

COLOSTRUM FEEDING AND ITS EFFECTS ON SERUM CORTISOL,
THYROXINE, IMMUNOGLOBULIN G AND CYTOSOLIC GLUCO-
CORTICOID RECEPTORS IN SKELETAL MUSCLE
IN THE BOVINE NEONATE

BY

David Kent Waggoner

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APPROVED:

F.D. McCarthy

F.D. McCarthy, CHAIRMAN

K.E. Webb, Jr.

K.E. Webb, Jr.

Craig D. Thatcher

C.D. Thatcher

G.L. Minish

G.L. Minish

January, 1987

Blacksburg, Virginia

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CHAPTER I
INTRODUCTION

The neonatal period, from birth until the calf is three to four months of age, is a period of time when severe economic losses occur in the cow-calf industry. Various surveys have revealed that the maximum potential number of calves born should be about 5.14 million with a total calf death loss of 11% in the first 60 d of life. This tragic death loss can in part be attributed to the overwhelming of the calf's immature immune system by an infectious agent.

The calf, born devoid of immunoglobulin in serum, relies on antibodies absorbed from colostrum via pinocytosis by villus epithelial cells of the small intestine (passive immunity). Intestinal permeability to immunoglobulin persists for only 24 to 36 h, after which "closure" is presumed. Calves failing to absorb adequate quantities of colostral immunoglobulin are predisposed to disease, death or minimal future productivity.

Passive immunity is complex because it involves a delicate balance of factors in the dam, the environment and the neonate. Knowledge of these interactions are necessary to give effective advice to the farming community so that mortality and morbidity of the bovine neonate can be reduced

to negligible proportions.

Of similar importance, skeletal muscle represents approximately 50% of the bovine body mass and its protein content contributes approximately 20% to the weight of the tissue. By being the largest body reservoir of amino acids and proteins, and because it responds to various hormonal and nutritional stimuli, skeletal muscle is highly adaptive and can regulate the supply of nutrients to other body tissues. Of the several hormonal factors that may influence the mass of skeletal muscle, glucocorticoids are clearly of major importance, however the physiologic and biochemical mechanisms involved in the response of muscle to glucocorticoids are ill-understood. Glucocorticoids, like other classes of steroid hormones, must bind to cellular receptors in order to exert their effects. Relatively few reports on glucocorticoid receptors in skeletal muscle are presently available. Since this type of information is essential for an understanding of the influence of glucocorticoids, as well as other steroids on muscle growth, an investigation of the quantitation and specificity of glucocorticoid receptors in neonatal bovine skeletal muscle has been undertaken. It is hopeful that the results of this particular study will contribute to the clarification of events in glucocorticoid

action that lie between cytoplasmic binding and the final biochemical response.

CHAPTER II
LITERATURE REVIEW

Colostrum - Composition and Immunologic Properties

Colostrum is a compositional source of nutrients and antibodies. The changes in composition associated with the progress of the lactational cycle in the cow are well documented (Larson et al. 1956; Hartmann, 1973). When compared to milk, colostrum contains more minerals, total proteins, casein, whey proteins and less lactose (table 1). Its fat content may vary however, being either higher or lower than normal milk depending on various factors. The total solids content of colostrum may be as high as 25% with the proteins synthesized by the mammary gland (caseins, alpha- and beta-lactoglobulins) double the midlactation level. Calcium, magnesium, phosphorus and chloride are present in higher concentrations in colostrum than in milk, however, the potassium concentration is lower.

In addition to the aforementioned colostrum constituents, colostrum contains a high percentage of three classes of immunoglobulins (Ig); IgA, IgM, and IgG (table 2). As illustrated by Butler (1971) and Duncan et al. (1972), all immunoglobulins are composed of polypeptide chains referred to as heavy (long) and light (short) chains

Table 1: APPROXIMATE NUTRITIVE VALUE OF COLOSTRUM
(FIRST 24 H AFTER CALVING) AND OF MILK^a

	Colostrum	Milk
Dry Matter (g/kg)	220.0	125.0
Apparent Digestability (%)	93.0	93.0
Gross Energy (MJ/kg DM)	20.6	23.6
Metabolic Energy (MJ/kg fresh material)	4.0	2.6
Fat (g/kg)	36.0	35.0
Non-fatty Solids (g/kg)	185.0	86.0
Protein (g/kg)	143.0	32.5
Casein (g/kg)	52.0	26.0
Albumine (g/kg)	15.0	4.7
Beta-lactoglobulin (g/kg)	8.0	3.0
Alpha-lactoglobulin (g/kg)	2.7	1.3
Lactose (anhydrous; g/kg)	31.0	46.0
Minerals		
Calcium (g/kg)	11.8	10.4
Phosphorous (g/kg)	10.9	8.8
Magnesium (g/kg)	1.8	1.0
Potassium (g/kg)	1.4	1.5
Chloride (g/kg)	1.2	0.7
Sodium (g/kg)	0.7	0.4
Iron (mg/kg)	2.0	0.1-0.7
Copper (mg/kg)	2.7	1.6
Manganese (mg/kg)	0.2	0.03
Cobalt (ug/kg)	5.0	0.5
Vitamin A (ug/g fat)	42-43.0	8.0
Vitamin D (mg/g fat)	23-45.0	15.0
Vitamin E (ug/g fat)	100-150.0	20.0
Riboflavin (mg/kg)	4.5	1.5
Vitamin B-12 (ug/kg)	10-50.0	5.0
Ascorbic Acid (mg/kg)	25.0	20.0

^aRoy (1980); Webster (1984).

Table 2: CONCENTRATION (mg/ml) OF IMMUNOGLOBULINS
IN BOVINE COLOSTRUM

<u>Immunoglobulins</u>				
IgG1	IgG2	IgA	IgM	Researchers
75.00	1.90	4.40	4.90	Mach & Pahud (1971)
31.90	3.59	1.61	3.62	Wilson et al. (1972)
88.24	2.49	3.40	9.18	Porter (1972)
41.76	4.01	3.63	3.22	Wilson & Jutila (1976)

that vary in composition both of component amino acids and prosthetic groups. The basic unit consists of two heavy and two light chains held together by disulfide bonds. Milstein and Feinstein (1968) have shown that there are two principal varieties of Ig in bovine serum, a fast and slow IgG1 and IgG2 (half-lives of 11 to 11.5 d; Fisher and Martinez, 1975; Sasaki et al., 1977b). Both have almost identical physical properties (MW-145,000; Milstein and Feinstein, 1968; Tewari and Mukkur, 1975) and share common antigenic determinants on the Fc (crystallizable fraction) portion of their heavy chains. However, the predominant Ig of bovine colostrum has been shown to be IgG1 (Klaus et al., 1969; Wilson et al., 1972; Lisowski et al., 1975) (Table 2) and the work of Sasaki et al. (1976) and Larson et al. (1980) indicates that IgG is selectively transported from blood serum to lacteal fluid before parturition. The colostrum IgG is elevated at parturition and decreases with each successive milking post-partum (Newstand, 1976; Oyeniya and Hunter, 1978; Stott et al., 1981).

Immunoglobulin A is a carbohydrate-rich immunoglobulin (MW-360,000; Macha and Pahud, 1971; half-life of 2.8 d; Porter, 1972) secreted by plasma cells in the submucosa of the gastrointestinal tract in response to local antigenic

stimulation. For this reason IgA is normally absent from newborn and germ-free animals (Butler et al., 1972). IgA is the major immunoglobulin in the intestinal secretions and although its mode of action is not completely clear, IgA is presumed to play an important role in immune defense of mucosal surfaces (Brown, 1978). Its effects within the intestinal lumen appears to be essentially passive by interfering with the binding of micro-organisms and their products to the epithelium. In support, Wu and Walker (1976) demonstrated antibodies such as IgA to be successful in excluding bacterial toxins (e.g. cholera) from binding the mucosal epithelium. In addition, Butler et al. (1972) found IgA to be the principal immunoglobulin in bovine tears and saliva.

Immunoglobulin M is an antigenically distinct immunoglobulin (MW-900,000; Butler, 1973; half-life of 4 d; Porter, 1972) comprising less than 10% of the serum immunoglobulins. The development of the IgM system seems to occur more rapidly than that of any other class of immunoglobulins. Although there seems to be no placental transfer, IgM is synthesized by the fetus and low levels of this fetally produced protein are usually demonstratable at birth. Butler et al. (1972) established IgM to be predom-

inantly synthesized by peripheral lymph nodes and spleen. Despite its predominantly intravascular location IgM occurs in many external secretions, frequently in association with secretory components. Allen and Porter (1975) suggested that at least for intestinal secretions, IgM is locally produced and is then presumably transported in the same manner as IgA. Generally, the level of secretory IgM is less than that of IgA except in cases of IgA deficiency when IgM seems able to take over the role as the major secretory immunoglobulin. IgM remains to be the most effective agglutinating agent (Rose et al. 1964). Despite its impressive lytic efficiency a more important biological function of IgM is complement mediated immune adherence to phagocytic cells (Klaus and Jones, 1968). In terms of the presence of IgM in lacteal secretions, Newby and Bourne (1977) demonstrated that less than half of the IgM in cows milk was serum-derived. Calculation of relative occurrence (Butler et al., 1980) and total IgM output (Guidry et al., 1980) show that lacteal IgM decreases in early lactation in a manner similar to IgG rather than increasing as in the case of IgA. In addition, the bacteriocidal activity of serum and milk to mastitic pathogens often appear to be associated with IgM (Brock et al., 1976; Carroll and Crenshaw, 1976).

MACROMOLECULAR ABSORPTION IN THE BOVINE NEONATE

Calves are born hypogammaglobulinemic (Fallon, 1978) due to the absence of transplacental transfer of maternal antibodies to fetal circulation during gestation (Brambell, 1970). Thus, transmission of immunity after birth is by way of the neonatal intestinal cell which has the physiological capacity to absorb unaltered colostral proteins (Comline et al., 1951).

Staley and Bush (1985) examined the cytological events associated with Ig absorption in the neonatal calf intestine. They reported the absorptive process to occur in three steps as the Ig is transported from the gut lumen into the circulation. The first step involves binding of the Ig by the microvillus border followed by endocytosis of the binding site (receptor) and Ig. The endocytosed membrane may extend into the cytoplasm while maintaining its contact with the surface, presenting the appearance of a tubule form. The second step involves an enlargement of the tubular end piece to form a vacuole. After the vacuole distends, a condensation of vascular contents occur, the tubular connections are lost, and the vacuole is transported either to the lateral or basal cell membrane. Finally, once

the vacuole comes into contact with the cell membrane, the vacuole exocytoses the entrapped contents to the extracellular spaces. Upon extrusion from the epithelial cell, the Ig enter the villus lacteal and pass to the thoracic duct where they enter the blood stream 1 to 2 h after absorption (Balfour and Comline, 1962). Upon the closure event, there is a rapid disappearance of the tubular system of the absorptive cells, indicating cessation of pinocytotic capabilities (Staley et al., 1968).

In contrast to previous reports (Bangham et al., 1958; Pierce and Feinstein, 1965; Hardy 1969), the bovine neonate may possess some selectivity in macromolecular absorption. Staley et al. (1972) demonstrated ferritin-IgG was taken up by the apical tubular complex of the jejunal cell but not transported past the apical end of the cell. In the ileal cell ferritin solutions were transported into the base of the cell but did not pass out of the epithelial cell to the lamina propria. Thus, it was concluded that the intestinal epithelial may exert a degree of selectivity in transfer of proteins to the blood.

Closure

The intestinal epithelial cells of the newborn calf loses its permeability to large molecules during the first

24 to 36 h of postpartum life (Deutsch and Smith, 1957; Brambell, 1958). This termination of permeability and(or) transport of immunoglobulin out of the intestinal epithelial cells has been referred to as "intestinal closure" (Lecce et al., 1964). Lecce (1973) described closure to occur once the plasmalemma of the intestinal epithelial cells makes contact with the ingested nutrients and digestive fluid. This contact would stimulate nonspecifically the cells to discharge their finite amount of pinocytotic activity, thus the cell intake of macromolecules would then cease. This cessation of transfer of material through the cell membrane to the intracellular or subcellular space appears to be the dominant feature of closure, rather than the inability of substances to enter the tubular and vacuolar system within the absorptive cells (Staley, 1971).

In addition, Penhale et al. (1973) feeding pooled colostrum to groups of 10 calves at 1, 5, or 9 h after birth, concluded that there is a gradual and progressive closure of the absorption mechanism for immunoglobulin which operates independently for each class of Ig. Closure was calculated to be complete by approximately 16, 22 and 27 h for IgM, IgA, and IgG, respectively. However, Stott et al. (1979) estimated time of closure to colostrum immuno-

globulins by a joint point analyses on data from 210 calves and found no significant differences in closure time for IgA, IgM and IgG. Moreover, Brandon and Lascelles (1971) showed there was not a significant difference between the relative efficiency of absorption of IgG1, IgG2, IgM, or IgA.

ENVIRONMENTAL FACTORS WHICH INFLUENCE IG
ABSORPTION

There is wide variability in efficiency of immunoglobulin absorption. Ten to 40% of the calves which acquire colostrum during the first 24 h postpartum remain persistently hypogammaglobulinemic (McEwan et al., 1970; Logan and Gibson, 1975; Frerking and Aikens, 1978). The works of many researchers provide evidence that various environmental factors may influence passive immunity in the bovine neonate.

Mass and Concentration of Ig Consumed

The mass of colostrum Ig consumed (Kruse, 1970a; McEwan et al., 1970; Bush et al., 1973; Stott et al., 1979b) and the colostrum Ig concentration (Kruse, 1970b; Selman, 1973) are probably the major environmental factors affecting Ig absorption. Kruse (1970b) utilizing regression analyses, calculated a 50% increase variation in neonatal serum Ig over a 24 h period following colostrum feeding. He noted the increase to be primarily dependent on the mass of Ig acquired by the calf. In accordance, Bush et al. (1973) revealed 68% of the variation in blood serum Ig in calves could be accounted to differences in amount of Ig consumed per unit

body weight. In a more comprehensive experiment performed by Stott and Fellah (1983), 120 newborn calves were separated from their dams at birth and fed either 1 or 2 liters of the prepared colostrum at the appropriate concentration (range of 7.5 to 123.8 mg/ml) within 1 h after birth and again 12 h later. Feeding was repeated after 12 h. They concluded that the concentration of Ig in colostrum fed (within limits) is probably the major factor affecting colostral Ig absorption. In addition, Stott and Fellah (1983) suggested both volume and concentration of Ig be evaluated as separate entities, because the volume of colostrum that can be fed at initial feeding is limited, and absorption of colostral Ig from an equal volume but a different Ig concentration may vary.

Delayed Colostral Ingestion

The time after birth in which ingestion of colostrum occurs is rendered as a vital factor in determining the immunological status of the neonate (Stott et al., 1979a; Patt, 1977; Stott and Fellah, 1983). Kruse (1970b) found the absorption coefficient (absorbed fraction of a given amount of Ig) to be reduced linearly to approximately half by delaying feeding from 2 to 20 h. In agreement, Staley et al. (1972) noted the concentration of heterologous proteins in serum following oral administration to calves 24 h of age

to be almost half that resulting from administration immediately postpartum.

The time delay effect is attributable to three different factors. The first important factor is the phenomenon of decreasing absorption capabilities of the intestinal epithelium with increasing age (Kruse, 1970b; Stott et al., 1979a). The second factor is probably just as important, if not more in terms of morbidity and mortality, delayed colostrum ingestion results in prolonged exposure of the intestinal epithelia of the calf to microorganism invasion before colostrum pinocytotic activation of the cells and cessation of indiscriminate intake of macromolecules can occur (Corely et al., 1977). Finally, the concentration of Ig in the colostrum decreases with time after calving, even with no withdrawal of colostrum (Lomba et al., 1978; Oyeniya and Hunter, 1978), which obviously reduces the calf's chances of obtaining the required quantity of Ig.

Compounds Present in Colostrum

Certain compounds in bovine colostrum may be involved in the absorption of immunoglobulin by the small intestine. Balfour and Comline (1962) reported that colostrum whey may contain substances which accelerate the absorption of globulin by the neonatal calf. They reported that the colostrum

factors required for absorption were; a small molecular weight protein, inorganic phosphate and glucose-6-phosphate.

Hardy (1969) investigated the chemical factors that are possibly involved in the absorption of macromolecules by the neonate. He found a stimulation of absorption by other compounds in colostrum whey such as lactate, pyruvate and VFA salts, especially potassium isobutyrate. It was substantiated that these compounds were not necessary for uptake but facilitated transport out of the cells, maybe by providing metabolic energy for the absorptive process.

Furthermore, the presence of colostrokinin (a naturally occurring or locally produced humoral "kinin" which exhibits smooth muscle stimulating activity; Guth, 1959) in colostrum may assist the transport of immunoglobulin across the intestinal epithelium. Schlagheck et al. (1983) suggested colostrokinin to have a definite role in the absorptive processes of the small intestine. In that study, calves fed colostrokinin in conjunction with pooled colostrum at 0 h had higher serum IgG concentrations at 16 h postpartum than calves not fed colostrokinin. It was further suggested that colostrokinin was involved in the cessation of IgG uptake and may have more than one physiological role.

Age and(or) Parity of Dam

Devery-Pocius and Larson (1983) suggested that the mammary transport system for IgG becomes fully developed when the cow reaches maximum capacity for milk production. This time of maximum gland development is when numbers of functional secretory cells are maximum that contain the active transport system for IgG. It is speculative whether this time of maximum development is a function of age or of the number of previous lactations or both.

