosteroids are used in diarrheal diseases because of this proabsorptive effect as well as the antiinflammatory effect of this drug. The Na^+ absorption involves a primary increase in Na^+ entry across the brush border membrane (BBM) of the intestinal epithelial cell, and also involves direct stimulation of sodium-potassium-adenosine triphosphatase activity (Na^+/K^+ ATPase) in the basolateral membrane. The mechanism by which glucocorticosteroids stimulate active intestinal Na^+ absorption is unclear, but in renal BBM glucorticosteroids stimulate the sodium/hydrogen (Na^+/H^+) exchange process [Donowitz et al., 1986]. Glucocorticoids increase the expression of the Na+/H+ exchanger in the ileum and proximal colon, but not in jejunum, distal colon or kidney [Cho et al., 1994; Yun et al., 1993]. Thus, in general, the glucocorticosteroids stimulate the fluid absorption in duodenum, jejunum and ileum. There is no stimulation by glucocorticoids in the colon. Usually, there is an increase in net Na^+ absorption in proportion to the increase in the fluid absorption.

Phosphate is effectively absorbed in the duodenum and jejunum. but phosphate absorption in ileum and colon is negligible. The administration of cortisone increases phosphate absorption in the small intestine but not in the colon. Thus. the glucocorticoids enhanced the phosphate absorption in rat jejunum. where passive transport predominates. This suggests that glucocorticoids increase phosphate uptake not by enhancement of fluid movement. but by a direct effect on mucosal membrane permeability [Yeh and Aloia. 1987]. In contrast. a different study reported decrease in phosphate transport in rats and chicks after glucocorticosteroid administration [Ferraro et al.. 1976]. The concentration of phosphate used in this ligated loop study was 2 mM. which could be considered as a condition where active transport of phosphate predominates. This discrepancy might be explained by the hypothesis that glucocorticosteroids would have different effects on phosphate absorption in rat intestine depending on the concentration of phosphate in the lumen. and therefore whether active or passive transport predominates [Yeh and Aloia. 1987].

The general glucocorticoid effect on calcium metabolism is a decrease in its intestinal absorption. However, the corticoids increase the passive transport of Ca^{-2} in the proximal regions of the small intestine. where they compensate for their inhibitory effect on the active transport of Ca^{-2}. The clear dissociation between Ca^{-2} and phosphate absorption efficiency in the ileum suggests that the absorptive processes for Ca^{-2} and phosphate are separate in this region [Yeh and Aloia. 1987].

3.23) Clinical Aspects of the New Non Systemic Steroids

Corticosteroids are widely used to treat a variety of gastrointestinal and hepatic conditions such as ulcerative colitis, Crohn's disease and chronic active autoimmune hepatitis [Bratssand, 1990; Truelove, 1956; Truelove, 1957; Watkinson, 1958; Matts, 1961; Mac Dougal, 1963; Danielson and Pritz, 1994; Tarpila et al., 1994; Nyman-Pantelidis et al., 1994; Lofberg et al., 1993; Hanauer et al., 1995]. However, the systemically active steroids are associated with numerous potentially serious adverse effects [Haynes and Murad, 1985; Haynes and Larner, 1975; Baxter, 1979; Girdwood and Petrie, 1987; Enrich et al., 1992; Loeb et al., 1973; Delemos et al., 1970; Motoyama et al., 1971; Ballard et al., 1974; Sambhi et al., 1965, Krakoff et al., 1975; Shimidt et al., 1972; David et al., 1970, Nicollof, 1969; Okabe et al., 1971]. Even topical treatment with systemic steroids using retention enemas or foams depresses plasma cortisol levels [Spencer and Kirsner, 1962; Farmer and Schumacher, 1970; Cann and Holdsworth, 1987; Reshef et al., 1992; Rodrigues et al., 1987].

The "perfect" steroid would have high topical glucocorticosteroid potency, low systemic bioavailability, rapid first pass metabolism in the intestine or liver, and rapid excretion. In this way, the numerous and potentially serious adverse effects might be avoided or minimized. To improve the poor topical anti-inflammatory action of systemic steroids such as betamethasone, dexamethasone and triamcinolone, lipophilic groups are introduced in the 16-α and/or 17-α positions of the steroid nucleus. Betamethasone 17-valerate and triamcinolone acetonide have been synthesized and used to treat patients with certain dermatological diseases as well as asthma (Mygind and Clark, 1980). Having

lipophilic groups, these corticosteroids are easily inactivated in the liver, which results in less circulating corticosteroids and consequently fewer systemic adverse effects [Mygind and Clark, 1980; Martin et al., 1975; Jewell and Phill, 1990]. Glucocorticosteroids with 16α, 17α acetyl groups, like budesonide and fluticasone propionate [Bratssand et al., 1982; Clissold and Hell, 1984; Bauer et al., 1988], have features which facilitate their topical anti-inflammatory activity and stability in extra-hepatic tissues such as the airways and intestinal walls. This confers about 90% first-pass hepatic metabolism of the parent drug to metabolites which posses minimal or no biological activity [Bratssand, 1990; Greenberg , 1994].

Tixocortol pivalate, a synthetic corticosteroid, has high first-pass metabolism, but does not have high topical corticosteroid potency [Juniem, 1988]. Fast and extensive transformation of tixocortol pivalate into inactive metabolites provides an explanation for the large dissociation between the topical and systemic activities of this drug [Chanoine et al., 1987; Chanoine and Juniem, 1984]. Reduced intestinal absorption of tixocortol pivalate may be an important factor to explain its low topical activity [Chanoine, 1988]. Tixocortol is an effective steroid for topical use in patients with ulcerative colitis, and is not associated with adverse reactions [Hanauer, 1988; Larochelle, 1983].

Beclomethasone dipropionate, fluticasone propionate and budesonide are called 17-α substituted glucocorticosteroids. These drugs have high topical activity (much greater than that of tixocortol pivalate), and have rapid first-pass metabolism in the liver. Fluticasone propionate has a systemic bioavailability which is extremely low as a result of extensive hepatic first-pass metabolism. Fluticasone has a topical potency two-times

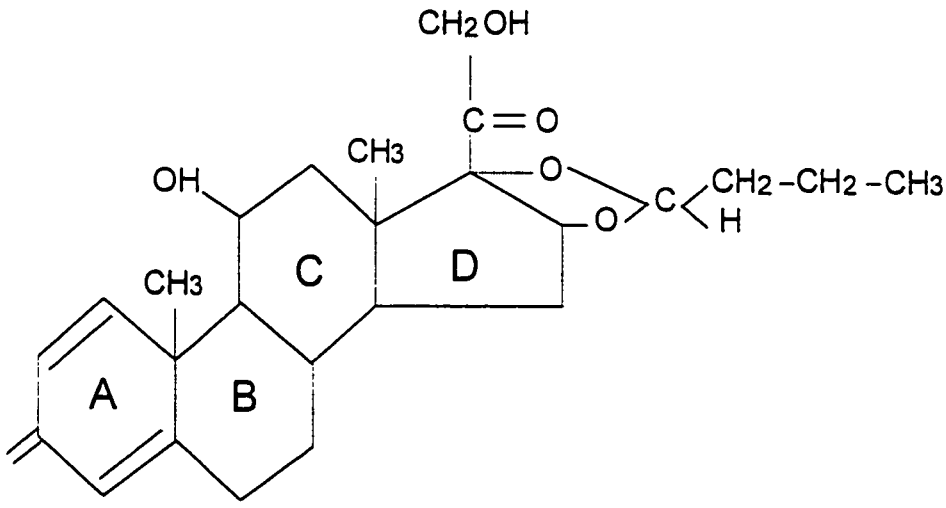
greater than that of beclomethasone dipropionate. Its oral bioavailability is low due to its low absorption from the intestine [Bratssand. 1990]. In an uncontrolled trial. the administration of an oral daily dose of 20 mg fluticasone propionate was shown to be effective to treat patients with mild to moderately active small intestinal and colonic Crohn's disease [Carpani de Kaski et al.. 1991]. In contrast. two controlled studies failed to show any benefit of fluticasone in the treatment of patients with ulcerative colitis [Hawthorne et al.. 1993. Wright et al.. 1993].

Beclomethasone dipropionate has a topical activity 100-times greater than that of tixocortol pivalate. Beclomethasone dipropionate is metabolized in the liver. resulting in oxidative products and beclomethasone which retain corticosteroid activity [Axelsson B. et al., 1984]. Beclomethasone is effective in the treatment of patients with proctosigmoiditis [Lofberg et al.. 1993. Van der Heide et al.. 1988; Kumana et al.. 1982; Levine and Rubin . 1985; Van der Heide et al.. 1987; Halpern et al.. 1991].

Budesonide. the new non-systemic glucocorticosteroid with substitution at the 17α ester position. has high topical activity. double that of beclomethasone dipropionate. It is 100-times more water soluble than beclomethasone and tixocortol. which facilitates its intestinal absorption [Bratssand. 1990; Greenberg. 1994; Edsbadker et al.. 1987a; Edsbadker et al..1987b]. Budesonide is metabolised in the liver. and has a 90% first-pass metabolism which allows only about 10 % of intact budesonide to reach the systemic circulation. Down-regulation of the expression of IL-6 mRNA in rat intestinal muscle cells and in splenic monocytes by budesonide *in vitro* illustrates the anti-inflammatory effect of this new non-systemic steroid [Deng et al.. 1996]. In addition. the inhibition *in*

vitro of the release of pro-inflammatory cytokines by intestinal lamina propria mononuclear cells from colonic biopsies as well as peripheral monocytes also demonstrates the budesonide's anti-inflammatory activity [Schreiber et al.. 1996].