Larson and Touchberry (1959) showed a positive correlation between age and serum beta-gamma-protein fractions (now classified as IgG1 and IgG2; Whitney et al., 1976). Since that time, Williams and Millar (1978) have confirmed that age is correlated with serum IgG1 and IgG2 of the cow. With this perspective, it seems logical that the loss of Ig from the cow's serum and the subsequent increase in colostrum Ig may be higher in older cows (Sasaki et al., 1977a). However, old cows (>8 pregnancies) appear to have a higher percentage of calves with low serum Ig levels than younger cows, due in part to pendulous utters, large teats and often a previous history of mastitis (Logan and Gibson, 1975).

With regard to parity, the colostrum from first-calf heifers is generally lower in Ig concentration than that of

cows with previous lactations (Smith et al., 1984). In agreement, Muller and Ellinger (1981) obtained colostrum samples from 70 females immediately postpartum and analyzed the Ig concentration in relation to parity per female. The colostrum Ig was lower from the first-calf heifers (5.58%) than from third (7.91%) or fourth (7.53%) parity cows. These results differ from Oyeniya and Hunter (1978) who found the amount of IgG in colostrum from females beginning their first, second, or third lactation did not differ and cows beginning their fourth through seventh lactations possessed more colostrum IgG. Despite the differing reports, considerable variation among cows with respect to Ig content of colostrum has been observed, thus jeopardizing the calf's chances of receiving needed amounts of colostrum Ig.

Method of Feeding

Calves nursing colostrum have been shown to have significantly higher Ig concentrations in serum than calves fed colostrum by hand (Selman et al., 1971). The reason for this is unknown. Nonetheless, the act of mothering, per se, seems to result in a greater absorption of Ig than in non-mothered calves (Selman et al., 1970; McBeath and Logan, 1974). Selman et al. (1971) reported higher Ig levels in

dairy calves kept with their dams for the first 24 h of life than in calves which were put with their dams only at feeding times. Even though both groups of calves were permitted to suckle at the same fixed times and consumed similar amounts of colostrum, it appeared that calves isolated from their dams could have impaired Ig absorption due to maternal deprivation. This phenomenon is due possibly to the stimuli from suckling which accelerates pinocytotic activity in the absorptive cells of the calf intestinal epithelium or increase the rate of transport of internalized Ig's through the cells into circulation, or both (Stott et al., 1979c). In addition, similar experiments utilizing young rats (Halliday, 1959) and puppies (Filkins and Gillette, 1966), suggest this phenomenon may be regulated by the adrenal cortex.

Despite the aforementioned evidence to support natural suckling, 20 to 30% of all calves left with their dams to suckle remain hypogammaglobulinemic (Klaus et al., 1969; Selman et al., 1970). Thus, calves that are Ig deficient have possibly failed to suckle or to suckle sufficient amounts of colostrum (Brignole and Stott, 1980). Calves which refuse to suckle should be given at least 2L (80ml per kg body-weight) immediately after birth via stomach tube

(Molla, 1978). Molla (1978) noted that tube-feeding of newborn calves is an effective, simple, and practical method of avoiding persistent hypogammaglobulinemia provided the dose of colostrum Ig is sufficient. Tubing narrows the interval between birth and possible infection by eliminating the need of depending on the unreliable appetite of the neonate.

Prepartum Nutrition

Work performed by Loh et al. (1971) indicates that protein restrictions on rat dams could possibly impair protein absorption by the pups, due to a marked decrease in the development of absorptive cells in the pups' jejunum. In accordance, Michalek et al. (1975) reported that a protein deficiency in rat dams resulted in a two-fold decrease in serum IgG2, through lactation and a 1.5 to twofold decrease in total serum IgG in the pups. Blecha et al. (1981) studied the effects of protein restrictions imposed upon beef heifers during the last trimester of pregnancy on (1) the serum and colostrum immunoglobulin concentration in the dam (2) the neonate's ability to absorb immunoglobulin. In their study, 62 yearling beef heifers (Hereford or Hereford-Angus Cross) were randomly assigned to six dietary treatments and given .52, .61, .71, .80, .89, or .98 kg crude

protein daily (dry matter basis) for the last 100 d of gestation. There were no significant correlations between concentration of immunoglobulins in the serum or colostrum of the cow and the prepartum crude protein consumption. The data did indicate, however, that there was a selective decrease in absorption of IgG in calves from heifers fed the low protein diets. In support of these studies, recent work by Burton et al. (1984) indicates that the prenatal cow nutrition may have a marked effect on the absorption of immunoglobulins by the neonatal calf. In that study, 26 yearling Holstein heifers were assigned to either a protein deficient (66% of estimated NRC requirement) or an adequate diet (115% of NRC) during the last trimester of pregnancy. The low protein intake for the heifers resulted in a significant reduction in the absorption of immunoglobulins by calves fed equal amounts of their dam's colostrum. There was no difference in colostrum immunoglobulin concentration regardless of the protein intake by the heifers, a conclusion which was also reported by DeLong et al. (1979) and Olson et al. (1981). There may be several reasons for the impaired immunoglobulin absorption by the neonate based on the results of these studies. First, the calves may have developmental differences due to the protein restriction of

the dam in late pregnancy (Loh et al., 1971). Second, variation in the nutritive value of the diet or nutrient intake of the pregnant cow may induce additional stress to the neonatal calf (Bull et al., 1974). Finally, the colostrum of the dam may be deficient in an unmeasured factor which may affect the calf's absorption of the colostral immunoglobulin. The latter explanation appears to be supported by work performed by Olson et al. (1981) which suggests that calves from dietarily restricted dams did not differ from control calves in their ability to absorb immunoglobulin from a common colostral source.

Seasonal Variation

Colostrum-derived passive immunity of the bovine neonate may be reduced by the effects of severely cold environmental temperatures that often prevail during late winter and early spring in parts of the United States (Olson et al., 1981). In efforts to explain this phenomenon, Olson et al. (1980a) and Woodard et al. (1980) observed that moderate cold stress of the neonate with no change in core body temperature should have minimal effects on intestinal immunoglobulin absorption, nonetheless there was a direct relationship between cold-induced hypothermia and the rate of immunoglobulin absorption capacity by the small intest-

ine. In support, Gay et al. (1983) reported a seasonal variation in IgG1 transfer to calves. In that study, commercial Holstein dairy cows were totally confined in free-stall housing without access to pasture and fed a corn silage/alfalfa hay mixture throughout the study period. One hundred twenty-three calves born during the 12-month study period were fed 2.8 L of colostrum via an esophageal tube feeder within 2 h postpartum and 48 h serum concentrations of IgG1 were determined. Mean monthly serum IgG1 concentrations were lowest in the winter and increased during the spring and early summer to reach their peak in September, after which they decreased. In addition, cold stress may also have an indirect impairment on the acquisition of colostral Ig by the neonate, due to an increased latency to rise, stand, and its willingness to nurse (Stauber, 1976; Olson et al., 1980b; Edwards et al., 1982).

GLUCOCORTICOID (CORTISOL) STRUCTURE, FUNCTION
AND LEVELS IN NEONATAL CALVES

In the bovine, like most mammals, the production of adrenal glucocorticoids is regulated by the action of pituitary adrenocorticotrophic hormone (ACTH) (Haynes and Berthet, 1957; Koritz and Kumar, 1970). The release of ACTH is in turn regulated by a hypothalamic corticotropin releasing factor (CRF) that reaches ACTH-producing cells of the anterior pituitary via the hypophyseal portal system (Yusuda et al., 1982). CRF specifically promotes depolarization of the corticotroph plasma membrane and rapidly elevates intracellular 3',5', cyclic AMP in the anterior pituitary cells (Labrie et al., 1982; Aguilera et al., 1983; Bileztkjian and Vale, 1983). Another mechanism controlling ACTH release is the negative feedback action of the glucocorticoids mediated at the level of the pituitary, hypothalamus, or even higher brain centers (Pollack and La Bella, 1966; Sieden and Brodish, 1971; Jones and Hillhouse, 1976; Vale et al., 1981; Rivier et al., 1982). Following ACTH release (concentrations as low as 10 M) a probable mode of action is the activation of membrane responses resulting in the production of adenylyl cyclase, thus increasing the production of cellular enzymes. ACTH also initiates the uptake of circu-

lating cholesterol, alters cell membrane and mitochondrial permeability, and increases the NADPH pool which permits the action of hydrolysis to progress (Gass and Kaplan, 1982).

The adrenal glucocorticoids (17-hydroxysteroids) are synthesized from cholesterol via a series of hydroxylations within the zona fasciculata and zona reticularis (Stachenko and Giroud, 1959; Brown et al., 1979; Carballera and Fishman, 1980). The rate-limiting step is considered to be the conversion of cholesterol to delta-5 pregnenolone (Weliky and Engel, 1963; Burstein and Gut, 1971). This substrate is then converted to progesterone and, through three successive hydroxylations, cortisol (11-beta, 17-alpha, 21-Trihydroxy-pregn-4-ene-3, 20-dione; MW-362), the major bovine glucocorticoid is formed (Gass and Kaplan, 1982).

In serum, less than 25% of cortisol is unbound ("free") and approximately 75% is bound to a corticosteroid-binding globulin (CBG or transcortin). Corticosteroid-binding globulin (MW - 52,000) is a glycoprotein (34 to 38 mg/ml in low serum concentrations) and has a high affinity (low capacity) for cortisol (Daughaday and Mariz, 1961; Slaunwhite et al., 1966). CBG serves as a carrier protein for cortisol and protects the steroid from renal clearance and metabolic degradation (Sandberg and Rosenthal, 1963; Lind-

ner, 1964; Matsui and Plager, 1966).

Cortisol, as well as other glucocorticoids, has numerous biological roles. The regulation of cortisol on cellular processes is both catabolic and anabolic in nature, depending on what specific message has been transcribed in the cell type activated (receptor recognition) by cortisol (Gass and Kaplan, 1982). Cortisol mediates alone and(or) in harmony with other hormones and cellular stimuli, the following processes: (1) inhibits both glucose and amino acid uptake in the periphery to increase blood glucose levels (Munck, 1971; Caldwell et al., 1977; Bailey and Flatt, 1982); (2) regulates adaptive responses to stress and fasting by maintaining blood glucose levels via gluconeogenesis stimulation (Friedman et al., 1965; Haynes, 1965); (3) mobilizes muscle tissue proteins and transports the derived amino acids to the liver (Kaplan and Schimizu, 1963; Schaeffer et al., 1969; Baxter and Forsham, 1972; Tomas et al., 1979); (4) increases the synthesis of enzymes involved in the transamination of amino acids in the liver (Korner, 1969); (5) promotes liver glycogen synthesis (Dorsey and Munck, 1962); (6) enhances degradation of fats (lipolysis) and release of fatty acids (Jeanrenaud, 1967; Exton et al., 1972); (7) maintains many other bodily functions.

Cortisol Levels

Ash and Heap (1975) concluded that the fetal pig was capable of corticosteroid production. This finding was determined by measuring corticosteroid levels in the sow and fetus. The maternal level of corticosteroid was 60 ng/ml of blood while the fetal levels for both male and female were 210 and 200 ng/ml, respectively. Blood was taken from the umbilical vein and contained only 40 ng/ml. Thus, it was concluded that the fetus is capable of producing its own corticosteroids and that the placental wall is impermeable to corticosteroids. Postpartum corticosteroid levels in pigs and sheep increase drastically as illustrated by Dvorak (1972) and, Bassett and Alexander (1971). Dvorak (1972) discovered values of 10 ng/ml for the pig fetus 10 days prepartum which elevated to 20 ng/ml one day prior to birth and peaked at 42 ng/ml 12 h after birth. Furthermore, 10 d postpartum the level dropped to 18 ng/ml and at 46 to 60 d postpartum the corticosteroid levels were 8.3 ng/ml. There were no differences in circulating levels between the intact male and female at any time before or after birth. Bassett and Alexander (1971) reported that the levels of corticosteroid in the neonatal sheep were as high as 81 ng/ml immediately postpartum but by d 20,

the levels had further fallen to 13 ng/ml which corresponded to adult sheep levels.

Khan et al. (1970) were the first to report a profile of cortisol concentrations in neonatal calves. They utilized nine calves which were bled at zero and 4 h postpartum with subsequent samples taken every 8 h until the third day. High cortisol levels at birth (100 ng/ml) were recorded followed by a steady decline during the sampling period. Glucocorticoid concentrations in calves during the first 2 d postpartum were assessed by Johnstone and Oxender (1979). The neonatal calves were fed 1 liter of pooled colostrum within the first 2 h after birth. Blood sampling was performed at 0, 0.5, 1, 1.5, 2, 2.5, 3, 6, 12, 18, 24, 30, 36, 42 and 48 h postpartum. The calves were born with a high glucocorticoid level (140 ng/ml) which was followed by a rapid decrease in serum concentrations over the next 3 h. It was determined that a decline in serum concentration occurred between 3 and 18 h postpartum, after which no alterations were recorded throughout the initial 48-h postpartum period. In a study by Stott (1980) newborn dairy calves were fed one liter of colostrum at 4, 16 and 28 h after birth, and blood samples were taken every 4 h from

birth to 40 h postpartum. He reported a steady decrease in serum cortisol concentrations within the first 8 h postpartum, after which the concentration stabilized and remained incessant throughout the 40-h sampling period. More recently, Schlagheck et al. (1983) removed 62 dairy calves from their dams immediately after birth and collected blood samples via jugular puncture at 0, 1, 2, 3, 4, 6, 8, 12, 13, 14, 15, 16, 18, 20, 24, 28, and 32 h postpartum. As expected, the calves were born with high cortisol concentrations (88 ng/ml) which appeared to decrease within 2 h postfeeding after birth.

Selye (1955) originally defined "stress" as a nonspecific response to a variety of physically traumatic events. Included in his first stage of stress response was the elevation of plasma glucocorticoid concentrations. In rodents, the acute glucocorticoid response to environmental stimulation is characterized by a very rapid rise (within 1-5 minutes) to levels many times higher than normal (Keith et al., 1978), which peak at approximately 30 minutes and can return to normal within an hour (Ader, 1969).

In rodents, a wide variety of stimuli can evoke elevations of glucocorticoids including five seconds of handling or just three minutes of exposure to a new environment

(Brown and Martin, 1974; Seggie and Brown, 1975). Hennessy et al. (1979) demonstrated that long term elevations in glucocorticoids can be provoked by prolonged exposure to stimuli. Shipping mice was shown to lead to high levels of corticosterone for 2 d, followed by a gradual return to normal after 13 d.

Livestock can claim no exception concerning stimuli-induced elevations of glucocorticoids. During the actual birth process and 24-28 h following parturition, the newborn undergoes numerous stressful factors, many of which would be expected to increase pituitary-adrenal activity (Stott, 1980). McNatty et al. (1972) reported the influence of a new environment on the plasma cortisol levels in sheep. The concentrations rose from normal values of 8 to 10 ng/ml to as high as 33 ng/ml. Only after 28 d did the concentrations return to normal. The stimulus of feeding has been shown to create rapid fluctuations in serum cortisol concentrations (Willett and Erb, 1972). In support of this finding, Purchas (1973) also demonstrated the effect of feeding and fasting. A three-fold increase in cortisol levels was noted in sheep fasted 18 to 20 h with a subsequent decrease in concentration occurring 2 to 4 h after feeding.

INTERACTIONS BETWEEN GLUCOCORTICOIDS AND IMMUNOGLOBULIN ABSORPTION

Stress has long been used as a ready explanation for poor performance in calves absorbing colostral Ig. There is growing evidence that certain stressful conditions imposed upon the dam prepartum may reflect upon the neonate's capabilities of absorbing macromolecules intestinally. When pregnant cows were injected with slowly-released corticosteroids during the last two months of gestation, calves from the treated cows had significantly lower serum immunoglobulin than calves from the untreated cows (Husband et al., 1973). It was not determined whether the corticosteroid treatments produced a precocious closure or if they reduced the efficiency of protein absorption. The study did suggest that corticosteroids can have an effect on intestinal absorption of colostral Ig if administered early enough in fetal development of the calf.

Brandon et al. (1975) produced conclusive results which demonstrated that the onset of copious milk secretion induced by Opticortenol (dexamethasone trimethylacetate) treatment was accompanied by a sharp decrease in the concentration of IgG1 in mammary secretions. There was a subsequent absence of the characteristic increase in the

selective index (i.e.):

$$\frac{\text{conc. of IgG1 in secretion}}{\text{conc. of IgG2 in secretion}} \times \frac{\text{conc. of IgG2 in serum}}{\text{conc. of IgG1 in serum}}$$

(Brandon et al., 1971b) for IgG1 observed in cows approaching a normal parturition. The decrease in serum IgG concentration was found to be in conformity with the failure of the mammary gland of the treated cows to selectively transfer adequate quantities of IgG. Thus, it seemed reasonable to postulate that glucocorticoid-treated cows in late pregnancy seriously diminished the availability of colostrum Ig to the neonatal calf. In accord with these results, earlier work by Gillette and Filkins (1966) reported that puppies from bitches treated with ACTH or hydrocortisone during the 24 h before birth absorbed colostrum immunoglobulin less efficiently.

Kruse and Buus (1972) reported relatively high concentrations of corticosteroids in the calf two hours after birth and suggested cortisol "shock" may induce changes in the intestinal epithelium leading to a hampered ability to absorb immunoglobulin. In support, Husband et al. (1973) and Stott et al. (1976) concluded that corticosteroids can influence cell permeability of the small intestine in post-natal livestock rendering them incapable of absorbing

immunoglobulins.

Just recently, Lentze et al. (1985) confirmed the presence of glucocorticoid receptors within the villus and crypt cells of the rodent small intestine. The team of researchers measured the glucocorticoid receptor activity in enriched villus and crypt cell fractions by use of [³H] dexamethasone. In normal rats the glucocorticoid activity was present in all cell fractions but was lowest in fully mature villus cells of the upper villus and greater in immature crypt cells. It was concluded that glucocorticoid hormones do influence a variety of epithelial cell functions (i.e. enhance sodium and water absorption; inhibit active calcium transport) in the small intestine and, furthermore, immature crypt cells may be more susceptible to glucocorticoid elicitation than fully mature villus cells in view of their higher glucocorticoid receptor activity. On this basis, it is assumed that physiological stress can activate the adrenal steroid output and restrict immunoglobulin absorption during the critical 24-h postnatal period in the neonate.

In contrast to the forementioned reports, Patt and Eberhart (1976) found the serum IgG concentration of ACTH-treated pigs did not differ significantly from the vehicle-injected control pigs at any of the times tested after

birth. They injected newborn cesarean-derived pigs with metyrapone, ACTH, or a vehicle to determine the effects of low, high or normal plasma cortisol concentrations on immunoglobulin absorption. The neonatal pigs were fed pooled bovine colostrum at birth. Serum samples were collected at 0, 6, 14, 22, 30 and 38 h after birth, and serum IgG concentrations were determined. It was suggested that maximal absorption of Ig could not occur unless plasma cortisol levels were adequate. It was also assumed that increased glucocorticoid concentrations would not affect the duration of intestinal permeability to globular proteins. In a similar experiment, Bate and Hacker (1985) investigated the influence of the sow's adrenal activity on the ability of the piglet to absorb bovine IgG from colostrum. Eighteen sows were injected intravenously with ACTH, metyrapone or saline between d 104 and 114 postbreeding. The newborn pigs were force-fed bovine colostrum at 30 minutes, 2, 4, and 6 h postpartum and bled at birth, 6 h, 1, 2, 4, 8, 12, 16 and 21 d of age. The pigs from ACTH treated sows had significantly higher serum IgG levels at 6 h postpartum and the pigs from the metyrapone treated sows maintained higher IgG levels throughout the experiment. In agreement, Johnstone and Oxender (1979) noted that increased serum glucocorticoid concentrations in newborn calves would not

interfere with absorption of immunoglobulins.

Neonatal calves subjected to a variety of nutritional or environmental circumstances have enabled several investigators to study the ability of neonates to absorb Ig following endogenous changes in corticoid levels.

Schlagheck et al. (1983) reported serum cortisol levels in neonatal calves increased between two and three hours after the calves had ingested a colostrum source of immunoglobulin. The time of initial feeding had no effect on the serum cortisol concentrations and there was no observable increase in serum cortisol in the neonates who consumed an immunoglobulin-free milk ration. They concluded the immunoglobulin fraction of colostrum was responsible for initiating an increase in cortisol secretion by the adrenal cortex.

In summary, documented effects of glucocorticoids on immune responses range from marked suppression to enhancement. These observations together with those of other workers indicate many factors interrelate to determine the nature of glucocorticoid effects on immune responses in the gut of the bovine neonate.

THYROXINE SYNTHESIS, FUNCTION,
AND LEVELS IN NEONATAL CALVES

The major secretions of the thyroid gland are the hormones, thyroxine (T4) and triiodothyronine (T3). Both T4 and T3 are synthesized from the principal iodoprotein, thyroglobulin of the thyroid gland (Eggo and Burrow, 1985) via stimulation by thyroid stimulating hormone (TSH) of the anterior pituitary, which is controlled by the hypothalamic thyrotrophin releasing hormone (TRH). The formation of thyroxin takes place in several successive but independent stages involving the participation of different structural components of the thyroid follicles: the transfer of iodine from the blood into the gland, protein synthesis and iodination, the formation of the thyroid hormones, and their subsequent secretion. These stages are integrated into a unified and coordinated endocrine process in the general cell metabolism of the follicular epithelium of the thyroid gland (Geiger et al., 1974). The level of thyroxine is normally maintained by its negative feedback effect on the anterior pituitary controlling the output of thyroid stimulating hormone.

Currently, the nuclear hypothesis of thyroid hormone action appears to be gaining the strongest experimental sup-

port (Baxter et al., 1979; Oppenheimer, 1979). The first significant interaction of thyroid hormones in the cell is with receptor proteins located in the nucleus. This interaction is thought to trigger specific alterations in the production of nuclear RNA. These alterations in RNA synthesis result in changes in protein synthesis and subsequently lead to altered cell function.

Thyroid hormones are well known to elicit responses in many different tissues (Gordon and Southern, 1977; Oppenheimer, 1979) as well as play an important role in numerous physiological and developmental processes in the neonate. Ottenweller and Hedge (1981) and Murakami et al. (1984) reported thyroxine to maintain the normal amplitude in circadian adrenocortical rhythm in the rat by affecting ACTH synthesis.

Thyroid hormones appear to be involved in the regulation of metabolic rate (Tata, 1963) and are important for normal growth and metabolism of skeletal muscle (Baldwin et al., 1978; Kahl et al., 1978), and may act synergistically with growth hormone to induce longitudinal longbone growth and protein metabolism in lambs (Wagner and Veenhuizer, 1978). Also, lipid metabolism has been shown to be influenced by thyroxine in several animal models. O'Kelley (1977) reported a positive correlation between thyroxine and choles-

trol levels in male and nonpregnant cattle.

Clinically, the dramatic effects of fetal and neonatal thyroidectomy on the development of several systems, including the central nervous system, indicate the importance of thyroxine in fetal growth and maturation (Hopkins and Thornburn, 1972). Lowered plasma thyroxine levels have been implicated in respiratory distress syndrome (Cuestas et al., 1976) and disease in the newborn calf and lamb (Cabello, 1980; Cabello, 1983), and in retrospect Cabello (1980) suggested that metabolic defects in dying calves which can affect the plasma level of thyroxine might be of many types: 1) reduction of the plasma concentration of thyroxine-binding globulin; 2) increased catabolism of thyroxine; 3) lack of iodine in the food of the mother; or 4) disturbances affecting the hypothalamo-pituitary-thyroid axis.

In the neonatal period, the fetal calf, lamb, and pig undergo a period of transient neonatal hypothyroxinemia. Immediately postpartum there is a marked increase in neonatal thyroxine (Slebodzinski, 1972; Nathanielsz and Thomas, 1973; Kahl et al., 1977; Slebodzinski et al., 1981). The high level of thyroxine at birth may be a reflection of high activity of the thyroid gland in utero, and that maternal and fetal thyroid hormone pools are relatively independent of one another (Hernandez et al., 1972; Strbak et al., 1976)

or is manifested in part by the stress of parturition (Nathanielsz et al., 1974).

To date, there is a wide range of reported average plasma thyroxine values in bovine neonates measured at birth: 12.8 ug/100ml (Nathanielsz, 1969), 12.9 ug/100ml (Grongnet et al., 1985), 14.0 ug/100ml (Kahl et al., 1977), 14.05 ug/100ml (Cabello, 1980), 17.04 ug/100ml (Cabello and Levieux, 1980), 18.0 ug/dl (Schlagheck et al., 1983), 19.0 ug/100ml (Hernandez et al., 1972), 19.5 ug/100ml (Cabello and Michel, 1974), 30.5 ug/100ml (Miller et al., 1967) and 122.87 - 150 ng/ml (Khurana and Madan, 1984). The individual variations in this period are probably due to some extrathyroidal factors (environmental and physiological), namely differences in body weight, thermal insulation and food supply (Slebozinski et al., 1981).

Various sequential studies have shown plasma thyroxine levels in bovine neonates to reach adult levels at approximately 24 to 28 d of age. Nathanielsz (1969) monitored the serum thyroxine levels in newborn Jersey calves and reported a 11.6 ug/100ml value at 3 to 24 h, with a subsequent fall to 4.2 ug/100ml on day 4 postpartum. A similar study comparing changes in plasma concentrations of thyroxine in Holstein calves from birth to 22 weeks of age

showed a very high level (140 ug/L) immediately after birth and a rapid decrease to 35 ug/L at one week, with a gradual increase to 67.0 ug/L at 22 weeks. Also, Hart et al., (1981) analyzed blood samples taken twice-weekly from newborn dairy heifers maintained under commercial conditions during the first 110 days of life. They found the average plasma concentration during the preweaning period (1 to 27 d postpartum) to be 75.2 ug/L, followed by a decrease to 66.4 ug/L at weaning (28 to 83 d postpartum).

In terms of more mature growing steers, (approximately 380 kg), Gopinath and Kitts (1984) reported an overall mean plasma thyroxine concentration of 4.9 ug/100ml, an obvious reduction from levels reported for neonates.

Also worth noting is a possible rhythmicity of circulating thyroxine in growing steers. Hammond et al. (1984) observed rhythmicity of circulating thyroid hormones in steers and suggested a trend towards increased plasma thyroxine in the afternoon, lower values in the morning and higher values at the beginning or just after feeding.

INTERACTION BETWEEN THYROXINE AND IG ABSORPTION

Thyroid hormones have been shown to have an effect on the gastrointestinal tract at all levels of organization and many researchers have long recognized the associations that exist between gastrointestinal symptoms and thyroid disease. However, the present knowledge of the mechanisms by which thyroid hormones act on the absorptive cells of the gastrointestinal tract is fragmentary and inconclusive.

There is evidence that thyroid hormones are necessary to ensure normal maturation in intestinal mucosal cells. For example, if the thyroid gland is removed in the immature rat the small intestine fails to develop and mature normally (Bronk and Parsons, 1965), and may be essential in stimulating cell mitosis and growth in the crypt zones of the intestinal mucosa (Carriere, 1966). In support, Chan et al. (1973) studied the effect of thyroxine administration on the cessation of macromolecular uptake by the neonate rat intestine. The experimental pups were injected at five days postpartum with a solution of thyroxine in saline. The neonates were sacrificed on d 9, 11, 12 or 13 postpartum and ^{125}I -PVP uptake was measured. It was determined that closure occurred approximately 5 to 6 d prematurely.

Several researchers have attempted to correlate the

acquisition of passive immunity via action of thyroxine. Cabello and Michel (1974) reported measurements of plasma levels of globulins in calves after ingestion of colostrum. They observed a negative correlation between the concentration of hormonal iodine at birth and the level of globulins after 48 h. In a subsequent trial, Cabello and Levieux (1978) studied the effects of thyroxine on the absorption of Ig in neonatal calves and once again noted a negative correlation between the plasma level of thyroxine at birth and the time at which the maximal concentration of IgG in the plasma of the calves was reached. It was thought that the increased thyroxine level shortened the absorption period and then decreased the maximal IgG level.

More recently, researchers have administered thyroxine to neonates during different stages of development and observed the subsequent effects on intestinal absorption of macromolecules. Cabello et al. (1980) administered intra-amniotic injections of thyroxine to three prenatal kids and saline to four control kids. The kids were bottle-fed a common source of colostrum at 2.5% of their body weight every 4 h until 32 h postpartum. Blood samples were collected at 4 h after birth and then every 4 h until 36 h postpartum. They reported no significant differences in

in plasma Ig concentrations between the control and treated kids, but did observe a reduced duration of IgG absorption in the treated kids. The treated kids exhibited a maximum IgG concentration at 20.7 h while the control kids peaked at 30.7 h postpartum. In a similar study, Cabello and Levieux (1980) injected six calves with thyroxine and six with saline at birth, and again at 24 h postpartum. The twelve calves were fed a common source of pooled colostrum. The authors detected no differences in plasma Ig concentration in the experimental calves. In summary, it has been suggested that either the thyroxine injection at birth occurred too late to be effective, or the correlation between thyroxine at birth and IgG levels was not a direct effect and reflected only an underlying common cause (Cabello and Levieux, 1978).

GLUCOCORTICOID RECEPTORS IN SKELETAL MUSCLE

Intracellular proteins which bind their hormones with high affinity and stereospecificity and which participate in the biochemical processes leading to biologically meaningful alterations in cellular function may be considered to be "hormone receptors" (Jensen and Jacobson, 1962). Over the past twenty years there have been significant advances in the understanding of the biochemical mechanisms by which glucocorticoids interaction with specific receptors elicits biological responses in target cells. A complete discussion of the available data on this subject is beyond the scope of this thesis. Thus, the primary focus will pertain to the physical and chemical properties, stereospecificity of binding, mode of action, and quantitation of the glucocorticoid receptor in skeletal muscle.

Physical and Chemical Properties

The glucocorticoid receptor is an asymmetric, slightly acidic phosphoprotein of about 100,000 daltons (Carlstedt-Duke et al., 1977; Housley and Pratt, 1983). Limited data is available involving glucocorticoid receptors purified to homogeneity due to the very low concentration of receptors in the cell (less than 0.01% of the cellular protein) and to

the lability of the steroid binding site (Failla et al., 1975; Govindan and Sekeris, 1976; and Schmid et al., 1976). However, a few physical characteristics and molecular properties of the glucocorticoid receptor from mouse fibroblasts are provided in table 3 (Middlebrook and Aronow, 1977).

Stereospecificity of Binding

The structural requirements for binding to the glucocorticoid receptor are less stringent than for other steroid hormone receptors (Rousseau et al., 1972; Rousseau and Schmidt, 1977). Thus, the number of steroids which could bind to the glucocorticoid receptor (given high enough concentrations) and elicit biological effects is quite large (Koblinski et al., 1972; Mayer and Rosen, 1975). In addition, there are glucocorticoid antagonists (compounds that bind to the receptor but do not evoke a glucocorticoid effect) which compete with the glucocorticoid hormone and therefore block its action (Raynaud et al., 1980; Chrousos et al., 1983; Rousseau et al., 1983). However, the glucocorticoid receptor can be distinguished from other glucocorticoid-binding proteins based on its steroid specificity. This specificity enables the target cell to respond to a hormonal signal without interference from other

Table 3: PHYSICAL CHARACTERISTICS OF THE
GLUCOCORTICOID RECEPTOR^{ab}

Tissue	Partial Specific Volume (cm/g)	Stokes Radius	Sed. Coef.	Mol. Wt.	Fract. Ratio	Isoel. Point
Mouse Fibro- blasts	0.735	59	4-4.5	109,000	1.72	5.9

^aAll parameters were determined at isotonic salt concentration. Molecular weight and fractional ratio were based on values in the preceding columns.

^bMiddlebrook and Aronow, 1977.

signals.

Litwack et al.(1973) found rat liver to contain three soluble proteins which bind natural glucocorticoids. One of these cytosolic proteins is identical to transcortin, referred to as binder II. Another liver cytosolic protein, component "G" also binds the natural glucocorticoids and cross reacts with antibodies prepared to transcortin, and could possibly be structurally related to it (Beato and Feigelson, 1972). Both transcortin and the component "G" bind naturally occurring glucocorticoids, such as hydrocortisone and corticosterone, but neither bind the synthetic fluorinated glucocorticoids, such as dexamethasone and triamcinolone, compounds which are highly potent glucocorticoids in vivo. The third cytosolic protein in the liver which binds the natural glucocorticoids and has a very high affinity for the synthetic fluorinated glucocorticoids is believed to be the "biologically significant" glucocorticoid receptor. Koblinsky et al.(1972) and Raynaud et al. (1980) discovered the protein to have a specific high affinity saturable binding for natural and (or) synthetic biologically active glucocorticoids. Beato et al.(1974) showed that both in vivo and in vitro its subcellular translocation from the cytoplasm to the nucleus was a

function of its saturation with glucocorticoids. In support, Kalami et al. (1975) and Grody et al. (1982) noted the steroid-receptor-complex undergoes a time-and-temperature calcium ion enhanced "activation" allowing it to bind to nuclei, chromatin, and possibly stripped DNA. To date, there is no clear-cut evidence that the glucocorticoid receptor differs from tissue to tissue (Baxter and Forsham, 1972; Snochowski et al., 1980; Lentze et al., 1985), and it is in fact very similar in various species (Ballard et al., 1974; Middlebrook et al., 1975). In short, the extensive tissue distribution of glucocorticoid receptors parallels the extensive role of glucocorticoids in regulation of bodily functions.

Mode of Action

From evidence accumulated in many laboratories, it is now possible to propose an acceptable model for the mode of action of the glucocorticoid hormone-receptor-complex. Higgins et al. (1973) proposed a nuclear-binding reaction sequence simulated in reconstituted, cell-free systems. This model has been recently modified by Schmidt and Litwack (1982). The sequence of events is essentially as follows: (1) After secretion, the hormone is distributed throughout the peripheral circulation by its association with a select-

ive binding protein in the plasma (i.e. CBG). (2) The hormone enters all cells to some extent either by passive diffusion or facilitated mechanisms of entry. (3) Extensive metabolism may occur within the target cells. (4) The hormone binds selectively and with high affinity to specific receptor proteins in the cytoplasm forming hormone-receptor complexes. (5) The receptor complex undergoes "activation" or some change in physiochemical configuration, thereby acquiring a particular propensity for interacting with nuclear chromatin. (6) The activated complex is translocated into the nucleus by passive diffusion through nuclear pores where it occupies a limited number of specific sites (Gorski and Gannon, 1976; Andre et al., 1978). It appears that translocation does not require energy (Shyamala and Gorski, 1969). (7) The receptor complex remains within the nucleus for a significant but not an infinite period of time, where it binds to DNA and non-histone protein "acceptor sites" (O'Malley et al., 1976; Chan and O'Malley, 1978) resulting in an increase in specific RNA messengers (mRNA), which elicit the cellular responses to the hormone. (8) The receptor complex finally leaves the nucleus and in the absence of further hormone secretion, the entire process rescinds. There is, however,

a replenishment of cytoplasmic glucocorticoid receptors which is probably due to the reappearance of the original receptor in the cytoplasm rather than new receptor synthesis (Munck and Foley, 1976; Raaka and Samuels, 1983; Munck and Holbrook, 1984).