Figure 7. Budesonide



BUDESONIDE

Physical Chemical Properties:

1. 90% fist-pass metabolism
2. High topical activity
3. 100 times more water soluble than tixocortol and beclomethasone

3.24) Topical Budesonide

Budesonide has been used for the treatment of patients with distal ulcerative colitis, proctitis and Crohn's disease (Table 2). Good clinical results have been observed with budesonide enemas (2 mg/100 ml) [Danielsson et al., 1992; Matzen, 1991; Hanauer et al., 1995; Pruitt et al., 1996]. When given as an enema, budesonide does not cause alterations in the levels of endogenous plasma cortisol [Halpern et al., 1991; Pruitt R. et al., 1996]. In distal colitis, budesonide enemas are equivalent in efficacy to 5-ASA enemas, or to enemas containing prednisone, methyl prednisolone or hydrocortisone [Mac Dougal, 1963, Danielsson et al., 1992, Lofberg et al., 1994; Leman et al., 1995; Lamers et al., 1991; Bianchi Porro et al., 1994; Bayless et al., 1995]. Low viscosity budesonide enemas are more effective in patients with ulcerative colitis and proctitis than are the high viscosity enemas, presumably because the former spread over a larger area [Nyman-Pantelidis et al., 1994]. It is suggested that a hydrophobic suppository with prednisolone should be used for patients in whom inflammation is confined to the rectum, and a hydrophilic suppository used for patients in whom inflammation reaches the rectum and the middle part of the sigmoid colon [Sadahiro, 1992].

In Canada, budesonide enemas are approximately the same cost as betamethasone or 5-ASA enemas. The comparative studies performed to date have not considered the total consumption of enemas over an extended time, such as 12 months. Because of the high recurrence rate of distal colitis/proctitis, and the need for some patients to use enemas continuously or intermittently, this becomes an important issue when considering cost and efficacy.

Table 2. Clinical Trials of Budesonide Eenemas in Distal Colitis

Budesonide enemas vs:	Author	Outcome
placebo	Danielsson 1992 Hanauer 1995	Bud > PL Bud > PL
prednisolone	Matzen 1991 Lofberg 1994	dose-ranging study Bud = Pred
methyl prednisolone	Bianchi Porro 1994	Bud = MPred
hydrocortisone	Tarpila 1994 Bayless 1995	Bud = Hc Bud = Hc
5-ASA	Lamers 1991 Leman 1995	Bud = 5-ASA Bud = 5-ASA

Abbreviations:Bud: budesonide; Hc: hydrocortisone: MPred: methyl prednisolone: P: prednisolone; PL: placebo; 5-ASA: 5-aminosalicyclic acid

In a recent meta-analysis of the efficacy of budesonide enemas in distal ulcerative colitis. this drug has shown to be as effective as conventional rectal steroids. with less suppression of endogenous cortisol production [Marshall et al.. 1996].

3.25) Oral Budesonide

The controlled ileal release (CIR) form of the budesonide taken by mouth is composed of a gelatine capsule involving multiple acid-stable microgranules which contain an inner sugar core surrounded by budesonide. and an outer coat of Eudragit L100-55 that dissolves in the small intestine at pH 5.5 or higher. Budesonide CIR is targeted for absorption in the ileo-cecal region. From 52% to 79% is absorbed. with a mean absorption time of 6.4 hours, and a systemic bioavailability of only 9% [Edsbacker et al.. 1987]. Budesonide CIR has been used in patients with Crohn's disease. with good clinical results [Lofberg et al., 1993. Greenberg et al.. 1994: Rutgeerts et al.. 1994;

Campieri et al., 1995]. For example, Greenberg and colleagues [Greenberg et al., 1994] tested the efficacy of varying doses of budesonide CIR in patients with active Crohn's disease: remission occurred in 51% of patients receiving 9 mg of budesonide, in 43% of those receiving 15 mg and in 33% of those receiving 3 mg of budesonide: the 9 and 15 mg doses were statistically superior to placebo. Although budesonide causes a slight alteration in basal and corticotropin-stimulated plasma cortisol concentrations, this drug was not associated with clinically important adverse effects [Greenberg et al., 1994]. Rutgeerts and co-workers (Rutgeerts et al., 1994; Campieri et al., 1995] assessed the efficacy of budesonide versus prednisolone in patients with active ileal or ileocecal Crohn's disease. The efficacy of prednisolone was greater than budesonide in terms of the patient's Crohn's disease activity index (CDAI): after 10 weeks of therapy, 53% of the patients receiving budesonide were in remission (determined as a CDAI score below or equal to 150), whereas 66% of patients receiving prednisolone were in remission. However, budesonide was associated with fewer side effects, as well as fewer effects on pituitary-adrenal function. Furthermore, two patients in the prednisolone group had serious complications of intestinal perforation and an abdominal-wall fistula [Rutgeerts et al., 1994].

In a thoughtful editorial [Sachar, 1994], Sachar raised three interesting points: firstly, in patients with active Crohn's disease, the peak remission rates were not achieved with budesonide before eight weeks, whereas in the European study the remission rates with prednisolone were maximal by four weeks. Secondly, in the absence of a unified study including budesonide, prednisolone and placebo groups, the clinical importance of

the lower incidence of systemic effects with budesonide is uncertain and difficult to assess: thirdly. if budesonide truly has fewer side effects than conventional corticosteroids. this may commensurate with a somewhat slower action and lower therapeutic efficacy .

Systemic effects have been reported with budesonide CIR 4.5 mg b.i.d. for 8 weeks and then with 4.5 mg once in the morning for 2 weeks. These systemic effects were assessed by cortisol excretion and plasma concentration of budesonide [Naber et al.. 1996]. In relation to the suppression of osteoblastic function. budesonide CIR is superior to conventional steroids. being considered safer for long-term use than systemic steroids [D' Haens et al.. 1996]. Systemic activity of CIR budesonide (200 μg/kg/day) in rats has also been demonstrated. However. this steroid was very effective in reducing intestinal inflammation [Boyd et al.. 1995].

Budesonide may also be useful to delay the time to recurrence of attacks of Crohn's disease [Greenberg et al.. 1996; Lofberg et al.. 1994]. However. by a year only one-third of the treatment and placebo groups were still free of relapses. This is important. since repeated relapses of Crohn's disease clearly increase morbidity of the disease [Bayless. 1996].

Oral budesonide (6 mg once daily) offers no benefit in preventing endoscopic recurrence after surgery for ileal/ileocecal fibrostenotic Crohn's disease. but it may be useful in patients who have undergone surgery for disease activity [Heller et al.. 1996]. Thus. budesonide is an effective therapy for the treatment of patients with Crohn's disease. without the risk of as many adverse effects.

3.26) Budesonide in Hepatitis , Ulcerative Colitis and Intestinal Transplantation

In an uncontrolled trial, it was shown that oral budesonide induces a remission in patients with autoimmune chronic active hepatitis, with low frequency of corticosteroid-related adverse effects [Lofberg et al., 1993]. Controlled colonic release budesonide may be useful in patients with active ulcerative colitis [Lofberg et al., 1994]. A controlled colonic release form of prednisolone metasulphabenzoate (Eudragit-coated prednisolone) may be useful for the treatment of extensive ulcerative colitis, without the risk of systemic steroid side-effects [Mc Intyre et al., 1985, Ford et al., 1992].

The administration of pH-modified release capsules of budesonide (Budenofalk 3mg) targeting colonic therapy has little effect on blood lymphocytes and granulocytes, indicators for systemic side effects [Moellmann et al., 1996].

An oral glucocorticoid-conjugate, budesonide-beta-D-glucuronide, which is not absorbed in the rat small intestine but is hydrolyzed by colonic bacterial and by mucosal beta-glucuronidase, releases free budesonide into the colon. This decreases the bioavailability of this drug, and consequently decreases its side effects [Mc Leod et al., 1994; Tozer et al., 1991; Cui et al., 1994]. More work is needed to determine whether this delivery system may be an alternative for treating patients with inflammatory bowel disease.

Budesonide has been demonstrated to be a useful immunosuppressive agent for clinical intestinal transplantation. This agent increases the immunosuppressive effect of cyclosporin A and prolongs small bowel allograft survival in rats without the expected

systemic activity of glucocorticosteroids (inhibition of normal ACTH release) [Ozcay et al., 1997].

3.27) Conclusions

The new non-systemic steroid budesonide is promising for the treatment of distal colitis when given by enema, and for Crohn's disease when given by mouth. Budesonide's adverse effect profile is superior to conventional glucocorticosteroids. Further applications may include the management of patients with ulcerative colitis or autoimmune chronic active hepatitis. Long-term safety studies using budesonide for inflammatory bowel disease are needed, and the results of cost-efficacy and quality life assessments are yet to be reported.

C) HYPOTHESES

I reviewed in detail the potential links between glucocorticosteroids. dietary lipids and the process of adaptation of nutrient transport. Based on this background I propose the following hypotheses:

- Systemically and locally acting glucocorticosteroids enhance the intestinal absorption of sugars and lipids.
- Feeding a saturated fatty acid diet will enhance the effect of steroids on the intestinal uptake of nutrients.

The rat animal model was used in the experiments based on the literature review where most of the studies describe the uptake and effect of steroids on rat small intestine. Weanling animals were chosen because this specific aging period involves dramatic changes in terms of intestinal uptake. Exactly at the third week. the animals need to obtain all their nutrients from a different source other than the mother's milk. This period of transition has been highly associated to the high peak of physiological corticosterone found at the period time. We speculate that administrating steroids to weanling animals until they reach adult life. that we would find more effects than administrating steroids in adult life in function of their higher sensitivity to steroids. This sensitivity to steroids has been correlated with the amount of glucocorticoid receptor present in the cell's cytoplasm.

D) METHODS

1) Animals and Diets

The principles for the care and use of laboratory animals, approved by the Canadian Federation of Biological Societies and by the Council of the American Physiological Society, were carefully observed in the conduct of this study. Weanling male Sprague Dawley rats, 21-23 days of age *post partum*, were obtained from the University of Alberta Vivarium. Pairs of rats were housed at a temperature of 21^{0}C, with 12 hours of light and 12 hours of darkness. Water and food were supplied *ad libitum*. The animals were fed one of three diets: a fed standard Purina^R rat chow, a semisynthetic diet with 20% (w/w) fat enriched in either polyunsaturated (PUFA) or saturated (SFA) fatty acids corresponding to a high or low polyunsaturated-to-saturated fatty acid ratio, respectively. The weaning rats were fed chow, SFA or PUFA for 4 weeks. These isocaloric semi-synthetic diets were nutritionally adequate, providing all known essential nutritional requirements. PUFA provided approximately 22 % of calories and 69 % of total fatty acids (% w/w) as 18:2ω6 (linoleic acid), whereas SFA provided 22 % of calories and 9.6 % of total fatty acids as 18:2ω6.

The animals were sacrificed by the injection of sodium pentobarbitol (240 mg/100 g body weight). The whole length of the small intestine was removed rapidly. The proximal third was termed the jejunum and the distal third the ileum; the middle third was discarded.

Table 3. Macronutrient Composition of the Semisynthetic Diets

INGREDIENTS	CONCENTRATION (g/kg DIET)
Fat	200.00
Cornstarch	378.00
Casein	270.50
Non-nutritive fiber	80.00
Vitamin mix*	10.00
Mineral mix#	50.00
L-methionine	2.50
Choline	2.75
Inositol	6.25

*A.O.A.C. vitamin mix (Teklad Test Diets. Madison. WI) provided the following per kilogram of complete diet: 20,000 IU of vitamin A; 2,000 IU of vitamin D; 100 mg of vitamin E; 5 mg of menadione; 5 mg of thiamine-HCl; 8 mg of riboflavin; 40 mg of pyridoxine-HCl; 40 mg of niacin; 40 mg of pantothenic acid; 2,000 mg of choline; 100 mg of myoinositol; 100 mg of p-aminobenzoic acid; 0.4 mg of biotin; 2 mg of folic acid; and 30 mg of vitamin B12.

#Bernhart Tomarelli mineral mix (General Biochemicals, Chagrin Falls, OH) was modified to provide 77.5 mg of Mn and 0.06 mg Se per kilogram of complete diet.

Table 4. Fatty Acid Composition of The Semisynthetic Diets

FATTY ACID (% of total)	SFA	PUFA
14:0	1.8	0.1
14:1ω9	0.0	0.0
15:0	0.2	0.0
16:0	21.8	5.5
16:1ω7	0.0	0.1
18:0	61.1	5.2
18:1ω9	2.8	16.3
18:1ω7	0.1	0.2
18:2ω6	9.6	69.2
18:3ω3	0.0	0.8
18:3ω6	0.0	0.1
18:4ω3	0.0	0.0
19:0	0.2	0.0
20:0	1.5	0.2
20:1ω9	0.0	0.3
20:1ω7	0.1	0.3
20:2ω6	0.1	0.2
20:3ω9	0.1	0.2
20:3ω6	0.0	0.0
20:4ω6	0.0	0.0
20:5ω3	0.0	0.3
22:0	0.0	0.4
22:1ω9	0.0	0.0
22:1ω7	0.3	0.3
22:4ω6	0.0	0.1
22:5ω6	0.0	0.0
22:5ω3	0.1	0.1
24:0	0.1	0.1
22:6ω3	0.1	0.0

2) Drugs

There were 24 animals in each of three drug groups: control (CON, 0.19% EDTA buffered saline), budesonide (BUD, 0.25 mg/kg body weight per day), and prednisone group (PRED, 0.75 mg/kg body weight per day). The doses of PRED and BUD were chosen on the basis of regimens which have been shown to be useful clinically [Bratssand, 1990; Rutgeerts et al., 1994; Greenberg et al., 1994]. These doses are similar to the doses used to treat trinitrobenzene sulphonic acid ileitis in rats [Boyd et al., 1995] and are lower than the doses used to prevent graft rejection in a rat model of intestinal transplantation (1.0 mg/kg/day) [Ozcay et al., 1997]. Within each drug group there were eight rats fed Chow, eight fed PUFA, and eight fed SFA. The drugs were administrated each day by oral gavage, and were dissolved in 0.19% EDTA buffered saline. The volume of vehicle given was 5 μl/g body weight. The oral dosing was performed at 12:00 h daily including weekends, and was continued for 4 weeks.

3) Morphology

To prepare the histological sections, the samples of the jejunum and ileum were dehydrated, embedded in paraffin, sectioned for light microscopy, and stained with hematoxylin and eosin using standard techniques. Multiple histological cross-sections were scanned for areas in which the section passed through the entire vertical length of villus and crypts. The areas of longitudinal cross-section of villi were measured using the Magiscan-GENIAS image analysis program (JOYCE-LOBEL, 1987 England) in at least 10 well-oriented crypt-villous systems for each animal group.

4) Probes and Marker Compounds

The [^{14}C]-labelled probes included lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18;1), linoleic acid (18:2), linolenic acid (18:3) (each at 0.1 mM), cholesterol (0.05 mM), L-glucose (16 mM), D-mannitol (16 mM), and varying concentrations of D-glucose and D-fructose (4, 8, 16, 32 and 64 mM). The long-chain fatty acids and cholesterol were solubilized in 10 mM taurodeoxycholic acid [Thomson and O'Brien, 1981]. Unlabelled and [^{14}C]-labelled probes were supplied by Sigma Co. (St Louis, Missouri) and by New England Nuclear, respectively. [^{3}H]-inulin was used as a non-absorbable marker to correct for the adherent mucosal fluid volume. Probes were shown by the manufacturer to be more than 99% pure by high performance liquid chromatography.

5) Tissue Preparation and Determination of Rates of Uptake

The animals were sacrificed by the injection of sodium pentobarbitol (240 mg/100 g body weight). The whole length of the small intestine was removed rapidly. The proximal third was termed the jejunum and the distal third the ileum; the middle third was discarded. The intestine was everted and cut into small rings of length of approximately 2-4 mm each. The rings were immersed immediately in preincubation beakers containing oxygenated Krebs-bicarbonate buffer (pH 7.2) at 37^{0}C, and were allowed to equilibrate prior to commencement of the uptake studies. Uptake was initiated by the timed transfer of tissue rings to a shaking water bath (37^{0}C) containing 5 ml plastic vials with gassed Krebs buffer, plus [^{3}H]-inulin and the [^{14}C]-labelled substrates. After incubation for 5 min. the uptake of nutrient was terminated by pouring the vial contents

onto filters immobilized on an Amicon vacuum filtration manifold maintained under suction. This was followed by washing jejunal or ileal rings with ice-cold saline. The tissue was dried and the weight was recorded prior to saponification with 0.75 N NaOH. Scintillation fluid was added, and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes.

6) Expression of the Results and Statistics

The rates of uptake were expressed as nmol of substrate taken up per 100 mg dry weight of tissue per minute (nmol*100 mg tissue^{-1}*min^{-1}). The values obtained from the two dietary groups (Chow, SFA or PUFA), and from the three treatment groups (control [CON], prednisone [PRED] or budesonide [BUD]), are reported as the mean ± SEM of results obtained from eight animals in each group.

The values of the maximal transport rate (Vmax) and the apparent Michaelis constant (Km) were estimated using non-linear regression software (Sigma Plot program, Jandel Scientific, San Rafael, CA). Because there was a linear relationship between fructose concentration and uptake, the values of Vmax and Km could not be calculated. For this reason, linear regression was used to obtain the value of the slope of this linear relationship. The ANOVA one way and Student Newman-Keul test were used to determine the significance of the differences among the means of animals fed Chow and treated with CON, BUD or PRED. The ANOVA two ways and Student Newman-Keul test were used to test the animals fed SFA and PUFA, and given CON, BUD or PRED. A p value of 0.05 or less was accepted as representing a statistically significant difference.