Investigations by Wira and Munck (1974) provide supportive evidence that the cytoplasmic receptor is required for nuclear binding of glucocorticoids and this process is due to the reversible association of the hormone-receptor complex with the nucleus. In fact, the cytosol-binding reaction was proven to be an intermediate for nuclear binding. To substantiate this finding, Baxter and Tomkins (1971) demonstrated that steroid specificity for nuclear binding was identical to that of cytosol binding.

The sequential model of glucocorticoid receptor binding leaves some unexplained questions, such as the hypothesized nuclear activation of the hormone-receptor complex, the nuclear localization of unfilled receptors in equilibrium with cytoplasmic receptors, and the nature of the acceptor site. In addition, more sound data needs to be generated concerning the identification of the nuclear acceptor sites and the specific sequences of DNA and genes involved in the hormonal response.

Quantitation of Receptors in Skeletal Muscle

As described by Baxter and Rousseau (1979), the interaction of glucocorticoids with the receptor site corresponds to a simple reversible reaction following the law of mass action:



where B and S designate the concentration of free receptor and free steroid, respectively, and BS corresponds to the bound receptor concentration. The apparent equilibrium dissociation constant K (in mol l^{-1}) or its reciprocal, the apparent equilibrium constant K (1 mole^{-1}) can be calculated either from the equilibrium concentrations of the reactants:

$$K = \frac{BS}{S \times B} \quad \text{Eq. (b)}$$

or else from the ratio of the association (K_a) and dissociation (K_d) rate constants:

$$K = \frac{K_a}{K_d} \quad \text{Eq. (c)}$$

If it is assumed that the hormonal effect (E) is a function of receptor occupancy (BS), it follows from equation (b) that $E=f(K - S \times B)$. Therefore, the steroid effect depends

on the concentration of both the steroid and the receptor as well as on the affinity of the binding.

The laboratory technique most commonly used for the study of glucocorticoid binding in skeletal muscle is the measurement of the amount of tritium-labeled natural or synthetic hormones bound at equilibrium to a high-speed supernatant fraction of muscle tissue. Hormone binding to the muscle cytosol is performed at 0 to 4 C to minimize steroid metabolism, binding capacity being lost in a few minutes at 37 C. As determined by Schmid et al. (1976) a basic standard procedure for preparing cytosol is to homogenize the tissue in no more than one to three volumes of a 20 mM Tris-HCl buffer, pH of 7.4, containing 50 mM 2-mercaptoethanol, and 2.5 mM EDTA.

The amount of bound hormone is determined by a method which employs activated charcoal to adsorb and remove the free (unbound) hormone. This method is rapid and ensures an efficient and instantaneous removal of the free labeled steroid as soon as equilibrium has been reached. The amount of macromolecular-bound radioactivity is then determined.

The classic work of Scatchard (1949) has enabled most investigators to adequately perform equilibrium studies whereby the total receptor site concentration may be obtained by graphic extrapolations, most often performed by

micro-computers. The most commonly used Scatchard equation results in:

$$\frac{(BS)}{S} = \frac{1}{-K_d} (BS) + \frac{B_{max}}{K_d}$$

where B_{max} is the total concentration of sites. If the binding reaction conforms to Eq.(a), the bound (BS) over free (S) steroid is linearly related to the bound. K_d can then be calculated from the slope and the intercept on the abscissa provides the concentration of sites B_{max} .

Since the early work of Long et al. (1940), it has been shown by several investigators that glucocorticoids exert a net catabolic effect on skeletal muscle proteins and play a major role in the regulation of protein turnover in skeletal muscle. However, to date there are relatively few reports on the equilibrium properties of the glucocorticoid receptor population. Thus, outlined in Table 4 is the limited cumulative data concerning receptor binding in muscle tissue.

These observations provide evidence that skeletal muscle is a glucocorticoid-responsive tissue. In fact, although the concentration of the glucocorticoid receptors per milligram protein in muscle is very low, relative to other responding cells, on a DNA basis (i.e. per cell), skeletal muscle contains the second highest concentration

Table 4: GLUCOCORTICOID RECEPTORS IN CYTOSOL FROM SKELETAL MUSCLE.

Specie	Muscle	K _d	B _{max} (fmol/mg protein)	B _{max} (fmol/mg tissue)	Author
Rat	" "	1.9x10 ⁻⁸	0.10	---	Mayer et al. (1974)
Rat	" "	1.9x10 ⁻⁸	0.10	---	Mayer & Rosen (1975)
Rat	Gasroc- nemius	1.7x10 ⁻⁷	.99	---	Mayer et al. (1975)
Rat	Extensor digitorum longus & soleus	7.13x10 ⁻⁹ 5.95x10 ⁻⁹	29.6 40.4	---	Shoji & Pen- nington (1977)
Rat	Gastroc- nemius	2.05x10 ⁻⁹	43.81	---	Dubois & Almon (1980)
Rat	Gluteus, Tensor, Biceps & Vastus	7.0x10 ⁻⁹	30.0	1900	Snochowski et al. (1980)
Rat	Same as Snochowski et al. (1980)	13.6x10 ⁻⁹	49.1	3100	Dahlberg et al. (1981)
Pig	Rectus femoris	12.4x10 ⁻⁹	---	2940	Snochowski et al. (1981)
Rat	Gastroc- nemius	2.1x10 ⁻⁹	42.45	1397	Dubois & Almon (1984)
Rat	Gastroc- nemius & Soleus	1.79x10 ⁻⁹	91.80	---	Danhaive & Rousseau (1986)

of receptor binding sites of all tissues measured thus far (Ballard et al., 1974).

Chapter III

OBJECTIVES

The objectives of this experiment were to:

- 1) Determine the effect of feeding colostrum or whole milk on the maintenance of serum cortisol, thyroxine and immunoglobulin G levels in the neonatal calf.
- 2) Analyze the quantity, binding kinetics and specificity of cytosolic glucocorticoid receptors in skeletal muscle of the neonatal calf.

Chapter IV

JOURNAL ARTICLE

COLOSTRUM FEEDING AND ITS EFFECTS ON SERUM CORTISOL, THYROXINE AND IMMUNOGLOBULIN G IN THE BOVINE NEONATE

SUMMARY

A study was conducted to determine the effect of colostrum or milk fed to newborn calves on serum cortisol, thyroxine and immunoglobulin G. Twenty-four calves (12 males and 12 females) were obtained immediately postpartum and were randomly assigned to one of two treatments after being blocked by breed and sex. The treatments consisted of: 1) pooled colostrum and 2) pooled whole milk, with both treatments being force-fed at birth, 12, 24 and 36 h postpartum. The calves were fed 10% of their body weight (BW) at birth and 5% of their BW at 12, 24 and 36 h postpartum. Blood sampling was performed at 0 time (prior to first feeding; <1 h postpartum), 1, 2, 3, 4, 6 and 12 h postfeeding with this regimen being followed for a 48-h period (4 feedings). Samples taken at 0, 1 and 2 h postpartum were collected via jugular puncture. Following the 2 h collection,

all calves were fitted with indwelling jugular catheters for the remainder of the collection period. Cows and calves were separated for the 48 h postpartum period and reunited after the final blood sample was collected. Serum cortisol, thyroxine and immunoglobulin G concentrations were quantitated by competitive single antibody radioimmunoassay and single radial immunodiffusion, respectively. The average initial cortisol concentration was highest at birth, 221.9 and 245.6 ng/ml for colostrum and milk-fed calves, respectively. Following the initial peak at birth, serum cortisol concentrations declined with time ($P < .05$) for both treatments. Cortisol levels between treatments were different ($P < .05$) at 2, 3, 12, 14, 18, 24, 37 and 48 h postpartum. There was also an apparent peak at each feeding with the average concentration ranging from 233 ng/ml at the first feeding, 136 ng/ml at 12 h, 56.7 ng/ml at 24 h, and 105 ng/ml at 36 h postpartum or at the last feeding. There was no sex difference observed in the average serum cortisol concentrations. Within 3 h postpartum, serum thyroxine concentrations increased ($P < .05$) to reach a peak of 23.3 and 21.0 ug/dl for colostrum and milk-fed calves, respectively. There was a noted sex difference in serum thyroxine concentration as the female calves exhibited the highest average concentration

over the entire trial. Both treatment groups were born with similar serum immunoglobulin G levels (~ 0.7 mg/ml). However, at approximately 4 h postpartum, the colostrum-fed calves acquired increases ($P < .001$) in serum immunoglobulin G, peaking at 24 h postpartum (26.83 mg/ml) and remaining much higher throughout the entire trial. There was a treatment difference ($P < .001$) between the two groups following the 4 h sample. The milkfed calves showed a lower peak at 24 h (1.35 mg/ml) and another peak ($P < .001$) at 72 h postpartum. Results indicate both cortisol and thyroxine are high at 0 to 4 h postpartum; the act of feeding may stimulate a cortisol surge; although not significant, there is a possible sex difference in the thyroxine levels; and the feeding of colostrum, rather than whole milk, has a drastic effect on serum immunoglobulin G levels in the newborn neonate.

(Key Words: Colostrum, Cortisol, Thyroxine, Immunoglobulin G, Neonatal Calves)

INTRODUCTION

The calf, born devoid of immunoglobulin in serum, relies on antibodies absorbed from colostrum via pinocytosis by villus epithelial cells of the small intestine (passive immunity). Intestinal permeability to immunoglobulin persists for only 24 to 36 h, after which "closure" is presumed.

There is wide variability in efficiency of immunoglobulin absorption. Ten to 40% of the calves which acquire colostrum during the first 24 h postpartum remain persistently hypogammaglobulinemic (McEwan et al., 1970; Logan and Gibson, 1975; Frerking and Aikens, 1978). Various environmental factors may influence passive immunity in the bovine neonate. They are: 1) mass and concentration of Ig consumed (Bush et al., 1973; Stott and Fellah, 1983); 2) delayed colostrum ingestion (Stott et al., 1979a); 3) compounds present in colostrum (Schlagheck et al., 1983); 4) age and(or) parity of the dam (Muller and Ellinger, 1981; Devery-Pocious and Larson, 1983); 5) method of feeding (Selman et al., 1971; Molla, 1978); 6) prepartum nutrition of the dam (Blecha et al., 1981; Burton et al., 1984) and 7) seasonal variation (Gay et al., 1983).

Minimal information is available concerning the role of

endogenous hormones on immunoglobulin absorption and gut closure. Early work by Halliday (1959) suggested the involvement of glucocorticoids. Kruse and Buus (1972) reported relatively high concentrations of corticosteroids in the calf 2 h after birth and suggested cortisol "shock" may induce changes in the intestinal epithelium leading to a hampered ability to absorb immunoglobulins. In support, Husband et al. (1973) and Stott et al. (1976) concluded that corticosteroids can influence cell permeability of the small intestine in postnatal livestock rendering them incapable of immunoglobulin absorption.

In contrast, Patt and Eberhart (1976) suggested maximal absorption of immunoglobulin could not occur unless plasma cortisol levels were adequate and increased glucocorticoid concentrations would not affect the duration of intestinal permeability (closure) to globular proteins. Concordantly, Johnstone and Oxender (1979) noted that increased serum glucocorticoid concentrations in newborn calves would not interfere with absorption of immunoglobulins. In short, documented effects of glucocorticoids on immune responses range from suppression to enhancement. These observations together with those of other workers indicate that many factors interrelate to determine the nature of glucocort-

icoid effects on immune responses in the gut of the bovine neonate.

Thyroid hormones have been shown to have an effect on the gastrointestinal tract at all levels of organization and numerous investigators have long recognized the associations which exist between gastrointestinal symptoms and thyroid disease. However, the present knowledge of the mechanisms by which thyroid hormones act on the absorptive cells of the gastrointestinal tract is fragmentary and inconclusive.

Thyroid hormones are necessary to ensure normal maturation in intestinal mucosal cells. For example, if the thyroid gland is removed in the immature rat the small intestine fails to develop and mature normally (Bronk and Parsons, 1965) and may be essential in stimulating cell mitosis and growth in the crypt zones of the intestinal mucosa (Carriere, 1966). There have been attempts to correlate the acquisition of passive immunity via action of thyroxine. Cabello and Levieux (1978) reported a negative correlation between the plasma level of thyroxine at birth and the time at which the maximal concentration of IgG in the plasma of the calves was reached. It was suggested the negative correlation was not a direct effect and reflected only an underlying common cause.

Passive immunity is complex because it involves a delicate balance of factors in the dam, the environment and the neonate. Knowledge of these interactions are necessary to give effective advise to the farming community so that mortality and morbidity of the bovine neonate can be reduced to negligible proportions. Thus, the following study was undertaken to investigate the effects of feeding colostrum or whole milk on serum cortisol, thyroxine and immunoglobulin G levels in neonatal beef calves.

MATERIALS AND METHODS

Twenty-four beef calves (12 males and 12 females; Angus, Hereford and Simmental Crossbred) weights ranging from 30 to 49 kg were obtained immediately postpartum and randomly assigned to one of two treatments after being blocked by breed and sex. The treatments consisted of pooled colostrum (65 mg/ml IgG) and pooled whole milk, both of which were acquired from a nearby commercial dairy¹ and the VPI&SU dairy. Both rations were deposited in 4 liter plastic containers and frozen at -20 C until utilized. All calves were force-fed 10% of their body weight (BW) at birth (<1 h postpartum) and 5% of their BW at 12, 24 and 36 h postpartum via an esophageal tube feeder² to assure uniform ingestion among all experimental calves. Both rations were warmed to room temperature prior to feeding. Collection of blood samples (~10 ml at each bleeding) was performed at 0 time (prior to first feeding; <1 h postpartum), 1, 2, 3, 4, 6 and 12 h postfeeding with this regimen being followed for a 48 h period (4 feedings). Samples taken at 0, 1 and 2 h postpartum were collected via jugular vac-
cutainers³. Following the 2 h collection, all calves were

¹Wall Brothers Dairy, Inc., Blacksburg, VA. 24060.

²Nasco, Fort Atkinson, WI. 53538.

³Fisher Scientific, Raleigh, NC. 27064.

fitted with indwelling jugular catheters for the remainder of the collection period. The cows and calves were separated for the 48 h postpartum period and reunited after the final blood sample was collected. The blood samples were kept at 4 C, permitted to clot and centrifuged⁴ for 15 min at 3000 x g. The serum was harvested and stored at -20 C for subsequent analysis.

LABORATORY ANALYSIS

The nonspecific serum IgG concentrations were determined by a modified radial immunodiffusion technique of Mancini et al. (1965). Serum cortisol and thyroxine were quantitatively measured via a competitive single antibody radioimmunoassay⁵. A pooled bovine serum sample was used to calculate intra- and interassay coefficients of variation, which were found to be 9.5% and 2.6% for cortisol, 7.4% and 2.1% for thyroxine, respectively.

STATISTICAL ANALYSES

Data were analyzed by least squares analysis of variance for a fixed effects model using the general linear

⁴Beckman model J2-21.

⁵Amersham Corp., Arlington Heights, IL. 60005.

models procedure of the Statistical Analysis Systems (SAS, 1979). Sources of variation for each parameter (cortisol, thyroxine and IgG) were treatment, block, collection time, and a treatment by collection time interaction. For each parameter where the treatment x collection time interaction was significant, a Student's t-test was used to test for significant differences between means at selected collection times within each treatment. In addition, each mean in the same treatment was tested against all means in a succeeding 4-h period.

RESULTS AND DISCUSSION

The average initial cortisol concentration was highest at birth, 221.9 and 245.6 ng/ml for the colostrum and milk-fed calves, respectively (table 5; figure 1). The elevated concentrations at birth are consistent with those found in pigs (Ash and Heap, 1975), sheep (Bassett and Alexander, 1977) and in newborn dairy calves (Khan et al., 1970; Johnstone and Oxender, 1979; Stott, 1980; Schlagheck et al., 1983). The absolute values at birth are somewhat higher than those recorded by the other investigators. This discrepancy may be best explained by the fact that several of the calves delivered were greatly traumatized due to difficult births, many of the calves were exposed to cold environmental temperatures at parturition (possible inducement of hypothermia), and another valid explanation could be systematic errors during the assay procedure.

Following the initial peak at birth, serum cortisol concentrations declined with time ($P < .05$) for both the colostrum and milk-fed calves. This is in agreement with the findings of Khan et al. (1970) and in partial agreement with Johnstone and Oxender (1979) who reported a rapid decline in serum concentration during the first 3 h, followed by a gradual decline between 3 and 18 h, after which no

Table 5: SERUM CORTISOL CONCENTRATIONS (ng/ml)
OF CALVES AT BIRTH AND AFTER FEEDING
AT 0, 12, 24 OR 36 H POSTPARTUM

Hours Postpartum	Treatment			
	Colostrum		Whole	Milk
	Mean ^a	SEM	Mean ^a	SEM
0	221.9 ^e	31.9	245.6 ^e	36.6
1	81.4 ^c	22.8	99.6 ^c	22.3
2	107.5 ^b	18.8	67.7 ^{cf}	12.6
3	74.3 ^{cf}	18.6	122.9 ^{bf}	9.7
6	83.4 ^{ce}	16.4	73.8 ^{ce}	8.8
12	123.6 ^{bef}	17.1	150.7 ^{bdf}	5.5
13	88.1 ^c	18.0	88.1 ^c	6.2
14	53.7 ^{cf}	12.3	97.6 ^{cf}	5.9
15	52.5 ^c	13.4	66.9 ^c	15.1
18	56.2 ^{ceg}	11.8	112.9 ^{bdg}	11.6
24	41.7 ^{cef}	19.6	71.5 ^{cf}	10.2
25	29.4 ^c	9.8	35.4 ^c	9.1
26	45.0 ^c	10.5	45.2 ^c	4.9
27	52.2 ^c	16.3	39.2 ^c	4.4
30	87.9 ^{ce}	8.7	92.7 ^c	12.3
36	111.0 ^{be}	6.6	98.1 ^{ce}	8.6
37	81.0 ^{cf}	18.1	121.0 ^{bf}	11.8
38	82.6 ^{cf}	14.3	126.7 ^{bf}	7.7
39	90.0 ^c	11.7	113.7 ^b	8.3
42	58.7 ^{ce}	18.0	35.2 ^{ce}	10.6
48	135.7 ^{bf}	10.8	104.3 ^{bf}	7.3

^aBeginning with the peak value after birth, the mean at each feeding in the same column was tested against the last mean in each seven-hour period.

^bMean differs from 0 h mean in the same column (P<.01).

^cMean differs from 0 h mean in the same column (P<.001).

^dMeans in the same column bearing the same superscript differ (P<.05).

^eMeans in the same column bearing the same superscript differ (P<.01).

^fMeans in the same row differ (P<.05).

^gMeans in the same row differ (P<.01).

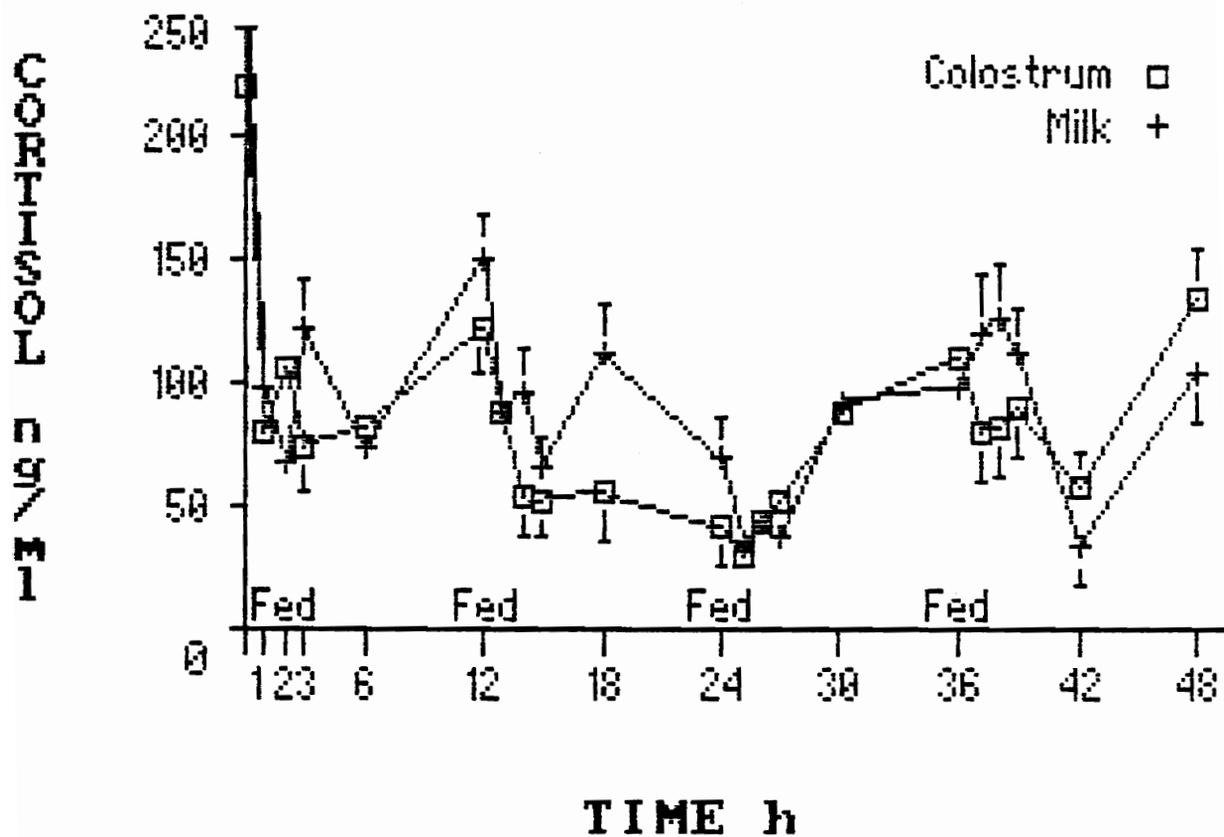


Figure 1. Mean serum cortisol concentrations of calves at birth and after feeding at 0, 12, 24 or 36 h postpartum.

alterations were recorded throughout the initial 48-h postpartum period.

Statistical comparisons were made at each collection time between treatment groups. Cortisol levels were significantly different ($P < .05$) at 2, 3, 12, 14, 18, 24, 37 and 48 h postpartum. There was also an apparent depression after each feeding with the average concentration of both treatment groups ranging from 233 ng/ml at the first feeding, 136 ng/ml at 12 h, 56.7 ng/ml at 24 h, and 105 ng/ml at 36 h postpartum (last feeding). In support, Nightengale and Stott (1981) reported an increase in serum cortisol due to feed intake following a period of inanition of at least 12 h. They further suggested it was the colostrum which was responsible for the subsequent rise in serum cortisol concentration. This hypothesis was supported by Schlagheck et al. (1983) as they demonstrated the immunoglobulin fraction of colostrum to be responsible for initiating an increase in cortisol secretion. The results of this study, however, were unable to substantiate such a finding. In addition, statistical comparisons revealed no sex differences in the average serum cortisol concentrations.

Within 3 h postpartum, serum thyroxine concentrations increased ($P < .05$) to reach levels of 23.3 and 21.0 ug/dl for colostrum and milk-fed calves, respectively (table 6;

Table 6: SERUM THYROXINE CONCENTRATIONS (ug/dl)
OF CALVES AT BIRTH AND AFTER FEEDING
AT 0, 12, 24 OR 36 H POSTPARTUM

Hours Postpartum	Treatment			
	Colostrum		Whole	Milk
	Mean ^a	SEM	Mean ^a	SEM
0	17.2 ^b	2.8	16.9 ^b	1.1
1	17.5	2.6	20.5	2.4
2	22.2	1.8	20.3	3.0
3	23.3 ^c	3.1	21.0 ^c	1.7
6	20.5	2.4	19.1	1.9
12	15.8	1.9	17.9	.9
13	17.3	2.2	17.8	1.1
14	16.7	2.3	17.9	2.6
15	16.9	2.4	17.6	2.2
18	18.8	1.8	18.5	1.0
24	14.8	4.3	14.9	1.6
25	17.7	3.7	14.9	1.6
26	14.2	2.7	15.9	2.1
27	16.1	1.8	15.6	1.9
30	13.9	4.4	13.6	2.2
36	12.3	1.6	13.3	1.4
37	12.4	1.7	10.3	.9
38	14.2	2.7	12.6	1.3
39	11.7	3.5	10.9	1.5
42	11.2	2.0	10.5	1.6
48	11.5 ^c	1.0	9.7 ^c	.8

^aMeans in the same column were tested against all means in a succeeding 7 h period.

^bSignificant reduction in concentration occurred with time ($P < .001$).

^cMeans differ from 0 h mean in the same column ($P < .05$).

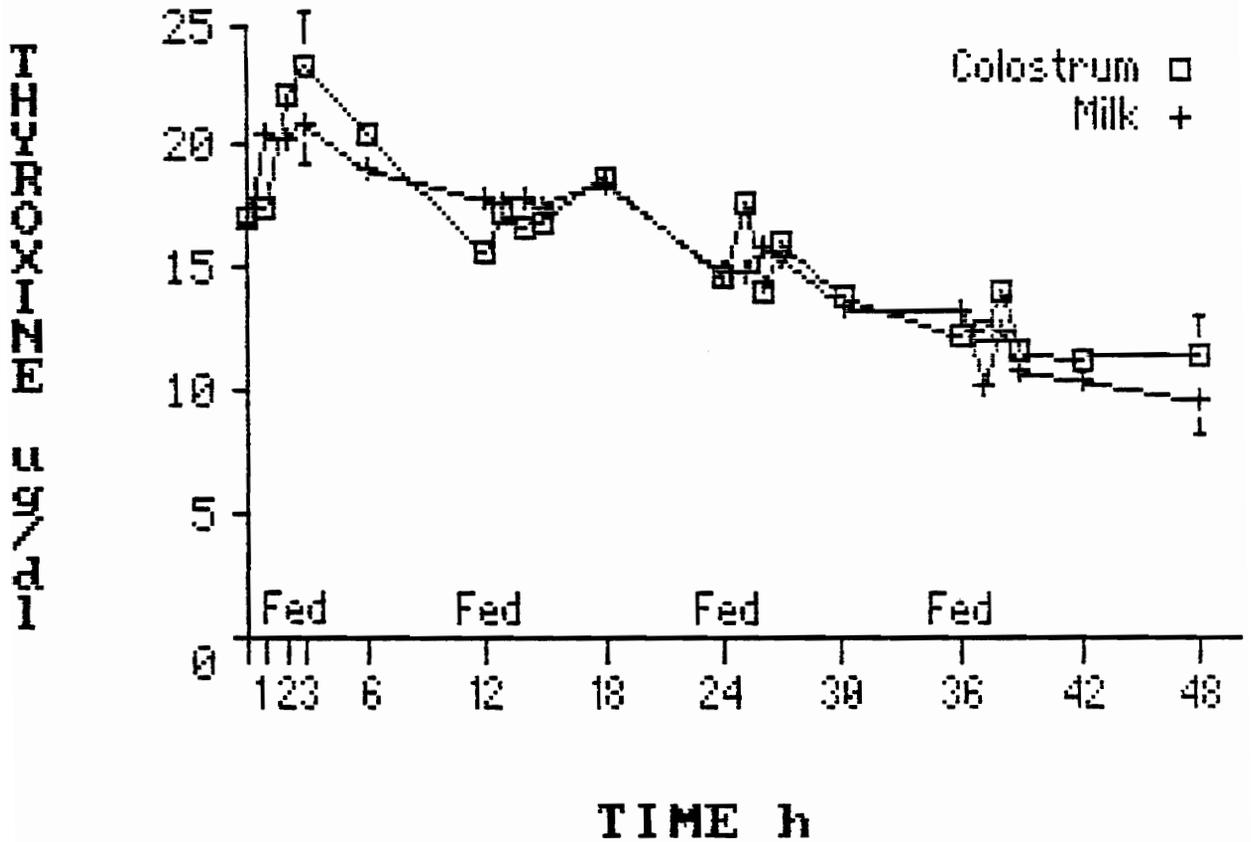


Figure 2. Mean serum thyroxine concentrations of calves at birth and after feeding at 0, 12, 24 or 36 h postpartum.

Table 7: SERUM THYROXINE CONCENTRATIONS
(ug/dl) OF MALE AND FEMALE CALVES
FROM 0 TO 48 H POSTPARTUM

Hours Postpartum	<u>Male</u>		<u>Female</u>	
	Mean	SEM	Mean	SEM
0	16.9	2.3	17.2	2.9
1	19.0	1.9	19.0	1.8
2	19.9	1.9	22.7	2.1
3	21.0	2.2	23.5	3.3
6	19.7	2.4	19.9	2.0
12	16.3	1.8	17.5	2.6
13	16.8	1.6	18.5	1.7
14	15.4	1.3	19.5	1.8
15	17.0	2.0	17.4	2.2
18	17.6	2.5	19.9	2.3
24	14.2	2.1	15.8	1.9
25	14.7	1.9	14.9	2.9
26	13.3	2.7	17.2	4.1
27	15.6	1.4	16.1	2.2
30	12.8	1.7	15.0	1.7
36	11.1	1.5	14.9	2.0
37	10.7	1.5	12.2	1.7
38	12.6	2.1	14.4	2.4
39	11.3	1.2	11.5	2.0
42	10.6	1.3	11.2	1.1
48	10.2	1.3	11.1	1.6

figure 2). Following the peak, thyroxine concentrations declined gradually throughout the duration of the sampling period. Our data differs from that reported by Hernandez et al. (1972) who reported elevated thyroxine concentrations at birth. However, the data compares favorably with Nathanielsz (1969) who monitored the serum thyroxine levels in newborn Jersey calves and reported an 11.6 ug/100ml value at 24 h, with a subsequent fall to 4.2 ug/100ml on d 4 postpartum. In additional support, Davicco et al. (1982) and Grongnet et al. (1985) both showed an increase in the plasma thyroxine level between 0 and 6 h postpartum.

Serum thyroxine levels of female calf fetuses have been reported higher ($P < .05$) than those of males during all three trimesters of gestation (Hernandez et al., 1972). Our results are similar with Kahl et al. (1977) and Grongnet et al. (1985) which also showed a noted sex difference in thyroxine concentration as the female calves exhibited the highest concentration (non-significant) over the entire trial.

As shown in table 8 and figure 3, both treatment groups were born with similar serum immunoglobulin G levels (~ 0.7 mg/ml). However, at approximately 4 h postpartum, the colostrum fed calves acquired increases ($P < .001$) in serum

Table 8: SERUM IMMUNOGLOBULIN G LEVELS (mg/ml)
OF CALVES AT BIRTH AND AFTER FEEDING
AT 0, 12, 24 OR 36 H POSTPARTUM

Hours Post- partum	Treatment			
	Colostrum		Whole	Milk
	Mean	SEM	Mean	SEM
0h	.07 ^b	.02	.06	.02
1h	.45 ^b	.01	.09	.04
4h	4.72 ^{ab}	.68	.40	.03
12h	19.75 ^{ab}	1.4	.76	.03
15h	19.41 ^{ab}	1.5	.18	.52
24h	26.83 ^a	2.0	1.35	.18
25h	24.55 ^a	1.8	1.02	.07
27h	22.96 ^{ab}	1.1	1.34	.38
36h	24.76 ^a	2.3	.96	.05
37h	22.65 ^{ab}	3.4	.75	.03
48h	19.70 ^{ab}	1.6	.75	.03
72h	22.00 ^{ab}	1.7	14.52 ^c	1.8

^aColostrum differ from Whole Milk (P<.001).

^bMean differs from 24 h mean (P<.01).

^cSignificant increase (P<.001).

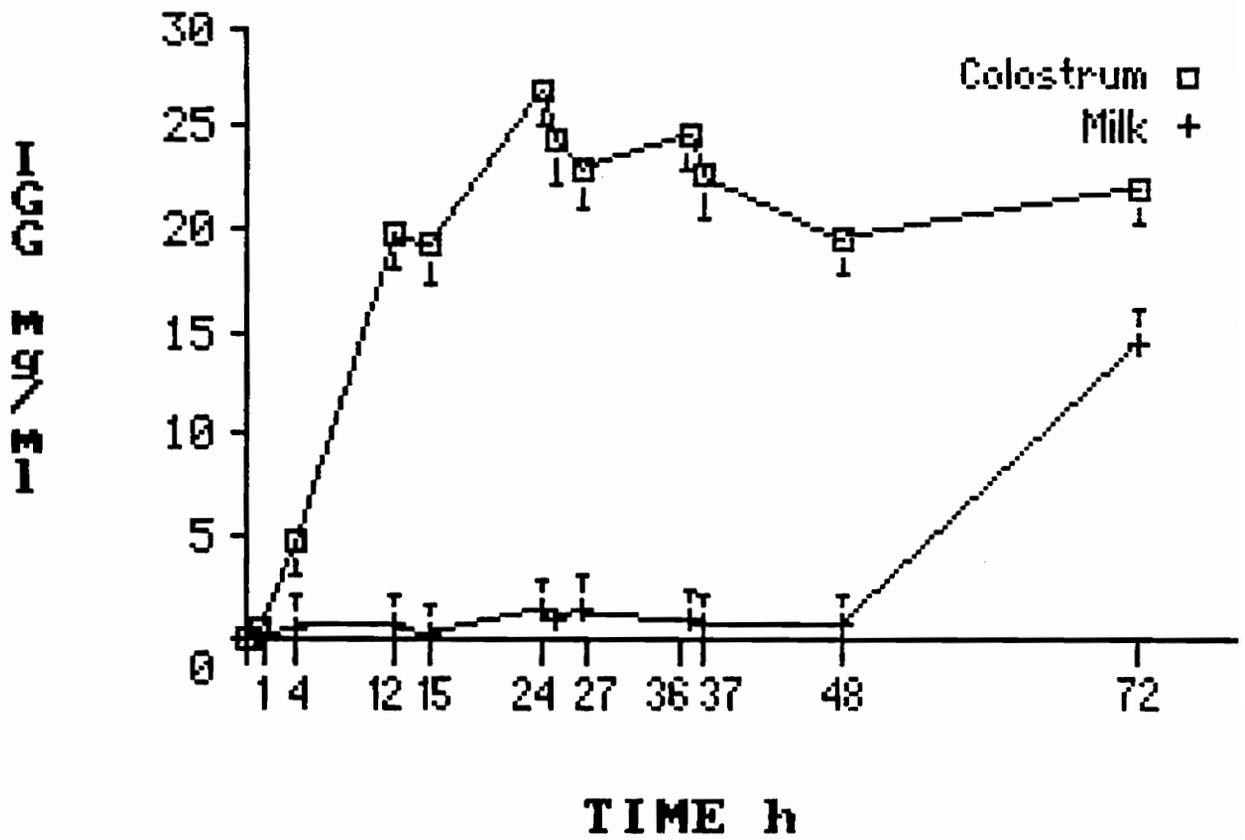


Figure 3. Mean serum immunoglobulin G levels of calves at birth and after feeding at 0, 12, 24 or 36 h postpartum.

immunoglobulin G, peaking at 24 h postpartum (26.83 mg/ml) and remaining much higher throughout the entire trial. There was a treatment difference ($P < .001$) between the two groups following the 4 h sample.