E) RESULTS

1) Animal Characteristics

Food intake was similar in the control (CON). budesonide- (BUD) and prednisone (PRED) - treated animals [Table 5]. Despite this, weight gain was lower ($p < 0.05$) in BUD than in CON or in PRED. This may have been a spurious result, since the body weight gain in rats given BUD 0.75 and 1.0 mg/kg was similar to controls [appendix I]. The percentage of weight gain (g/day) per food intake (g/day) was lower in BUD than in CON, and yet was higher in PRED than in CON, and was also higher in PRED than in BUD. The mean weight of the intestine (mg/cm length) and the percentage of the intestinal wall comprised of mucosa were similar in CON, PRED and BUD [Table 6].

Prednisone (PRED) and Budesonide (BUD) had no effect on food intake or body weight gain, but the weight gain was higher in the rats fed PUFA as compared with SFA [Table 7]. PRED and BUD had no effect on the total weight of the intestine, or on the % of the intestinal wall comprised of mucosa [Table 8]. Also, steroids had no effect on the villous height of jejunum or ileum of rats fed SFA, or for the jejunum of animals fed PUFA. In the ileum of rats fed PUFA, the villous height was higher ($p<0.05$) in animals given PRED as compared with BUD. In the ileum of rats given BUD, the height of the villi was less in those fed PUFA than SFA.

2) Uptake of Sugars

A curvilinear relationship was noted between the concentration of D-glucose in the bulk phase (4-64 mM) and the rate of glucose uptake [appendix II]. The estimated values of the maximal transport rate (Vmax) and of the apparent Michaelis constant (Km)

for glucose uptake were unaffected by treatment with PRED or with BUD (0.25 mg/kg) [Table 9]. BUD given in doses of 0.75 and 1.0 mg/kg also had no effect on glucose uptake [appendix III]. In addition. the jejunal and ileal rates of uptake of L-glucose and of D-mannitol were unaffected by PRED or BUD as compared with CON [Table 10].

A linear relationship was noted between increasing concentrations and the rate of uptake of fructose [appendix IV]. Because this relationship was linear, it was not possible to calculate values for Vmax or for Km. In the jejunum and ileum. the slope of this linear relationship was higher ($p<0.05$) in PRED and in BUD (0.25 mg/kg) as compared with CON [Table 11]. A dose of BUD 1 mg/kg increased the slope of fructose uptake into the jejunum to 13.9 nmol*100mg tissue^{-1}*min. as compared with 12.1 nmol*100 mg tissue^{-1}*min in CON.

There was a curvilinear relationship between the concentration and the rate of uptake of glucose [appendix II]. The values of the maximal transport rate (Vmax) and apparent Michaelis constant (Km) were unaffected by giving PRED or BUD [Table 12]. The value of the Vmax for ileal uptake of glucose was higher in control animals fed SFA versus PUFA. There was no further change giving PRED or BUD. The rates of uptake of L-glucose and D-mannitol were similar, and were unaffected by PRED or BUD in animals fed PUFA or SFA [Table 13].

The relationship between fructose concentration and uptake was linear [apendix IV]. As a result, it was not possible to calculate values of Vmax or Km. The slope of the linear relationship between fructose concentration and uptake into the jejunum or ileum was greater in animals fed SFA as compared with PUFA [Table 14]. In animals fed SFA.

giving PRED or BUD increased jejunal and ileal uptake of fructose as compared with those given CON. This enhancing effect of PRED and BUD was not observed in animals fed PUFA. In rats fed SFA, the uptake of fructose was lower in the jejunum but higher in the ileum of those given BUD as compared with PRED.

3) Uptake of Lipids

As compared with CON, PRED increased jejunal uptake of cholesterol as well as ileal uptake of lauric (12:0), palmitic (16:0), linoleic (18:2) and linolenic (18:3) acids [Table 15]. BUD (0.25 mg/kg) increased jejunal uptake of oleic acid (18:1), and increased ileal uptake of linoleic acid (18:2). In BUD as compared with PRED, the jejunal uptake of 18:1 was higher and cholesterol was lower, and ileal uptake of 18:3 was lower. BUD in doses of 0.75 and 1.0 mg/kg also had no effect on the jejunal or ileal uptake of 16:0, 18:2 or cholesterol.

In rats fed PUFA, PRED increased the jejunal uptake of 12:0, 18:1 and 18:2, and increased the ileal uptake of 18:3. In contrast, in rats fed SFA and given PRED, there was no change in the jejunal uptake of lipids, whereas the rates of ileal uptake of 12:0 and 18:2 were increased and the uptake of 16:0 was decreased. Giving BUD to rats fed PUFA increased the jejunal uptake of 16:0, 18:1 and 18:2, and enhanced the ileal uptake of 18:1. In rats fed SFA, BUD increased the jejunal uptake of 12:0, decreased the jejunal uptake of cholesterol, and reduced the ileal uptake of 16:0, as compared with control vehicle [Table 16]. Thus, the enhanced uptake of 18:1 and 18:2 into the jejunum of rats fed PUFA was not observed in those fed SFA.

In rats fed PUFA, the jejunal uptake of 16:0 and the ileal uptake of 18:1 was higher with BUD than PRED. In those fed SFA, the rates of ileal uptake of 12:0, 18:0 and 18:2 was lower with BUD than with PRED.

F) DISCUSSION

1) Effects of Steroids

Animals fed BUD had a reduced rate of weight gain which was not explained by a change in food intake, or in the rate of uptake of glucose, fructose or lipids. In fact, animals given BUD had increased intestinal uptake of fructose [Table 11], and increased jejunal uptake of 18:1 and ileal uptake of 18:2 [Table 15]. The mechanism responsible for this lower weight gain in rats given BUD was not established in this study. However, in rats the hepatic metabolism is not quite as efficient as in humans, allowing up to 25% of BUD to reach the systemic circulation after oral administration [Chanoine et al., 1991]. Furthermore, BUD has less of an effect on cortisol; than does PRED. The systemic activity of budesonide would then provide the expected negative effect on the weight gain. It is interesting to note, however, that the dose of PRED used in this study did not alter body weight gain, despite its systemic nature. The effect of BUD on weight gain may have been spurious, however, because at higher doses (0.75 and 1.0 mg/kg), weight gain was similar to controls, and because BUD (0.25 mg/kg) has no effect on body weight gain in animals fed a semisynthetic diet enriched with saturated or polyunsaturated fatty acids [unpublished observations, 1997].

PRED acts both locally as well as systemically on the intestine, in contrast to the largely local action of BUD [Bratssand, 1990]. In adult animals PRED increases glucose absorption [Batt and Peters, 1976a; Batt and Peters, 1976b; Batt and Scott, 1982]. The lack of effect of PRED on glucose uptake in this study may be due to the younger age of the animals (in this study the animals were post-weanings; and in the other studies the

animals were adults). The lack of effect of PRED or BUD on the jejunal or ileal uptake of L-glucose or D-mannitol [Table 10] suggests that the paracellular contribution to sugar uptake also is unaffected by these steroids. The lack of effect of either PRED or BUD on the value of the Vmax of glucose uptake in these four week post-weaning rats [Table 9] suggests that there was no change in the expression of the activity of the sodium-dependent glucose transporter in the brush border membrane, SGLT1. The linear relationship between fructose concentration and uptake precluded the calculation of values for Vmax or Km. Fructose uptake is mediated by GLUT5 (the sodium-independent fructose transporter in the brush border membrane). It remains to be established whether the increased fructose uptake with BUD or PRED is associated with enhancement in the abundance of GLUT5 protein and mRNA in the brush border membrane of the enterocyte.

Intestinal lipid uptake occurs mostly by a process of passive permeation [Thomson et al., 1993], but a component of the uptake of long-chain fatty acids also is mediated by the sodium/hydrogen exchanger [Schoeller et al., 1995] and/or by fatty acid binding proteins in the brush border membrane [Poirier et al., 1996; Schoeller et al., 1996; see section 1.2.b]. The increased jejunal and ileal uptake of lipids in animals given BUD or PRED [Table 15] does not distinguish between which of these passive or mediated steps may have been affected by steroids. The enhanced uptake of lipids as a result of giving BUD or PRED was not explained by alterations in the animal's food intake or mucosal mass [Tables 5 and 6]. Adaptations in lipid uptake may be due to alterations in the lipid content of the brush border membrane (BBM) [Keelan et a., 1996], but these measurements were not performed in this study. However, any steroid-

associated change in the lipophilic properties of the BBM would be expected to modify the uptake of all lipids, and this did not occur [Table 15]. Thus, while it is unknown whether PRED or BUD affect the lipid composition of the BBM, we speculate that steroids also modify the protein-mediated component of lipid uptake.

In summary, giving weanling rats four weeks of oral budesonide or prednisone in doses equivalent to those used in clinical practice (ileitis: 0.20 μg/kg/day: intestinal transplantation: 0.1 to 1.0 μg/kg/day; these experiments: 0.25 μg/kg/day) up-regulates GLUT5 but not SGLT1, and also enhances the uptake of some lipids. These changes are not explained by variations in food intake or in the weight of the mucosa or in the villous height. The similar effects of a locally and a systemically acting steroid on fructose uptake suggests that the effect was produced at the level of the enterocyte, whereas the greater effect of PRED than BUD on lipid uptake raises the possibility that the mechanism was related to some systemic effect. One could speculate that the alterations in the expression of GLUT5 might occur due to up-regulation of GLUT2, the basolateral sugar transporter. However, if this possibility were true, alterations in the expression and activity of SGLT1 would also have been expected, and this was not observed.

2) Interaction Between Steroids and Lipids

In adult rats, feeding SFA increases the value of the Vmax for glucose uptake in the jejunum and ileum [Thomson and Rajotte, 1983]. This was observed only for the ileum in these young animals [Table 12]. Changing dietary lipids also had no effect on the passive uptake of D-mannitol or L-glucose [Table 13]. The reason for the lack of change in glucose uptake by SGLT1 in the jejunum in response to feeding SFA was not

established in this study. We suggest that this is not due to a lack of response of SGLT1 to dietary lipids, because the ileal uptake of glucose was greater in animals fed SFA as compared with PUFA [Table 14]. Neither prednisone nor budesonide alter glucose uptake in weaning rats fed chow [Table 9], SFA or PUFA [Table 12], suggesting that while the SGLT1 in the intestine of young animals is capable of responding to the stimulus of changes in dietary lipids, it is not responsive to the effects of steroids.

Fructose is transported by GLUT5, the sodium-independent transporter in the brush border membrane. Fructose transport is increased similarly by BUD and PRED in weanling rats fed chow, SFA but not PUFA [Table 11 and 14]. We propose that fructose uptake was modified by GLUT5 and not by passive permeation, because unlike the markers of passive permeation, fructose uptake was modified by dietary lipids and by steroids. This effect of steroids appears to be specific for GLUT5 and not for SGLT1, and the enhancing effect of steroids on fructose uptake observed in rats fed SFA can be prevented by feeding a polyunsaturated diet. The mechanisms involved in this inhibitory action observed with PUFA might be associated to the same negative effect observed in resected animals fed PUFA when compared to those fed SFA [Keelan et al., 1995]. It would appear that there is a degree of specificity of the diet effect (GLUT5 but not SGLT1), but it is unclear why the diet effect may be different for the jejunum than for the ileum.

When adult rats are fed isocaloric diets varying in their type of lipids (SFA and PUFA), there is no difference in food intake or body weight gain [Thomson et al., 1986]. In contrast, when post weanling rats are fed the same saturated (SFA) or a

polyunsaturated (PUFA) diet for four weeks, weight gain is approximately 30% higher in those fed PUFA as compared with SFA [Table 7]. The explanation for the greater weight gain is unclear; this is not due to a difference in food intake, or to any alteration in the passive or carrier-mediated jejunal uptake of glucose [Tables 12 and 13] or fructose [Table 14]. Indeed, ileal uptake of glucose was lower in PUFA than in SFA, and jejunal as well as ileal uptake of fructose was lower in PUFA than SFA. The differences in weight gain are also not explained by changes in lipid uptake *in vitro* [Table 16]. These studies do not provide an explanation for the greater weight gain in rats fed PUFA than SFA, but the explanation likely relates to variations in metabolism because food intake was similar and absorption was lower, not higher. Although SFA stimulate lipid uptake, it is possible that these lipids are not as well delivered to the blood and lymphatic system and that the absorption of lipids *in vivo* would be less when rats are fed SFA than PUFA, even though the initial step was greater. It is important to remember that the uptake was measured *in vitro*, meaning that *in vivo* uptake might have been different from that represented in the experiments. Therefore, alterations of uptake *in vivo* might explain the alterations observed in the weight gain in animals fed SFA versus PUFA.

The effect of BUD on lipid uptake depends upon the lipid composition of the diet: in animals fed PUFA, BUD increases the rate of jejunal uptake of the long-chain length fatty acids 16:0, 18:1 and 18:2, as well as the ileal uptake of 18:1 [Table 16]. In animals fed SFA, giving BUD reduces the jejunal uptake of cholesterol and the ileal uptake of 16:0. By the same token, giving PRED to animals fed PUFA increases the jejunal uptake of 12:0, 18:1 and 18:2, and as well increases the ileal uptake of 18:3. However, giving

PRED to rats fed SFA has no effect on lipid uptake in the jejunum, and reduces ileal uptake of 16:0. Thus, the ability of steroids to alter intestinal lipid uptake depends upon whether the animal is fed a diet enriched with PUFA or SFA. Feeding PUFA increases the effect of these steroids on the absorption of lipids. The mechanism of this interactive effect needs to be clarified.

The increased jejunal and ileal uptake of lipids in rats given PRED or BUD [Table 16] does not distinguish between which of these passive or protein-mediated steps may be affected by giving these steroids. The enhanced uptake was not associated with alterations in food intake, mucosal mass or villous height [Tables 7 and 8]. Adaptations in lipid uptake due to changes in the fat content of the diet are associated with alterations in the phospholipid or cholesterol content of the BBM [Thomson et al., 1986]. However, any steroid-associated alteration in the lipophilic properties of the BBM would be expected to change the uptake of all lipids, and this did not occur [Table 16]. Thus, we speculate that steroids modify the protein-mediated component of lipid uptake.

We did not establish the mechanism by which a polyunsaturated diet prevents the PRED- or BUD-associated enhanced uptake of fructose observed in rats fed a saturated diet, nor did we establish the mechanism by which a polyunsaturated diet increases the effect of those steroids on the absorption of lipids. Clearly, these locally and systemically active steroids, when given in doses which are effective clinically, modify the intestinal absorption of fructose and lipids by a process which is modified by the dietary content of lipids.

Budesonide has been increasingly used rather than prednisone for the treatment of inflammatory diseases in the intestine, because of its superior adverse effect profile and compromable clinical efficacy. In these experiments. the doses of budesonide and prednisone are comparable to those used in humans. I accept that there may be differences in the responsiveness of the rat and human intestine to glucocorticosteroids and to dietary lipid changes. It is important to emphasize therefore. that the results obtained in my studies with rats cannot be directly applied to humans. Within these limitations, however. the results of the first part of my experiments with chow (low fat diet) demonstrate that budesonide might be of potential benefit improving fructose and lipid uptake, and thereby potentially improving the malnutrition which occurs in patients with inflammatory bowel disease. In rats in a high fat diet (PUFA and SFA). budesonide had the advantage over prednisone in improving the uptake of some lipids. Again this might have some clinical benefit in humans.

G) FUTURE STUDIES

1) Mechanisms and Signals

These experiments support the first hypothesis (systemically and locally acting glucocorticosteroids enhance the intestinal absorption of sugars and lipids), but do not support the second hypothesis (feeding a saturated fatty acid diet will enhance the effect of steroids on the intestinal uptake of nutrients). This suggests either that the mechanisms and signals for intestinal adaptation are different for steroids and for dietary lipids, or that a different model of intestinal adaptation needs to be used to test the second hypothesis. I would like to propose several future studies in which I will test the following hypotheses:

- The mechanisms and signals of intestinal adaptation are different for glucocorticosteroids and dietary lipids;
- The up-regulation of lipid and sugar uptake in resected animal is due to increased abundance of SGLT1, GLUT5, GLUT2, Na^+/K^+ ATPase, NHE, I-$FABP_c$, L-$FABP_c$, and their respective mRNAs;
- The up-regulation of sugar and lipid uptake in resected rats can be enhanced by feeding SFA, by giving PRED or BUD, or by giving both SFA and steroids;
- This diet- and steroid-associated alteration in nutrient absorption in resected rats is due to increased values of maximal transport rate (Vmax) of SGLT1 and GLUT5, increased abundance of transport proteins and message of SGLT1, GLUT5, GLUT2, Na^+/K^+ ATPase, NHE, I-$FABP_c$ and L-$FABP_c$ and increased lipophilic properties of the BBM;

- The weight loss expected after resection is prevented by feeding SFA and giving PRED/BUD because of the enhanced nutrient absorption:
- The absorption enhancing effects of BUD/PRED are not due to an effect on systemic concentrations of blood sugar or corticosterone. but instead are signalled by proglucagon. GLP-1 and GLP-2, and by the early response genes (c-myc. c-jun and c-fos).

Because the uptake of fructose by GLUT5 is affected by diet and steroids. I would anticipate that there would be increased abundance of GLUT5 and GLUT5 mRNA. Also. because glucose uptake did not change. I would speculate that SGLT1 and SGLT1 mRNA would be unaffected by these treatments. Performing Western blots of isolated brush border membranes (BBM) and Northern blots on isolated mRNA would be an appropriate avenue to test this speculation. Finally, because fructose and glucose in the diet mat be supplied by sucrose. it would be interesting to determine the effect of diet and steroids on the activity of BBM sucrase-isomaltase (SI). as well as the abundance of the protein and mRNA for SI. I would speculate that SI abundance and activity would be increased by steroids. and by feeding a saturated lipid diet.

Because the uptake of some but not all lipids was modified by diet and by steroids, it would be interesting to measure the lipid composition of the BBM. anticipating that the lipophilic properties of the BBM would adapt. Also. because the lipid binding proteins in the BBM ($FABP_{pm}$) and cytosol (L-$FABP_c$ and I-$FABP_c$) may influence fatty acid uptake, I would be interested in determining the abundance of mRNA for NHE. I- and L-FABP in these animals [see figure 2]. If and when the mRNA for

$FABP_{pm}$ becomes available. I would propose to undertake similar measurements. I speculate that steroids do not change BBM lipid composition, since the uptake of only some lipids was changed. I would also speculate that the fatty acid binding proteins were altered by steroids. These proteins may have varying affinities for the lipids, thereby, explaining the variable effect of steroids on the uptake of only some lipids.