These data are in agreement with the works of Johnstone and Oxender (1979) and Stott (1980) which suggested increased serum glucocorticoid concentrations did not interfere with the immunoglobulin absorptive process of the neonate. The colostrum-fed calves possessed a relatively high serum cortisol concentration throughout the duration of the trial, yet still absorbed approximately 27 mg/ml of IgG.

The results indicate both cortisol and thyroxine are high at 0 to 4 h postpartum; the act of feeding may stimulate a cortisol surge; although not significant, there is possibly a sex difference in the neoantal serum thyroxine levels; and the feeding of colostrum, rather than whole milk, has a drastic effect on serum immunoglobulin G levels in the newborn neonate.

Chapter V

JOURNAL ARTICLE

GLUCOCORTICOID CYTOSOLIC RECEPTORS IN SKELETAL MUSCLE OF THE BOVINE NEONATE

SUMMARY

A study was undertaken to investigate the quantitation, binding kinetics, and specificity of binding of glucocorticoid receptors in neonatal bovine skeletal muscle. Muscle samples (20-30 g) were surgically removed from the right semitendinosus at 36 h postpartum from 14 neonatal beef calves (male and female). Immediately (<15-20 s) following removal, each sample was placed in a plastic poly-bag and frozen in liquid nitrogen. Samples were later ground to a fine powder at -20 C and stored at -80 C. Twenty-five g of muscle were added to 1 volume [v/w] of buffer (5mM Tris-HCl; 1mM EDTA; .1mM dithioerythritol; 10% [v/v] glycerol; distilled H₂O to volume) and homogenized for periods of 5 s separated by 30 s intervals of cooling. The homogenate was centrifuged at 105,000 x g at 4 C for 60 min, and the supernatant (cytosol) was removed avoiding the lipid phase and stored at -20 C until analyzed. Receptor quantitation

assay was performed via [1,2,4,³H] dexamethasone at six concentrations either in the absence or presence of a 100-fold excess of unlabeled dexamethasone. Binding kinetics were ascertained via 5.0 mM of [1,2,4,³H] dexamethasone, either in the presence or absence of a 100-fold excess of unlabeled dexamethasone. Binding incubations were carried out in duplicate at 4 C for varying time periods until terminated by the addition of charcoal suspension. Ligand specificity was determined via a buffer solution concentrated at 9.1 mM [1,2,4,³H] dexamethasone and 100 ul of an ethanolic solution of each ligand competitor (cortisol, corticosterone, estradiol-17, beta, testosterone, triamcinolone and progesterone). Calculation of binding data from modified Scatchard plots was employed via an equilibrium binding data analysis program by way of an IBM Personal Computer. There were no binding differences between the colostrum and milk-fed calves' muscle samples. The average protein content of the cytosol fraction was 50.82 mg/ml. The binding component displayed a high apparent equilibrium dissociation constant for the binding of [³H] dexamethasone ($K_d = 2.34 \times 10^{-8}$). The apparent maximum number of binding sites (B_{max}) determined from Scatchard plots was approximately 37.61 fmol/mg of protein in the case of the dexamethasone receptor. The effect of time of incubation on [³H] dexamethasone binding

to muscle cytosol revealed total binding occurred rapidly and reached almost maximum equilibrium at 4 h (47.7%). Maximum binding appeared to be reached between 16 and 24 h (48.5 and 48.2 %, respectively). Competition assays indicated that all of the ligands tested had an affinity for the glucocorticoid receptor. The percent of specific binding for each was: dexamethasone (66+/-14), corticosterone (52+/-10), cortisol (58+/-13), estradiol-17, beta (37+/-7), progesterone (29+/-9), testosterone (10+/-3), and triamcinolone (41+/-11).

(Key Words: Skeletal Muscle, Dexamethasone, Glucocorticoid Receptors, Binding Kinetics, Ligand Specificity)

INTRODUCTION

Skeletal muscle represents approximately 50% of the bovine body mass and its protein content contributes approximately 20% to the weight of the tissue. By being the largest body reservoir of amino acids and proteins, and because it responds to various hormonal and nutritional stimuli, skeletal muscle is highly adaptive and can regulate the supply of nutrients to other body tissues. Of the several hormonal factors that may influence the mass of skeletal muscle, glucocorticoids are clearly of major importance, however the physiological and biochemical mechanisms involved in the response of muscle to glucocorticoids are ill-understood.

Glucocorticoids, like other classes of steroid hormones, must bind to cellular receptors in order to exert their effects. However, the structural requirements for binding to the glucocorticoid receptor seem to be less stringent than for other steroid receptors (Rousseau et al., 1972; Rousseau and Schmidt, 1977). Thus, the number of steroids which could bind to the glucocorticoid receptor (given high enough concentrations) and elicit biological effects is quite large (Koblinski et al., 1972; Mayer and Rosen, 1975). In addition, there are glucocorticoid antag-

onists which compete with the glucocorticoid hormone and potentially block its action (Chrousos et al., 1983; Rousseau et al., 1983). To date, there is no clear-cut evidence that the glucocorticoid receptor differs from tissue to tissue (Snochowski et al., 1980; Lentze et al., 1985), and it is in fact very similar in various species (Ballard et al., 1974; Middlebrook et al., 1975).

The laboratory technique most commonly used for the study of glucocorticoid binding is the measurement of the amount of tritium-labeled natural or synthetic hormones bound at equilibrium to a high-speed supernatant fraction of tissue. The classic work of Scatchard (1949) has enabled most investigators to adequately perform equilibrium studies whereby the total receptor site concentration may be obtained by graphic extrapolations. However, there are few current reports on the equilibrium properties of the glucocorticoid receptor population in skeletal muscle. Since this type of information is essential for an understanding of the influence of glucocorticoids, as well as other steroids on muscle growth, an investigation of the quantitation, binding kinetics and specificity of binding of glucocorticoid receptors in skeletal muscle of the bovine neonate has been undertaken. It is hopeful the results of this particular study will contribute to the clarification

of events in glucocorticoid action that lie between cytoplasmic binding and the final biochemical response.

MATERIALS AND METHODS

The determination of glucocorticoid cytosolic receptors in skeletal muscle was estimated by the modified methods of Snochowski et al. (1981).

Muscle Tissue

Muscle samples (20-30 g) were surgically removed from the right semitendinosus at 36 h postpartum from 14 neonatal beef calves (male and female). Immediately (<15-20 s) following removal each sample was placed in a poly-bag¹ and frozen in a liquid nitrogen tank (-196 C)². The samples were later transferred to a freezer³(-80 C) until time of assay.

Preparation of Muscle Cytosol Fraction

The frozen tissue samples (stored at -80 C) were ground to a powder with the use of a Bel-Art tissue mill⁴ at -20 C with the addition of dry ice to the grinding head to minimize the heat resulting from the friction of grinding. The samples were then returned to -80 C storage until homogenization.

Homogenization buffer was prepared to contain the fol-

¹Fisher Scientific, Raleigh, NC. 27604.

²Minnesota Valley Engineering model Apollo SX-35.

³Revco Ultra Low Blast Freezer.

⁴Bel-Art Products, Pequannok, NJ. 07444.

lowing: 5mM Tris-HCl⁵; 1mM EDTA⁵; .1mM dithioerythritol⁵; 10% [v/v] glycerol¹; and distilled H₂O to volume. The pH was adjusted to 7.4 with .5 N NaOH and the buffer was then stored in 2 liter plastic containers until used.

A 25 g sample of powdered muscle was added to 1 volume [v/w] of ice-cold buffer, homogenized thoroughly with a Tekmar Tissuemizer⁶ for periods of 5 s separated by 30 s intervals of cooling. The homogenate was centrifuged⁷ at 105,000 x g at 4 C for 60 min, and the supernatant (cytosol) was removed avoiding the lipid phase. The portions of cytosol were stored at -20 C for subsequent receptor analysis, binding kinetics, ligand specificity and protein determinations, respectively.

Receptor Quantitation

Aliquots of .2 ml of cytosol were placed in 12 x 75 mm borosilicate tubes¹. Also added to the tubes was .1 ml of buffer solution of [1,2,4,³H] dexamethasone⁸ at six concentrations (.2, .4, .8, 1.6, 3.2, and 6.4 nM) either in the absence or presence of a 100-fold excess of unlabeled

⁵Sigma Chemical, St. Louis, MO. 63178.

⁶Tekmar, Inc., Cincinnati, O. 45222.

⁷Beckman model L5-75B Ultracentrifuge.

⁸Amersham Corp., Arlington Heights, IL. 60005.

dexamethasone⁵. The incubations were carried out in duplicate at 4 C for 20 h until terminated by the addition of .4 ml of charcoal¹ suspension [.625% (w/v) of charcoal in homogenization buffer] to separate unbound and protein bound steroids. The suspension was mixed on a Vortex mixer¹ for 15 s, incubated at 4 C for 20 min, followed by centrifugation⁹ at 1500 x g for 5 min. Total amount of radioactivity (total count tubes) was determined from similar incubations, but in which the cytosol and the charcoal suspension was replaced by .6 ml of buffer. Following centrifugation, aliquots of .5 ml of the supernatant were combined with 4.5 ml of scintillation fluid¹ in 7 ml plastic vials¹ and radioactively counted via a liquid scintillation counter¹⁰ possessing a 65.3% counting efficiency for tritium.

Binding Kinetics

Aliquots of .2 ml of cytosol were added to two series of 12 x 75 mm borosilicate tubes. Also added to the tubes was .1 ml of buffer solution concentrated at 5.0 nM of [1,2,4,³H] dexamethasone, either in the presence or absence of a 100-fold excess of unlabeled dexamethasone. Incubations were carried out in duplicate at 4 C for varying

⁹Beckman model LS-1800.

¹⁰Beckman model J2-21.

time periods (0, 1, 2, 4, 8, 16, 20, and 24 h) until terminated by the addition of .4 ml of charcoal suspension [.625% (w/v) of charcoal in homogenization buffer]. The suspension was mixed on a Vortex mixer for 15 s, incubated at 4 C for 20 min, followed by centrifugation at 1500 x g for 5 min. Total amount of radioactivity (total count tubes) was determined from duplicate samples of each concentration of [³H] dexamethasone, but in which the cytosol and the charcoal suspension was replaced by buffer. Radioactivity was determined as previously outlined in the receptor quantitation section.

Ligand Specificity

Aliquots of .2 ml of cytosol were added to two series of 12 x 75 borosilicate tubes. Also added to the tubes was .1 ml of buffer solution concentrated at 9.1 mM [³H] dexamethasone and 100 ul of an ethanolic solution of each ligand competitor³ (cortisol, corticosterone, estradiol-17 beta, testosterone, triamcinolone, and progesterone). The incubations were carried out in duplicate at 4 C for 24 h. Termination of binding via the addition of a charcoal suspension and the radioactive counting was performed as described in the previous sections. The total binding in the absence of the competing ligands was determined from incu-

bations of .2 ml cytosol, .1 ml [^3H] dexamethasone suspension and .1 ml of ethanol¹.

Calculations

Calculation of binding data from modified Scatchard plots (Scatchard, 1949) was carried out via the 1984 version of the logit-log radioimmunoassay data processing computer program (Equilibrium Binding Data Analysis) written by McPherson (1982) and Ligand, a general purpose computer program designed for analysis of binding data developed by Munson and Rodbard (1980). The programs were obtained from the Biomedical Computing Technology Information Center, Vanderbilt Medical Center, Department of Radiology, Nashville, TN.

RESULTS AND DISCUSSION

Steroid binding to the cytosolic receptor in skeletal muscle is the pivotal step in the general model of steroid hormone action. Due to its importance several investigations have been performed to discern the mechanism of the binding reaction via the analization of its kinetic properties (Snochowski et al., 1981; Danhaive and Rousseau, 1986). On the basis of these observations, as well as others, we undertook a valid approach to demonstrate the quantitation, binding kinetics over time, and the binding specificity of the glucocorticoid receptor in neonatal bovine skeletal muscle (in vitro) using synthetic steroids as ligand competitors.

The binding reactions described herein were performed in vitro by incubating the 105,000 x g supernatant fraction (cytosol) of bovine skeletal muscle with [³H] dexamethasone and nonlabeled synthetic steroids at 4 C. Incubation at a low temperature minimizes possible bio-transformations of the hormone and eliminates changes in binding which could result from differences in cellular metabolism, cellular uptake, or binding by extracellular sites (Mayer et al., 1974). The synthetic steroids chosen for this study have advantages compared to their naturally occurring counter-

parts of not binding specifically to contaminating blood proteins (abundant in muscle) and not being susceptible to enzymatic attacks (Snochowski et al., 1980).

The quantitative binding parameters are given in table 9. The K_a (equilibrium association constant), K_d (equilibrium dissociation constant) and B_{max} (maximum receptor-site concentration) were calculated according to Scatchard (1949). The equilibrium dissociation constant ($K_d = 2.34 \times 10^{-8}$) for the formation of the dexamethasone-receptor complex in the bovine muscle cytosol was in general agreement with earlier published values obtained for cytosol from rat skeletal muscle (Mayer et al., 1974 [$K_d = 1.9 \times 10^{-8}$]; Mayer and Rosen, 1975 [$K_d = 1.9 \times 10^{-8}$]), however, our value was approximately one order of magnitude greater than the more recently published data (Dubois and Almon, 1984 [$K_d = 2.1 \times 10^{-9}$]; Danhaive and Rousseau, 1986 [$K_d = 1.79 \times 10^{-9}$]). Our data were also expressed in a Scatchard plot (figure 4) in order to estimate the number of hormone binding sites and the binding affinity. The plot of the ratio of bound to free hormone as a function of bound hormone yielded a straight line, indicating that only a single class of specific binding sites exists for dexamethasone (Scatchard, 1949). B_{max} (intercept of the Scatchard line with the x-axis) was determined

Table 9: GLUCOCORTICOID RECEPTOR ANALYSIS OF BOVINE SEMITENDINOSUS MUSCLE AT 36 H POSTPARTUM VIA [1,2,4,³H] DEXAMETHASONE*

Cytosol Protein (mg/ml)	K_a [mole]	K_d [mole]	B_{max} [mole]	Receptor Conc. (fmol/mg protein)
50.82	4.28×10^7	2.34×10^{-8}	2.02×10^{-8}	37.61

*Data from Scatchard analysis of equilibrium studies. Each value is the mean of 14 determinations.

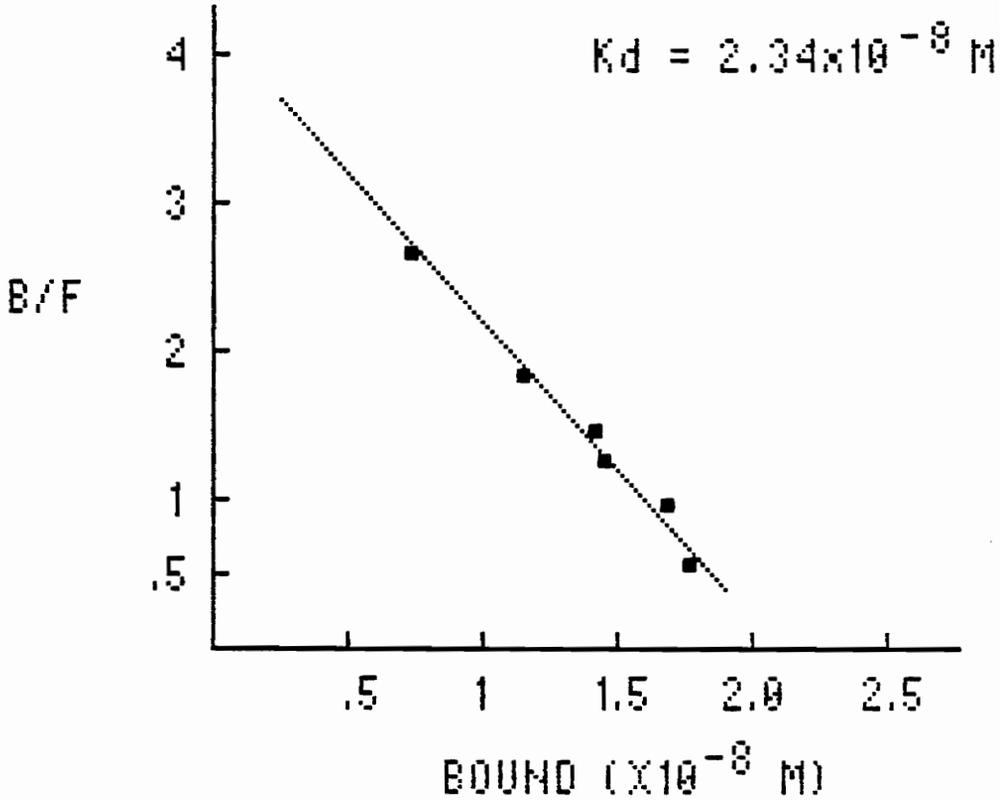


Figure 4. Scatchard plot of the binding of [1, 2, 4, ³H] dexamethasone in neonatal bovine semitendinosus muscle cytosol. ■ = mean of 14 determinations. K_d = apparent equilibrium dissociation constant.

to be 2.02×10^{-8} M, and expressed as binding sites per milligram of cytosolic protein yielding 37.61 fmol/mg. Our greater K_d and B_{max} values may be due to the greater cytosolic protein content in the muscle of the rapidly growing calf (50.82 mg/ml) compared to mature rats (~35 mg/ml), increased muscle turnover due to the physiological stage of growth (Dubois and Almon, 1984), increased intracellular turnover of glucocorticoid-sensitive binding proteins resulting from the traumatization of the birth process, or the systematic errors present in the charcoal assay procedure. There were no differences in apparent binding affinity observed in muscle cytosols from male ($K_a=4.21 \times 10^7$) and female ($K_a=4.30 \times 10^7$) calves, a finding consistent with the works of Dahlberg et al. (1981) and Dubois and Almon (1984). Also, as expected, there were no binding differences between the colostrum ($B_{max}= 2.0 \times 10^{-8}$) and milk-fed ($B_{max}= 2.04 \times 10^{-8}$) calves' muscle samples.