Proglucagon, GLP-1, GLP-2 and the early response genes (c-jun, c-myc and c-fos) may play a role in the signalling of intestinal adaptation [Reimer et al., 1996]. I would propose examining the abundance of selected signalling proteins in rats given PRED, BUD, SFA and PUFA.

In my literature review I outlined what is know about the mechanism of action of glucocorticosteroids. To determine whether the mechanism of effect of PRED and BUD were related to changes in blood sugar (known to up-regulate GLUT2), or to serum corticosterone, these measurements would be undertaken. From data obtained in humans, we know that BUD has much less of a systemic effect than does PRED [Greenberg et al., 1994; Rutgeerts et al., 1994]. I would speculate that BUD had less of an effect than did PRED on blood glucose and corticosterone concentrations. It is likely that the different effect of BUD than PRED on uptake was not explained by variations in their effects on these blood measurements, but rather by differences in their direct effect on the steroid receptors or steroid response elements in the enterocyte.

2) Another Model: Intestinal Resection

In my studies reported in this thesis, I used the model of dietary lipid modification to examine the influence of steroids on sugar and lipid absorption in young animals.

Intestinal adaptation was first explored in animals subjected to a bowel resection. The dietary content lipid influences the adaptation of the intestine seen after resection [Keelan et al., 1996]. Patients with Crohn's disease often require bowel resection to be performed surgically, and they often require treatment with steroids. Accordingly, an animal model of interest would be the bowel resection, model in which the expected adaptation could be stimulated or depressed by feeding SFA or PUFA, respectively. I propose studying the effect of PRED and BUD on nutrient absorption in rats fed SFA or PUFA, and having a 50% small bowel resection.

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Table 5. Food Intake and Body Weight Gain in Chow Fed Rats

	Food Intake (g/day)	**Weight Gain (g/day)**	**Weight Gain per Food Intake, %**
Control	22.3 ± 0.8	9.1 ± 0.2	40.8 ± 0.4
Prednisone	21.1 ± 1.1	9.0 ± 0.2	42.6 ± 0.7*
Budesonide	21.0 ± 0.8	8.2 ± 0.3*	39.0 ± 0.4*#

Mean ± SEM. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

*, $p<0.05$. budesonide or prednisone vs control.

#, $p<0.05$. budesonide vs prednisone.

Table 6. Characteristics of Intestine in Chow Fed Rats

Drug	Intestinal Weight (mg/cm)	% of Intestinal Wall Comprised of Mucosa
Jejunum		
Control	20.0 ± 0.7	53.4 ± 3.2
Prednisone	22.6 ± 2.1	45.7 ± 6.0
Budesonide	18.3 ± 1.2	53.3 ± 3.6
Ileum		
Control	15.4 ± 2.8	47.6 ± 6.1
Prednisone	13.5 ± 1.1	37.5 ± 6.2
Budesonide	10.7 ± 0.7	38.9 ± 6.4

Mean ± SEM. The dose of budesonide was 0.25 mg/kg. and the prednisone dose was 0.75 mg/kg.

None of these differences were statistically significant.

Table 7. Body Weight Gain and Food Consumption in SFA and PUFA Fed Rats

Diet	Drug	Food Intake (g/day)	Body Weight Gain (g/day)
Saturated	Control	17.5 ± 1.1	6.8 ± 0.6
	Prednisone	16.3 ± 0.7	6.2 ± 0.5
	Budesonide	16.6 ± 0.9	6.0 ± 0.4
Polyunsaturated	Control	16.1 ± 0.4	8.8 ± 0.3#
	Prednisone	16.9 ± 0.9	8.7 ± 0.3#
	Budesonide	16.4 ± 0.4	8.7 ± 0.3#

Mean ± SEM

#, $p<0.05$, polyunsaturated (PUFA) versus saturated fatty acid diet (SFA).

Table 8. Characteristics of Intestine in SFA and PUFA Fed Rats

Diet	Site	Drug	Weight (mg/cm)	% of Mucosa	Villous Height (μm)
SFA	Jej	Control	23.1 ± 1.9	49.3 ± 2.6	224 ± 37
		Prednisone	18.0 ± 1.0	48.7 ± 3.3	309 ± 23
		Budesonide	20.9 ± 1.5	56.8 ± 3.9	252 ± 17
	Ile	Control	11.4 ± 1.1	44.9 ± 4.4	211 ± 36
		Prednisone	9.3 ± 0.6	52.3 ± 5.6	261 ± 49
		Budesonide	10.4 ± 0.8	45.3 ± 2.5	239 ± 32
PUFA	Jej	Control	17.7 ± 1.8	52.2 ± 2.8	257 ± 31
		Prednisone	19.0 ± 2.5	44.1 ± 4.9	269 ± 15
		Budesonide	17.2 ± 1.5	54.4 ± 2.8	262 ± 16
	Ile	Control	11.9 ± 1.7	49.8 ± 3.8	270 ± 71
		Prednisone	12.5 ± 1.2	62.1 ± 6.2	314 ± 14~
		Budesonide	13.6 ± 1.5	57.8 ± 6.8	181 ± 07#

Mean ± SEM

~, $p<0.05$, prednisone vs budesonide

#, $p<0.05$, polyunsaturated (PUFA) vs saturated diet (SFA)

Table 9. Kinetic Constants of Intestinal Uptake of D-glucose in Chow Fed Rats

Drug	Maximal Transport Rate Vmax (nmol.100 mg-1.min-1)	Apparent Michaelis Constant Km (mM)
Jejunum		
Control	1492 ± 84	47 ± 5
Prednisone	1366 ± 150	43 ± 9
Budesonide	1468 ± 58	44 ± 3
Ileum		
Control	1652 ± 272	80 ± 21
Prednisone	1659 ± 382	85 ± 30
Budesonide	1808 ± 373	82 ± 26

Mean ± SEM. The dose of budesonide was 0.25 mg/kg. and the dose of prednisone was 0.75 mg/kg.

None of these differences was statistically significant.

Table 10. Rates of Uptake of L-glucose and D-mannitol in Chow Fed Rats

Drug	Control	Prednisone	Budesonide
Jejunum			
L-Glucose	14 ± 1	16 ± 2	14 ± 1
D-Mannitol	17 ± 1	18 ± 1	16 ± 1
Ileum			
L-Glucose	10 ± 1	10 ± 1	10 ± 1
D-Mannitol	12 ± 1	14 ± 2	13 ± 1

Mean ± SEM. The rates of uptake are expressed as nmol*100 mg tissue^{-1} *1 mM^{-1}.

The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

None of these differences were statistically significant.

Table 11. Slopes of Relationship Between Concentration and Rates of Uptake of D-Fructose in the Jejunum and Ileum in Chow Fed Rats.

	Jejunum	Ileum
Drug	**Slope**	**Slope**
Control	12.1 ± 0.3	10.1 ± 0.3
Prednisone	13.2 ± 0.1*	11.7 ± 0.2*
Budesonide	12.8 ± 0.1*	12.1 ± 0.8*

Mean ± SEM. The values of the slopes are expressed as nmol * 100 mg tissue^{-1} * min^{-1}. The dose of budesonide was 0.25 mg/kg. and the dose of prednisone was 0.75 mg/kg.

*, $p<0.05$. budesonide or prednisone vs control.

Table 12. Kinetic Constants of Intestinal Uptake of D-glucose in SFA and PUFA Fed Rats

Diet	Site	Drug	Vmax (nmol*100 mg^{-1}*min^{-1})	Km (mM)
PUFA	Jej	Control	1454 ± 242	72 ±15
		Prednisone	1224 ± 151	54 ± 9
		Budesonide	1596 ± 250	62 ± 13
	Ile	Control	1233 ± 257	77 ± 20
		Prednisone	1352 ± 246	72 ± 17
		Budesonide	1090 ± 167	51 ± 11
SFA	Jej	Control	1285 ± 148	35 ± 6
		Prednisone	1696 ± 171	45 ± 6
		Budesonide	1461 ± 154	39 ± 6
	Ile	Control	2256 ± 423#	100 ± 23
		Prednisone	3514 ± 838#	185 ± 60
		Budesonide	2165 ± 355#	93 ±19

Mean ± SEM

Prednisone and budesonide had no effect on the values of Vmax or Km.

#, $p<0.05$. polyunsaturated (PUFA) versus saturated fatty acid diet (SFA).

Table 13. Rates of Uptake of L-Glucose and D-Mannitol in SFA and PUFA Fed Rats

Diet	Site		Control	Prednisone	Budesonide
PUFA	Jej				
		L-Glucose	10 ± 1	13 ± 1	10 ± 1
		D-Mannitol	12 ± 1	13 ± 1	10 ± 1
	Ile				
		L-Glucose	12 ± 1	11 ± 1	8 ± 1
		D-Mannitol	11 ± 1	10 ± 1	10 ± 1
SFA	Jej				
		L-Glucose	12 ± 1	13 ± 1	12 ± 1
		D-Mannitol	14 ± 1	15 ± 1	13 ± 1
	Ile				
		L-Glucose	12 ± 1	11 ± 1	13 ± 1
		D-Mannitol	14 ± 1	13 ± 1	13 ± 1

Mean ± SEM. The rates of uptake are expressed as nmol*100 mg tissue^{-1}*min^{-1}*mM^{-1}

None of these differences were statistically significant.

Table 14. Slopes of Linear Relationships Between Concentrations and Rates of Uptake of D-fructose in SFA and PUFA Fed Rats

Diet	Drug	Jejunum	Ileum
Polyunsaturated	Control	12.4 ± 0.2	12.2 ± 0.5
	Prednisone	11.7 ± 0.4	10.8 ± 0.1
	Budesonide	12.7 ± 0.2	11.8 ± 0.6
Saturated	Control	13.7 ± 0.1#	17.3 ± 0.4#
	Prednisone	16.8 ± 0.2*#	16.5 ± 0.5#
	Budesonide	15.3 ± 0.2#*~	20.5 ± 1.0#*~

Mean ± SEM. The values of the slopes are expressed as nmol * 100 mg tissue^{-1} * min^{-1}. The dose of budesonide was 0.25 mg/kg. and the dose of prednisone was 0.75 mg/kg.

~, $p<0.05$. budesonide vs prednisone

#, $p<0.05$. polyunsaturated (PUFA) vs saturated diet (SFA)

*, $p<0.05$. budesonide or prednisone vs control

Table 15. Rates of Uptake of Fatty Acids and Cholesterol in Chow Fed Animals

Drug	Control	Prednisone	Budesonide
Jej			
12:0	64 ± 11.9	86 ± 16.6	70.8 ± 11.8
16:0	9.5 ± 0.9	9.7 ± 1.1	10.9 ± 1.5
18:0	13.7 ± 2.1	12.1 ± 1.6	8.9 ± 0.9
18:1	8.8 ± 0.6	9.2 ± 0.7	18.0 ± 1.2*#
18:2	7.3 ± 1.0	10 ± 0.9	7.7 ± 0.8
18:3	11.1 ± 0.8	13.5 ± 2.1	11.6 ± 1.0
Chol	4.4 ± 0.6	10.6 ± 2.2*	5.4 ± 1#
Ile			
12:0	49.8 ± 10.6	114 ± 1.57*	87.3 ± 1.41
16:0	4.2 ± 0.6	9.7 ± 0.15*	6.7 ± 0.09
18:0	7.3 ± 0.8	8.9 ± 0.10	8.5 ± 0.09
18:1	5.7 ± 1.1	6.3 ± 0.08	5.4 ± 0.05
18:2	5.5 ± 0.9	12.2 ± 0.09*	11.0 ± 0.08*
18:3	7.3 ± 1.2	12.1 ± 0.12*	8.5 ± 0.07#
Chol	3.2 ± 0.2	4.2 ± 0.6	3.8 ± 0.4

Mean ± SEM. The rates of uptake of fatty acids are expressed as nmol * 100 mg tissue^{-1} *min^{-1}* 1 mM^{-1}. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

*, $p<0.05$, budesonide or prednisone vs control

#, $p<0.05$, budesonide vs prednisone

Table 16. Rates of Uptake of Fatty Acids and Cholesterol in SFA and PUFA Fed Rats

DIET	SITE	CONTROL	PREDNISONE	BUDESONIDE
Polyunsaturated	Jejunum			
	12:0	50 ± 2	66 ± 4 *	60 ± 4
	16:0	12 ± 1	11 ± 1	15 ± 1~*
	18:0	11 ± 1	12 ± 1	13 ± 1
	18:1	9 ± 1	12 ± 1 *	13 ± 1 *
	18:2	8 ± 1	13 ± 1 *	14 ± 1 *
	18:3	6 ± 1	6 ± 1	7 ± 1
	Cholesterol	16 ± 2	14 ± 2	18 ± 2
	Ileum			
	12:0	50 ± 3	53 ± 3	59 ± 3
	16:0	13 ± 1	13 ± 1	16 ± 1
	18:0	14 ± 2	12 ± 1	11 ± 1
	18:1	9 ± 1	8 ± 1	12 ± 1 *~
	18:2	8 ± 1	11 ± 1	11 ± 1
	18:3	5 ± 1	6 ± 1 *	6 ± 1
	Cholesterol	18 ± 2	14 ± 2	14± 2
Saturated	Jejunum			
	12:0	67 ± 4#	78 ± 4 #	84 ± 4 # *
	16:0	17 ± 1 #	17 ± 1 #	16 ± 1
	18:0	17 ± 2 #	18 ± 1 #	16 ± 1 #
	18:1	15 ± 1 #	17 ± 1 #	14 ± 2
	18:2	19 ± 2 #	16 ± 1	15 ± 2
	18:3	9 ± 1 #	8 ± 1 #	8 ± 1
	Cholesterol	28 ± 4 #	22 ± 2 #	18 ± 2 *
	Ileum			
	12:0	67 ± 8	129 ± 7 # *	83 ± 6 # ~
	16:0	19 ± 2	14 ± 1 *	13 ± 1 *
	18:0	16 ± 1	18 ± 2	12 ± 1 # ~
	18:1	14 ± 2 #	15 ± 1	11 ± 1 #
	18:2	12 ± 1 #	16 ± 2 *	10 ± 1 # ~
	18:3	7 ± 1 #	9 ± 1	7 ± 1 #
	Cholesterol	26 ± 2 #	22 ± 2 #	3 ± 1 #

Mean ± SEM. The rates of uptake are expressed as nmol*100 mg tissue^{-1}*min^{-1}*mM^{-1}

~. $p<0.05$. budesonide vs prednisone

#. $p<0.05$. polyunsaturated (PUFA) vs saturated diet (SFA)

*. $p<0.05$. budesonide or prednisone vs control

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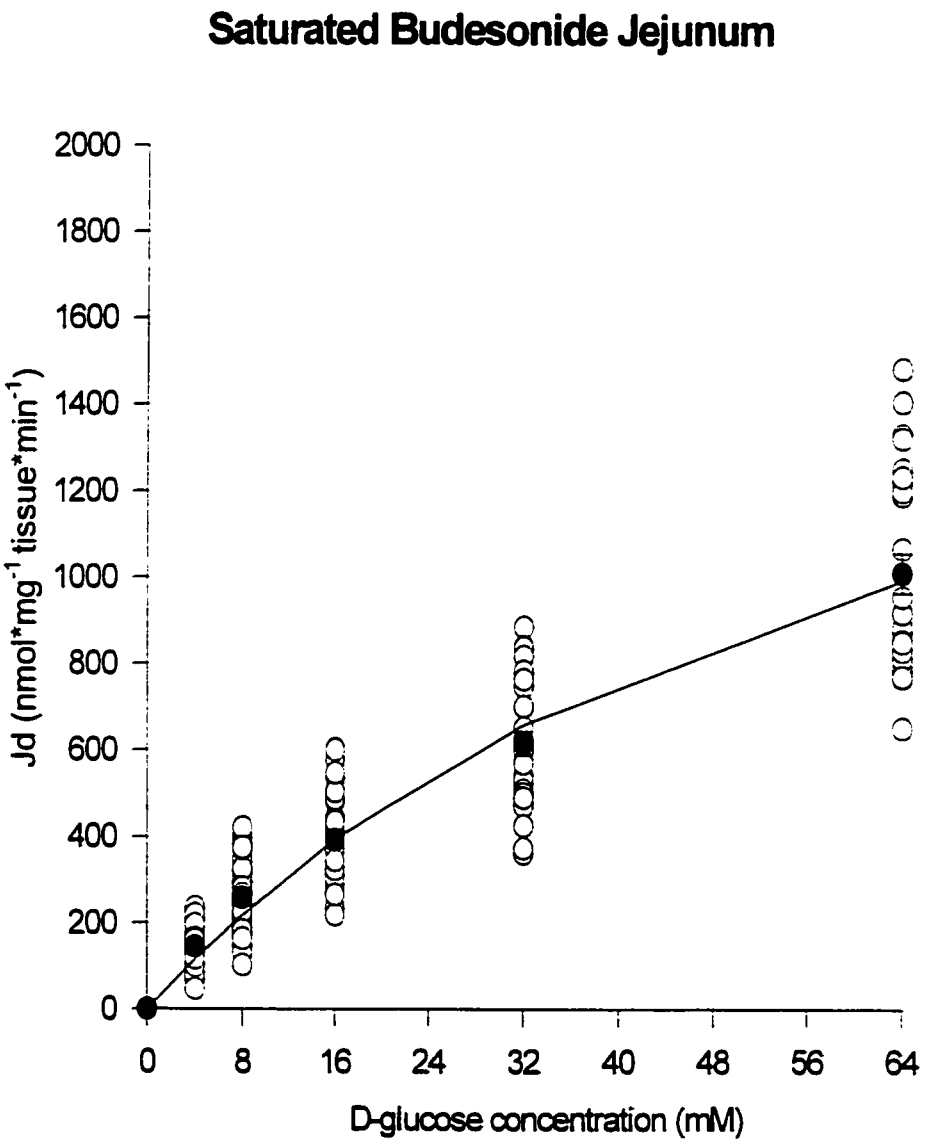
I) APPENDIX I: Body Weight Gain and Food Intake in Animals Given Budesonide at the doses 0.50, 0.75 and 1.00 mg/Kg

	Weight gain (g/day)	Food Intake (g/day)	Weight gain per Food Intake %
Bud 0.50 mg/Kg	4.95 ± 0.32	23.59 ± 0.84	21.34 ± 2.05
Bud 0.75 mg/Kg	5.00 ± 0.22	24.68 ± 0.19	19.83 ± 0.84
Bud 1.00 mg/Kg	5.12 ± 0.30	25.04 ± 0.28	20.41 ± 1.12
Control	5.34 ± 0.51	24.24 ± 0.80	21.86 ± 1.57

Mean ± SEM.