Figure 5 illustrates the effect of time of incubation on [3 H] dexamethasone binding to muscle cytosol. The total binding occurred rapidly and reached almost maximum equilibrium at 4 h (47.7%). Maximum binding appeared to be reached between 16 and 24 h (48.5 and 48.2%, respectively). These findings are in agreement with those of Snochowski et

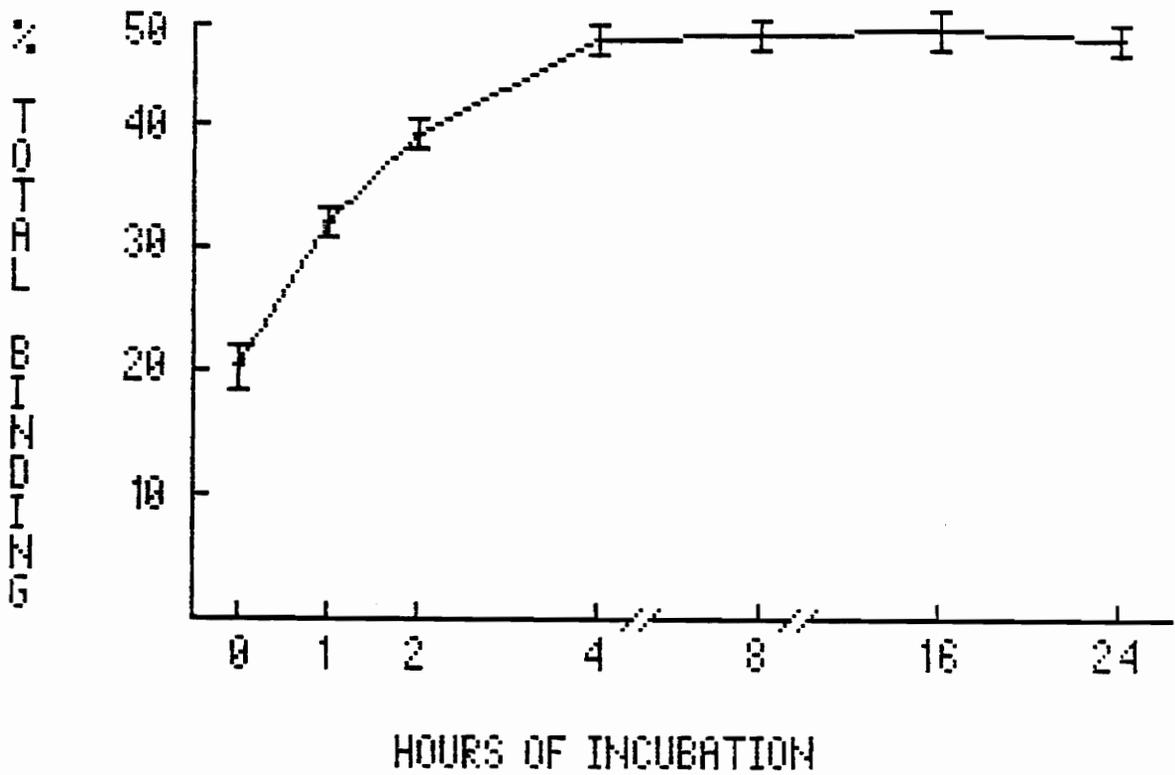


Figure 5. Binding kinetics (association and degradation) of the specific dexamethasone receptor complexes in neonatal bovine semitendinosus muscle cytosol. Points represent percent of total counts bound and are means \pm SD for duplicate tubes.

al. (1981).

The inhibition of hormone binding by a steroid reflects binding of the steroid to the hormone-binding site. Thus, the structural requirements for specific binding can usually be derived from determinations of the ability of various synthetic steroids to inhibit binding of a labeled glucocorticoid. Therefore, the ability of various steroids to compete for glucocorticoid-specific binding sites was also tested. The relative effectiveness of the different steroids to prevent the *in vitro* binding of [³H] dexamethasone was quite apparent (table 10). The order of relative binding specificity for the hormones tested in this study was as follows (in descending order): dexamethasone (66%), cortisol (58%), corticosterone (52%), triamcinolone (41%), 17-Beta estradiol (37%), progesterone (29%) and testosterone (10%).

As our data suggests, the four glucocorticoids tested were competitive with the [³H] dexamethasone binding sites in the skeletal muscle cytosol which is in relative agreement with the works of Snochowski et al. (1980), Snochowski et al. (1981) and Dubois and Almon (1984). We did acquire a slightly lower value for triamcinolone (41%) than expected, an occurrence we find difficult to explain biologically.

Our results are in accord with other workers (Dahlberg et al., 1981; Snochowski et al., 1981) which reveal skeletal

Table 10. EFFECT OF UNLABELED LIGANDS ON SPECIFIC BINDING OF [³H] DEXAMETHASONE IN BOVINE SKELETAL MUSCLE CYTOSOL*

Unlabeled Ligand	% Specific Binding
None	100
Dexamethasone	66 +/- 14
Corticosterone	52 +/- 10
Cortisol	58 +/- 13
Estradiol-17, Beta	37 +/- 7
Progesterone	29 +/- 9
Testosterone	10 +/- 3
Triamcinolone	41 +/- 11

*Cytosol from the semitendinosus muscle of bovine neonates at 36 h postpartum incubated with 9.1 nM of [1,2,4,³H] dexamethasone and a 100-fold excess of unlabeled ligand. Each value is the mean of 14 determinations +/- SD.

muscle to possess binding sites for 17-Beta estradiol. However, as shown by Dahlberg et al. (1981) there may be binding differences between anatomically separate muscles, an interesting point which has yet to be substantiated.

Progesterone exhibited some affinity for the dexamethasone receptor, but as pointed out by several texts it lacks substantial catabolic activity in muscle. As proposed earlier by Mayer and Rosen (1975), progesterone's antiglucocorticoid effects may be due in part to its binding to the receptor, thereby preventing the binding of active glucocorticoids to the same sites.

The binding specificity of testosterone (10%), though it may be relatively low, adds support to a number of studies which suggest there may be an antagonistic relationship between androgens and glucocorticoids in several tissues. For example, Singer et al. (1973) observed testosterone to be a potent competitor for [³H] cortisol binding to a glucocorticoid-binding protein in liver. Another valid explanation for the decreased specificity of testosterone may be that skeletal muscle contains about 40 times less androgen receptors than glucocorticoid receptors. It has been suggested that differentiated muscle cells contain only the glucocorticoid receptor, while the androgen receptors are restricted to satellite cells since the latter

contribute only about 2-5% of the nuclei in muscle (Snow, 1977; Danhaive and Rousseau, 1986). Thus, it has been hypothesized that androgen-dependent inhibition of glucocorticoid binding, either by direct competition for binding to receptor sites or by induced conformational changes in the glucocorticoid binding molecules, could result in a reduced response to glucocorticoids (Mayer and Rosen, 1975).

It is assumed the glucocorticoid-receptor complex, as a result of the rather specific binding of other steroid hormones to the skeletal muscle cytosol, is involved in the catabolic actions of these tested hormones on skeletal muscle. The existence of specific intracellular receptors suggests the interaction of steroids with muscle is of a "primary" type (like that observed in other target tissues), in which the hormones directly interact with the muscle tissue, and it is the binding which triggers the catabolic biological response (Mayer et al., 1974). Thus, on the basis of the findings reported in our study, we suggest the dexamethasone-binding protein in neonatal bovine skeletal muscle is a specific receptor for biologically active glucocorticoids, 17-Beta estradiol, progesterone and testosterone, and subsequently, these tested steroids could possibly be potential anabolic agents when administered to cattle with free receptor sites present to mediate the hormone action.

CHAPTER VI

SUMMARY

A study was conducted to determine the effect of colostrum or milk fed to newborn calves on serum cortisol, thyroxine and immunoglobulin G. Twenty-four calves (12 males and 12 females) were obtained immediately postpartum and randomly assigned to one of two treatments (pooled colostrum or whole milk) after being blocked by breed and sex. Blood sampling was performed at 0 time (prior to first feeding; <1 h postpartum), 1, 2, 3, 4, 6 and 12 h postfeeding with this regime being followed for a 48 h period (4 feedings). The average initial cortisol concentration was highest at birth for both treatments. Cortisol levels between treatments were different ($P < .05$) at 2, 3, 12, 14, 18, 24, 37 and 48 h postpartum. There was also an apparent peak at each feeding. There was no sex difference observed in the average serum cortisol concentrations. Within 3 h postpartum, serum thyroxine concentrations reached a peak ($P < .05$) for both treatment groups. There was a sex difference in serum thyroxine concentration as the female calves exhibited the highest average concentration over the entire trial. Both treatment groups were born with similar immunoglobulin G levels. However, at approximately 4 h post-

partum, the colostrum-fed calves acquired increases ($P < .001$) in serum immunoglobulin G, peaking at 24 h postpartum and remaining higher throughout the entire trial. The results herein indicate both cortisol and thyroxine are elevated at 0 to 4 h postpartum; the act of feeding may stimulate a cortisol surge; there was a noted difference in serum thyroxine between male and female calves; and the feeding of colostrum, rather than whole milk, has a drastic effect on serum immunoglobulin G levels in the newborn neonate.

Another study was undertaken to investigate the quantitation, binding kinetics, and specificity of binding of glucocorticoid receptors in bovine skeletal muscle. Muscle samples (20-30g) were surgically removed from the right semitendinosus at 36 h postpartum from 14 neonatal beef calves (male and female). The average protein content of the muscle cytosolic fraction was 50.82 mg/ml. The apparent equilibrium dissociation constant (K_d) for the dexamethasone receptor was 2.34×10^{-8} . The apparent maximum number of binding sites (B_{max}) determined from Scatchard plots was approximately 37.61 fmol/mg. The effect of time of incubation on [3H] dexamethasone binding to muscle cytosol revealed total binding occurred rapidly and reached maximum equilibrium between 16 and 24 h.

Competition assays indicated that all of the tested glucocorticoids, 17-Beta estradiol, testosterone and progesterone had an affinity for the glucocorticoid (dexamethasone) receptor.

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APPENDIX A

Experimental Rations

Approximately 96 liters of colostrum from first and second milkings postpartum (65 mg/ml IgG) were acquired from a nearby commercial dairy¹ and the VPI&SU dairy. Also, approximately 100 liters of whole milk were acquired from the holding tank of the VPI&SU dairy. Both the colostrum and whole milk were separately pooled (uniform concentration attained), deposited in 4 liter plastic containers, and frozen at -20 C.

Prior to birth, each calf was randomly assigned to a ration, colostrum or whole milk. Immediately after birth, every calf was removed from its dam and fed the assigned ration at birth (< 1 h postpartum), 12, 24 and 36 h postpartum. All calves were fed 10% of their body weight (BW) at birth and 5% of their BW at 12, 24 and 36 h postpartum. Both rations were warmed to room temperature prior to feeding. Force-feeding was implemented via a calf esophageal tube feeder² to assure uniform ingestion among all experimental calves.

¹ Wall Brothers Dairy, Inc., Blacksburg, VA. 24060.

² Nasco, Fort Atkinson, WI. 53538.

Appendix B

Blood Sampling

Collection of blood samples (~10 ml at each bleeding) was performed according to the following schedule:

0 time (prior to first feeding; <1 h postpartum)
Feed
1 h post-feeding
2 h "
3 h "
4 h "
6 h "
12 h (prior to second feeding)
Feed
1 h post-feeding
2 h "
3 h "
4 h "
6 h "
12 h (prior to third feeding)
Feed
↓
(same 24 h bleeding pattern as previous 24 h)
↓
48 h postpartum

Samples taken at 0, 1 and 2 h postpartum were collected via jugular vacutainers¹. Following the 2 h collection, all calves were fitted with indwelling jugular catheters for the remainder of the collection period. The catheterization procedure consisted of shearing and disinfecting² the neck

¹Fisher Scientific, Raleigh, NC. 27064.

²Betadine, Purdue Frederick Co., Norwalk, CN. 06068.

and inserting a sterilized 12-gauge needle into the jugular vein. Using a total of 25 cm of Tygon microbore tubing¹, approximately 12 cm was introduced through the needle into the jugular vein with the remainder (~13 cm) attached to an adapter and stopper³. The catheter was sutured to the skin of the neck (~6 cm cranio-laterally from point of entry) and was achieved by doubling a piece of surgical cloth tape⁴ around the adapter making certain the adapter was located in the center. The tape was then sutured to the skin and .2% nitrofurazone⁵ was applied liberally. The necks of the calves were wrapped with an elastic, adhesive bandage⁶ and surgical cloth tape. Maintenance of the catheters between blood samplings involved flushing with approximately 3 ml of 4% sodium citrate⁷ after each blood withdrawal.

The blood samples were kept at 4 C, permitted to clot and centrifuged⁸ for 15 min at 3000 x g. The serum was saved and stored at -20 C until analyzed.

³Becton Dickerson & Co., Rutherford, NJ. 07070.

⁴Parke-Davis & Co., Detroit, MI. 48215.

⁵Clay-Parks Labs, Bronx, NY. 10469.

⁶Elastikon, Johnson and Johnson, Inc.,
New Brunswick, NJ. 08903.

⁷40 g Sodium Citrate (Fisher Scientific)/L double
distilled deionized H₂O; Autoclaved.

⁸Beckman model J2-21.

Appendix C

Serum Cortisol Quantitation

Serum cortisol was quantitatively measured via Amerlex Cortisol RIA kits¹. The standards¹ were set up in duplicate placing 25 ul aliquots into 12 x 75 mm borosilicate tubes² 1 through 10. Serum samples (25 ul) were pipetted in duplicate into the tubes using an Eppendorf digital pipetter². Using a Hamilton repeating syringe², 100 ul aliquots of cortisol ¹²⁵I derivative¹ and cortisol antibody suspension¹ were added to the tubes. All samples were then mixed for 5 s on a Vortex mixer², covered with plastic film and incubated in a water bath (37 C) for 1.0 h. Following incubation the samples were centrifuged³ at room temperature for 15 min at 1500 x g.

After centrifugation the tubes were placed in test tube racks and the supernatant was decanted by inverting the tubes up-side down on absorbant paper for 15 min. After decanting, the racks were carefully reinverted and the tubes placed in a gamma counter⁴ and radioactively counted.

¹Amersham Corp., Arlington Heights, IL. 60005.

²Fisher Scientific, Raleigh, NC. 27604.

³Beckman model J2-21.

⁴Beckman model 5500.

In addition to the steps previously described, a 100 ul aliquot of the cortisol ^{125}I derivative was placed into two tubes (total count tubes) and were set aside until required for radioactive counting.

The calculations were automatically computed by way of a stored computerized program within the Beckman gamma counter. The computer utilized a log-logit plot analysis to determine the concentrations of the unknown samples (i.e. % Bound = $B/B_0 \times 100$; where, B = sample counts, B_0 = average zero standard counts). A pooled bovine serum sample was used to calculate intra- and interassay coefficients of variation, which were found to be 9.5% and 2.6% respectively.

Appendix D

Serum Thyroxine Quantitation

Total serum thyroxine (T4) was quantitatively measured via Amerlex T-4 RIA kits¹. The standards¹ were set up in duplicate placing 10 ul aliquots into 12 x 75 mm borosilicate tubes² 1 through 10. Serum samples (10ul) were pipetted in duplicate into the tubes using an Eppendorf digital pipetter². Using a Hamilton repeating syringe², 200 ul aliquots of thyroxine ¹²⁵I derivative¹ and thyroxine antibody suspension¹ were added to the tubes. All samples were then mixed for 5 s on a Vortex mixer², covered with plastic film and incubated in a water bath (37 C) for 1.5 h. Following incubation the samples were centrifuged³ at room temperature for 15 min at 1500 x g.