None of these differences were statistically significant.

L) **APPENDIX II:** Curvilinear Relationship between D-Glucose Concentration and Uptake



M) **APPENDIX III:** Kinetic Constants of Intestinal Uptake of D-Glucose in Animals Given Budesonide at the doses 0.50, 0.75 and 1.00 mg/Kg

	Maximal Transport Rate Vmax ($nmol*100\ mg^{-1}*min^{-1}$)	Apparent Michaelis Constant Km (mM)
Jejunum		
Bud 0.50 mg/kg	1870 ± 138	38 ± 6
Bud 0.75 mg/kg	1852 ± 129	31 ± 4
Bud 1.00 mg/kg	1989 ± 209	35 ± 4
Control	2231 ± 213	46 ± 7
Ileum		
Bud 0.50 mg/kg	1709 ± 139	32 ± 4
Bud 0.75 mg/kg	1302 ± 97	23 ± 3
Bud 1.00 mg/kg	1413 ± 112	28 ± 4
Control	1326 ± 128	28 ± 4

Mean ± SEM.

None of these differences were statistically significant.

M) APPENDIX IV: Linear Relationship between D-Fructose Concentration and Uptake

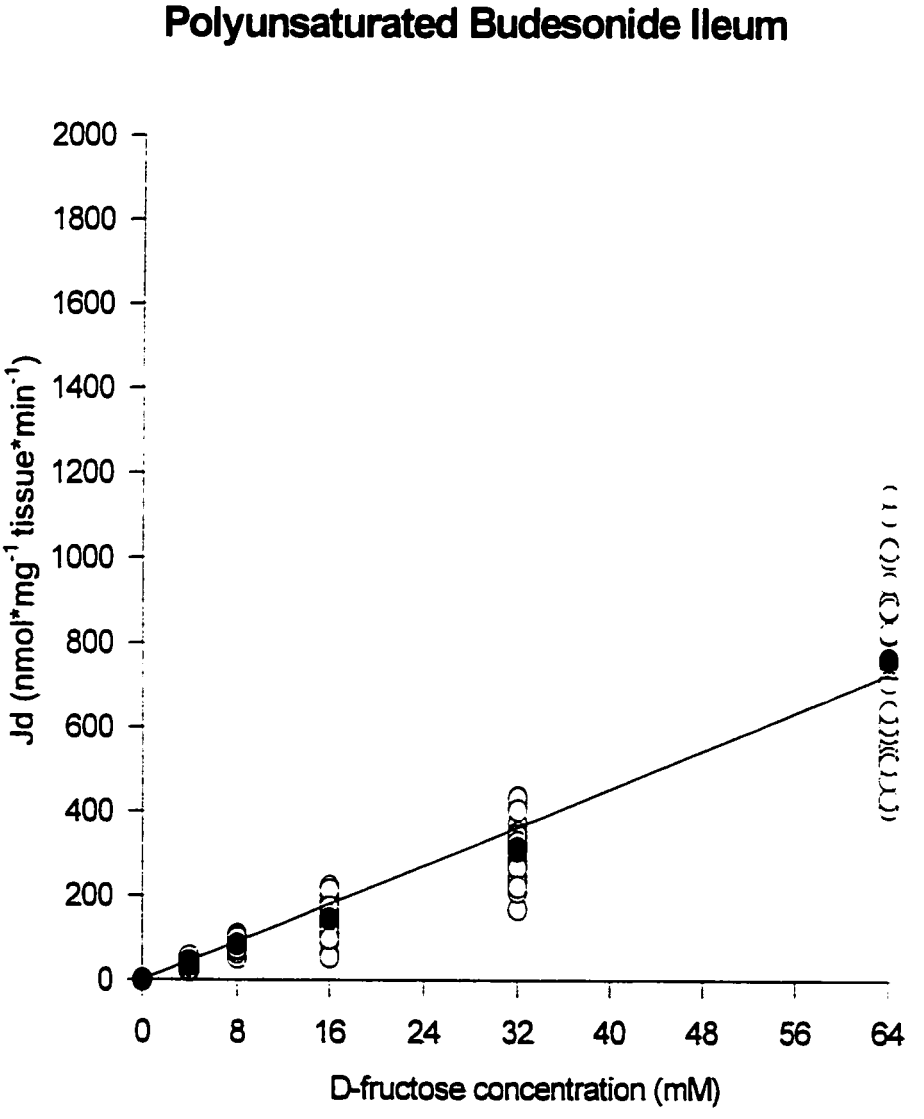
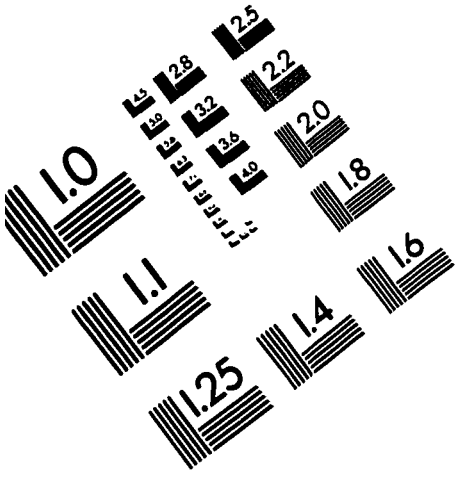
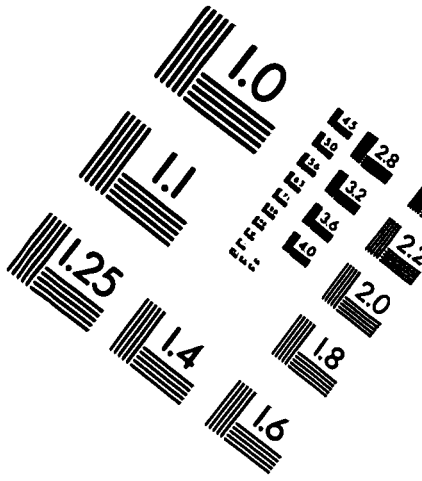
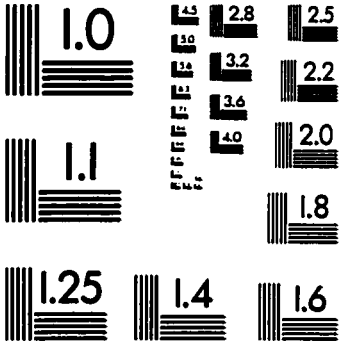
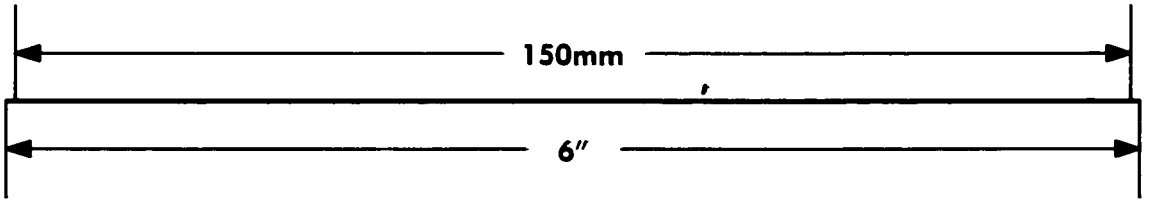


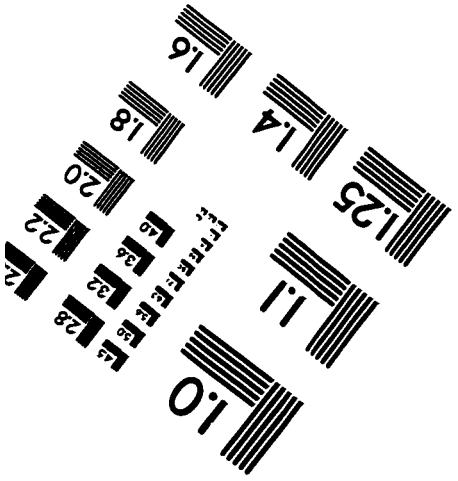
IMAGE EVALUATION
TEST TARGET (QA-3)

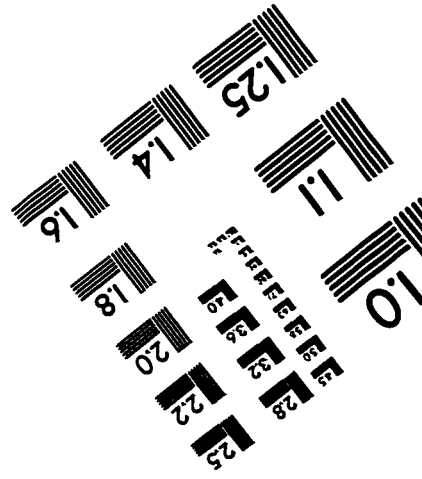












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