After centrifugation the tubes were placed in test tube racks and the supernatant was decanted by inverting the tubes up-side down on absorbant paper for 15 min. After decanting, the racks were carefully reinverted and the tubes placed in a gamma counter⁴ and radioactively counted.

¹Amersham Corp., Arlington Heights, IL. 60005.

²Fisher Scientific, Raleigh, NC. 27604.

³Beckman model J2-21.

⁴Beckman model Gamma 5500.

In addition to the steps previously described, a 200 ul aliquot of the thyroxine ^{125}I derivative was pipetted into two tubes (total count tubes) and were set aside until required for radioactive counting.

The calculations were automatically computed by way of a stored computerized program within the Beckman gamma counter. The computer utilized a log-logit plot analysis to determine the concentrations of the unknown samples (i.e. % Bound = $B/B_0 \times 100$; where, B = sample counts, B_0 = average zero standard counts). A pooled bovine serum sample was used to calculate intra- and interassay coefficients of variation, which were found to be 7.4% and 2.1%, respectively.

Appendix E

Serum IgG Determination

Preparation of Buffered Gel Solution

Seven grams of agarose powder¹ were added to 500 ml of phosphate buffered saline (PBS)² in a 1000 ml Pyrex beaker³. The agarose solution was placed in a boiling water bath in order to completely dissolve the agarose powder and to obtain a homogeneous solution. However, care was taken to avoid boiling of the agarose solution. Continual stirring via a magnetic stirrer was maintained to prevent the formation of clumps. The dissolved agarose solution (1.5%) was distributed into several 40 ml test tubes³, each tube two-thirds filled. After gelling at room temperature, the tubes were sealed with pieces of parafilm³ and stored at 4 C.

Preparation of Standards

Standard serum was prepared by adding 5 mg of bovine IgG⁴ to 1 ml of PBS. This stock solution was then serially

¹Type III; Sigma Chemical, St. Louis, MO. 63178.

².25 M (7.40 g) NaCl, .02 M (1.80 g) NaH₂PO₄, .372 g EDTA, 0.5 g NaN₃, filled to volume (1L) with distilled H₂O; final conc. 0.01 M, pH 7.4.

³Fisher Scientific, Raleigh, NC. 27604.

⁴Miles Scientific, Naperville, IL. 60566.

diluted to obtain concentrations of 5.0, 4.0, 3.0, 2.0, 1.0, and .5 mg/ml IgG.

Preparation of Gel Plates

Two test tubes containing the agarose gel (~ 60 ml) was refluxed in a boiling water bath until the gel became totally liquified. The liquified agarose was then placed in a 56 C water bath until it had cooled to this temperature. An aliquot of this solution (40 ml) was pipetted into a 100 ml Pyrex beaker also suspended in a water bath (56 C). Added to the 40 ml of liquid agarose was 38 ml of PBS and 2.0 ml of purified goat anti-bovine IgG (H + L)⁴. Extreme care was taken to stabilize the temperature of the agar-antiserum solution. Onto separate 3 1/4 by 4 inch clean glass plates⁵ atop a leveling table⁵ was pipetted 7 ml of the agar-antiserum solution. It was made certain that each plate was covered evenly. The agarose was allowed to solidify at room temperature.

Inoculation of Gel Plates

Wells (2.4 mm in diameter and 12 mm apart) were cut into the agarose gel utilizing a tubular cutter and a plastic template⁵. To remove the small gel cylinders after the cutting, suction by a water jet pump was used in con-

⁵Gelman Sciences, Ann Arbor, MI. 48106.

nection with a Pasteur pipette³ whose tip diameter was slightly less than that of the well area. To these wells was placed 5 ul of the serum samples diluted 1:20 with PBS, or standard solutions via an Eppendorf digital pipetter³. The wells were filled to the top without allowing the sample to overflow onto the agarose gel. The four corner holes in each plate were not used because of possible distortion of precipitin rings. The diffusion plates were placed into work-shop made plexiglass boxes containing moisture pads with closely fitting lids. The plates were incubated for 48 h at 4 C. The boxes were kept humid to avoid evaporation and drying of the antibody-gel layers. Opaque antigen-antibody precipitate rings formed around the antigen wells and the diameter of the rings was measured via a calibrated ruler⁵. The diameter of the precipitate rings was easiest to read when they were viewed against a black background and illuminated by a small fluorescent desk lamp, thus producing a dark-field effect.

Linear regression analysis was used to establish a calibration curve by plotting the concentrations of the standards against the precipitin ring diameters and, likewise, the concentration in the unknown samples were determined from the calibration curve via linear regression.

Appendix F

Glucocorticoid Receptor Assay

The determination of glucocorticoid cytosolic receptors in skeletal muscle was estimated by the modified methods of Snochowski et al. (1981).

Muscle Tissue

Muscle samples (20-30 g) were surgically removed from the right semitendinosus at 36 h postpartum from 14 neonatal beef calves (male and female). Immediately (<15-20 s) following removal the samples were placed in a poly-bag¹ and frozen in a liquid nitrogen tank (-196 C)². The samples were later transferred to a freezer³(-80 C) until time of assay.

Preparation of Muscle Cytosol Fraction

The frozen tissue samples (stored at -80 C) were ground to a powder with the use of a Bel-Art tissue mill⁴ at -20 C with the addition of dry ice to the grinding head to minimize the heat resulting from the friction of grinding. The samples were then returned to -80 C storage until homogenization.

¹Fisher Scientific, Raleigh, NC. 27604.

²Minnesota Valley Engineering model Apollo SX-35.

³Revco Ultra Low Blast Freezer.

⁴Bel-Art Products, Pequannok, NJ. 07444.

Homogenization buffer was prepared to contain the following:

- 1) 5 mM (.7880 g/l) Tris-HCl⁵
- 2) 1 mM (.2922 g/l) EDTA⁵
- 3) .1 mM (.0154 g/l) dithioerythritol⁵
- 4) 10 % [v/v] (100 ml/l) glycerol¹
- 5) distilled H₂O to volume

The pH was adjusted to 7.4 with .5 N NaOH and stored in 2 L plastic containers until used.

A 25 g sample of powdered muscle was added to 1 volume [v/w] of ice-cold buffer, homogenized thoroughly with a Tekmar Tissuemizer⁶ for periods of 5 s separated by 30 s intervals of cooling. The homogenate was centrifuged⁷ at 105,000 x g at 4 C for 60 min, and the supernatant (cytosol) was removed avoiding the lipid phase. The portions of cytosol were stored at -20 C for subsequent receptor quantitation, binding kinetics, ligand specificity and protein determinations, respectively.

Receptor Quantitation

Aliquots of .2 ml of cytosol were placed in 12 x 75 mm borosilicate tubes¹. Also added to the tubes was .1 ml of buffer solution of [1,2,4-³H] dexamethasone⁸ at six

⁵Sigma Chemical, St. Louis, MO. 63178.

⁶Tekmar, Inc., Cincinnati, O. 45222.

⁷Beckman model L5-75B Ultracentrifuge.

⁸Amersham Corp., Arlington Heights, IL. 60005.

concentrations - .2 to 6.4 nM, either in the absence or presence of a 100-fold excess of unlabeled dexamethasone⁵. The incubations were carried out in duplicate at 4 C for 20 h until terminated by the addition of .4 ml of charcoal¹ suspension [.625% (w/v) of charcoal in homogenization buffer] to separate unbound and protein bound steroids. The suspension was mixed on a Vortex mixer¹ for 15 s, incubated at 4 C for 20 min, followed by centrifugation⁹ at 1500 x g for 5 min. Total amount of radioactivity (total count tubes) was determined from similar incubations, but in which the cytosol and the charcoal suspension was replaced by .6 ml of buffer. Following centrifugation, aliquots of .5 ml of the supernatant was combined with 4.5 ml of scintillation fluid¹ in 7 ml plastic vials¹ and radioactively counted via a liquid scintillation counter¹⁰ possessing a 65.3% counting efficiency for tritium.

Binding Kinetics

Aliquots of .2 ml of cytosol were added to two series of 12 x 75 mm borosilicate tubes. Also added to the tubes was .1 ml of buffer solution at 5.0 nM concentration of [1,2,4,³H] dexamethasone, either in the presence

⁹Beckman model LS-1800.

¹⁰Beckman model J2-21.

or absence of a 100-fold excess of unlabeled dexamethasone. Incubations were carried out in duplicate at 4 C for varying time periods (0, 1, 2, 4, 8, 16, 20, and 24 h) until terminated by the addition of .4 ml of charcoal suspension [.625% (w/v) of charcoal in homogenization buffer]. The suspension was mixed on a Vortex mixer for 15 s, incubated at 4 C for 20 min, followed by centrifugation at 1500 x g for 5 min. Total amount of radioactivity (total count tubes) was determined from duplicate samples of each concentration of [³H] dexamethasone, but in which the cytosol and the charcoal suspension was replaced by buffer. Radioactivity was determined as previously outlined in the receptor quantitation section.

Ligand Specificity

Aliquots of .2 ml of cytosol were added to two series of 12 x 75 borosilicate tubes. Also added to the tubes was .1 ml of buffer solution concentrated at 9.1 mM [³H] dexamethasone and 100 ul of an ethanolic solution of each ligand competitor³ (cortisol, corticosterone, estradiol-17 beta, testosterone, triamcinolone, and progesterone). The incubations were carried out in duplicate at 4 C for 24 h. Termination of binding via the addition of a charcoal suspension and the radioactive counting was performed as

described in the previous sections. The total binding in the absence of the competing ligands was determined from incubations of .2 ml cytosol, .1 ml [^3H] dexamethasone suspension and .1 ml of ethanol¹.

Calculations

Calculation of binding data from modified Scatchard plots (Scatchard, 1949) was carried out via the 1984 version of the logit-log radioimmunoassay data processing computer program (Equilibrium Binding Data Analysis) written by McPherson (1982) and Ligand, a general purpose computer program designed for analysis of binding data developed by Munson and Rodbard (1980). The programs were obtained from the Biomedical Computing Technology Information Center, Vanderbilt Medical Center, Department of Radiology, Nashville, TN.

Appendix G

Protein Determination

The total protein content of the cytosol was determined by the Bio-Rad microassay, a dye binding method by Bradford (1976). The assay involved the binding of Coomassie Brilliant Blue G-250¹ to protein to form a protein-dye complex.

The protein standards were prepared in triplicate using bovine serum albumin¹ solution containing 10 to 100 ug of protein. The protein reagent was formed by dissolving 50 mg of the Coomassie dye in 25 ml of 95% ethanol³. Added to this solution was 50 ml of 85% phosphoric acid³ (w/v) and the final solution was filled to volume (500 ml) with distilled H₂O, resulting in 0.01% (w/v) of Coomassie dye, 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid. Vigorous stirring was employed via a magnetic stirrer throughout the combining of the reagents.

An aliquot of .1 ml of cytosol was pipetted into 12 x 75 mm borosilicate tubes³ (in triplicate). Five ml of the protein reagent was added to the tubes and mixed on a Vortex mixer² for 15 s. Absorbance was measured for 10 min.

¹Sigma Chemical, St. Louis, MO. 63178.

²Fisher Scientific, Raleigh, NC. 27064.

using a spectrophotometer³ at a wavelength of 595. The spectrophotometer was zeroed against blank solutions containing .1 ml of distilled H₂O and 5 ml of protein reagent.

Calculation of the protein content was estimated by way of linear regression using an IBM Personal Computer. The mean values of each cytosol sample were computed using a dilution factor of 10 and a recovery rate of 1. The correlation coefficient was calculated at .992 .

³Perkin-Elmer model Lambda 3B UV/VIS.

Appendix H: Treatment, Date of Birth, Sex, Birth Weight and Breed of Experimental Calves

Calf #	TRT ¹	Birth Date	Sex ²	Birth Wt. (kg)	Breed ³
9	M	2/24	M	37.2	Angus
10	C	2/26	F	38.1	Angus
15	C	3/1	M	35.4	Angus
16	M	3/3	F	34.0	Angus
27	C	3/23	F	38.6	Angus
28	C	3/23	M	39.5	Angus
32	M	4/2	F	35.0	Angus
35	M	4/17	F	39.0	Angus
38	M	4/26	F	35.4	Simm. x
62	M	2/29	M	39.5	Hereford
68	C	3/23	F	34.5	Hereford
69	C	3/23	M	31.8	Hereford
70	C	3/25	M	49.0	Hereford
71	M	3/26	M	38.6	Hereford
85	C	4/6	F	37.7	Simm. x
86	C	2/18	M	48.5	Simm. x
89	M	3/20	F	41.0	Simm. x
90	C	2/24	M	42.6	Angus x
91	M	2/24	M	41.0	Simm. x
92	C	2/24	F	36.3	Simm. x
94	M	2/27	M	30.8	Simm. x
96	M	2/29	F	34.0	Simm. x
98	M	3/1	M	39.0	Simm. x
99	C	3/1	F	33.1	Simm. x

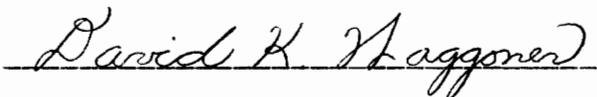
¹ TRT = Treatment; C = Colostrum, M = Whole Milk

² M = Male F = Female

³ Angus, Hereford, or Simmental Crossbred

VITA

David Kent Waggoner was born May 2, 1960 in Lubbock, Texas to Kelley D. and Bene J. Waggoner. He has three brothers; Dan, Lance, and Wade. He grew up in Athens, Texas and graduated "with honors" from Athens High School in May, 1978. In 1983 he graduated from Texas Tech University, "Cum Laude", receiving three B.S. degrees; Animal Business, Animal Production, and Animal Science. During his tenure at Texas Tech he was a student assistant within the Department of Animal Science, a member of numerous clubs and organizations, and a member of two outstanding collegiate livestock and horse judging teams. He initiated his graduate studies in September, 1983, at Virginia Polytechnic Institute and State University in Animal Science. While at Virginia Tech he assisted with the livestock judging program and served as official judge at several livestock shows. Prior to the completion of his Master's thesis, he served one year as the Agricultural Legislative Assistant for U.S. Congressman Byron L. Dorgan in Washington, D.C., a rare opportunity which was truly educational and thoroughly enjoyed.



David Kent Waggoner

COLOSTRUM FEEDING AND ITS EFFECTS ON SERUM CORTISOL,
THYROXINE, IMMUNOGLOBULIN G AND CYTOSOLIC GLUCO-
CORTICOID RECEPTORS IN SKELETAL MUSCLE
IN THE BOVINE NEONATE

by

David Kent Waggoner

Committee Chairman: F.D. McCarthy
Animal Science

(ABSTRACT)

The effect of feeding colostrum or milk to newborn calves on serum cortisol, thyroxine and immunoglobulin G was investigated. Twenty-four calves (12 males and 12 females) were obtained immediately postpartum and randomly assigned to one of two rations after being blocked by breed and sex. Both rations were force-fed at birth, 12, 24 and 36 h postpartum. Blood sampling was performed at 0 time, 1, 2, 3, 4, 6 and 12 h postfeeding with this regime followed for a 48 h period (4 feedings). The average serum cortisol concentration was highest at birth, 221.9 and 245.6 ng/ml for colostrum and milk-fed calves, respectively. Cortisol levels between treatments were different ($P < .05$) at 2, 3, 12, 14, 18, 24, 37 and 48 h postpartum. The sex of the calf did not affect the mean cortisol concentrations. No treatment difference was observed for serum thyroxine. A sex difference was observed with the female calves exhibiting higher average thyroxine concentrations over the entire trial. A reduction

in thyroxine concentration occurred with time ($P < .001$) as mean concentrations peaked at 4 h postpartum (22.1 ug/dl) and declined to 10.6 ug/dl by 48 h postpartum. Both treatment groups were born with similar serum immunoglobulin G levels (~0.7 mg/ml). However, at approximately 4 h postpartum, the colostrum-fed calves acquired an increase ($P < .001$) in serum immunoglobulin G, peaking at 24 h postpartum (26.83 mg/ml) and remaining much higher throughout the entire trial. There was a treatment difference ($P < .001$) between the two groups following the 4 h sample.

Muscle samples (20-30g) were surgically removed from the right semitendinosus at 36 h postpartum from 14 neonatal beef calves (male and female), homogenized, and centrifuged at 105,000 x g at 4 C for 60 min. The supernatant (cytosol) was harvested and receptor quantitation, binding kinetics and ligand specificity assays were performed via [1,2,4,³H] dexamethasone. There were no binding differences between the colostrum and milk-fed calves' muscle samples. The average protein content of the muscle cytosol fraction was 50.82 mg/ml. The binding component displayed a high apparent equilibrium dissociation constant for the binding of [³H] dexamethasone ($K_d = 2.34 \times 10^{-8}$). The apparent maximum number of binding sites determined from Scatchard plots was approximately 37.61 fmol/mg of protein in the case of the dexamethasone receptor. Maximum binding appeared to be reached between 16 and 24 h (48.5 and 48.2 %, respectively).

Competition assays indicated all of the ligands tested had an affinity for the glucocorticoid receptor. The percent of specific binding for each was: dexamethasone (66+/-14), corticosterone (52+/-10), cortisol (58+/-13), estradiol-17, beta (37+/-7), progesterone (29+/-9), testosterone (10+/-3), and triamcinolone (41+/-11).