INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

University
Microfilms
International
300 N. Zeeb Road
Ann Arbor, MI 48106

Schlagheck, Thomas Gerard

PHYSIOLOGICAL EFFECTS OF THE COLOSTRAL PEPTIDE, COLOSTROKININ, AND INANITION ON IMMUNOGLOBULIN ABSORPTION AND ADRENAL/THYROID RESPONSE IN THE BOVINE NEONATE

The University of Arizona PH.D. 1983

PHYSIOLOGICAL EFFECTS OF THE COLOSTRAL PEPTIDE, COLOSTROKININ, AND INANITION ON IMMUNOGLOBULIN ABSORPTION AND ADRENAL/THYROID RESPONSE IN THE BOVINE NEONATE

by

Thomas Gerard Schlagheck

A Dissertation Submitted to the Faculty of the

COMMITTEE OF ANIMAL PHYSIOLOGY (GRADUATE)

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 8 3

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Thomas Gerard Schlagheck entitled Physiological Effects of the Colostral Peptide, Colostrokinin, and Inanition on Immunoglobulin Absorption and Adrenal/Thyroid Response in the Bovine Neonate.

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

[signature]	March 2, 83 Date
[signature]	2 MARCH 1983 Date
[signature]	2 March 1983 Date
[signature]	2 March 1983 Date
[signature]	2 March 1983 Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

[signature] Dissertation Director

July 19, 1983 Date

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgement the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Thomas G. Schlagheck

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to his wife, Grace, for her undying support and patience throughout the duration of this graduate program. He would also like to thank her for the flawless preparation of all the illustrations.

The author would like to express his gratitude to Dr. G. H. Stott for his help and guidance throughout the course of this study and the preparation of this dissertation. Gratitude is also expressed to Dr. R. Chiasson, Dr. O. Koldovsky, Dr. W. Calder, and Dr. D. Vleck for serving as committee members and for their invaluable aid in writing the dissertation.

A special thanks is extended to the Animal Physiology graduate students for their assistance in collection of the data.

Appreciation is expressed to my parents, Walter and Louise Schlagheck, for their thoughtfulness, support, and encouragement during my course of study. Thanks is also extended to my wife's parents for their support. Finally, I would like to dedicate this dissertation to my son, Chris, who was born during my graduate studies at Arizona and who added, in his own way, something special to the entire endeavor.

TABLE OF CONTENTS

TABLE OF CONTENTS - Continued

LIST OF TABLES

LIST OF TABLES - Continued

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS - Continued

ABSTRACT

Sixty-two newborn Holstein-Friesian calves were used to study the role of colostrokinin, serum cortisol, and serum thyroxine in the absorption of maternal immunoglobulin. Calves were removed from their dams prior to suckling and assigned one of four rations: colostrum, whole milk, milk plus colostral immunoglobulin, and milk plus immunoglobulin plus colostrokinin. Calves were fed their assigned ration either at birth or after twelve hours inanition. All calves were fed pooled colostrum at 24 hours postpartum. Blood samples were collected at seventeen times during the first 32 hours postpartum.

Calves were born with high cortisol concentrations (88 ng/ml) which decreased ($P<.05$) within two hours postfeeding. Serum cortisol levels increased ($P<.05$) between two and three hours after calves ingested a colostral source of immunoglobulin. Time of initial feeding had no effect on the cortisol surge. No such increase was observed in neonates consuming an immunoglobulin-free milk ration. These results demonstrate that the immunoglobulin fraction of colostrum is responsible for initiating an increase in cortisol secretion by the adrenal cortex.

Within four hours postpartum, serum thyroxine concentrations increased ($P<.05$) at least 50% in all treatment groups regardless of whether the calves were fed or fasted. After peaking at 18 µg/dL, the serum thyroxine concentrations fell gradually throughout the duration of the collection period.

Colostrokinin exhibited a biphasic effect on serum immunoglobulin concentrations which was dependent on the initial time of feeding. Calves exposed to colostrokinin in 0 hour feedings had serum immunoglobulin G concentrations significantly higher ($P<.05$) after 16 hours postpartum than animals not fed colostrokinin. Fasted calves, exposed to colostrokinin at 12 hours postpartum, had no increase in serum immunoglobulin G concentrations following a colostrum feeding at 24 hours postpartum. Fasted calves fed a ration not containing colostrokinin exhibited a two-fold increase in serum immunoglobulin G concentrations after the 24 hour colostrum feeding. Colostrokinin did not have an immediate effect on serum immunoglobulin G concentrations, but required an approximate twelve hour period to manifest its regulatory function. The presence or absence of colostrokinin in the experimental rations did not have any effect on the cortisol or thyroxine profiles. The variable serum immunoglobulin G profiles suggest that colostrokinin is involved in the acquisition of passive immunity by the calf, but colostrokinin may have more than one physiological role.

CHAPTER 1

REVIEW OF LITERATURE

The calf is born with little or no serum antibody due to an immature immunological system and the failure of immunoglobulin (Ig) to be transferred from maternal serum to the fetus. To compensate, the neonate acquires passive immunological protection by consuming maternal colostrum within the first twenty-four hours of life. During this time, the intestinal epithelium of the calf is able to absorb colostral immunoglobulin intact. Recent studies suggest that the mechanisms by which the gut absorbs these relatively large protein molecules for a limited period of time are stringently controlled. The endogenous endocrine system of the neonate, certain humoral substances found in colostrum, and the dietary regime have been implicated as regulatory factors. A more comprehensive understanding of the mechanisms controlling the acquisition of passive immunological protection would be beneficial to the producer of animals born hypogammaglobulinanemic. High rates of postnatal morbidity and mortality may be reduced by application of future findings to managerial practices.

Glucocorticoid Levels in Newborn Calves

The prevalence of a high serum glucocorticoid concentration, 100-140 ng/ml, in calves at birth has been reported by numerous investigators (Khan, Dickson, and Meyers, 1970; Hudson et al., 1976; Johnstone and Oxender, 1976; Stott, 1980; Nightengale and Stott, 1981). However,

there are a limited number of studies which present a thorough profile of cortisol, the major bovine serum glucocorticoid, during the first two days postpartum.

The first reported profile of cortisol concentrations in neonatal calves was presented by Khan et al. (1970). Using nine calves kept at 16 C, high cortisol levels at birth (100 ng/ml) were recorded followed by a steady decline during the sampling period. Calves were bled at 0 and 4 hours postpartum with subsequent samples taken every 8 hours until the third day. Johnstone and Oxender (1979) measured glucocorticoid concentrations in calves during the first two days postpartum. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 6, 12, 18, 24, 30, 36, 42, and 48 hours postpartum. All calves were fed one liter of pooled colostrum within the first two hours postpartum. Calves were born with a high glucocorticoid level (140 ng/ml) which was followed by a rapid decrease in serum concentrations over the next three hours. Between 3 and 18 hours postpartum the slope of the decline decreased, after which no changes were recorded through 48 hours postpartum. Stott (1980) bled newborn calves every four hours from birth to 40 hours postpartum, and fed one liter of colostrum at 4, 16, and 28 hours postpartum. Serum cortisol concentrations steadily decreased over the first eight hours postpartum, and then stabilized and remained constant through the 40 hour sampling time. At no time in the three previous studies were the glucocorticoid concentrations observed to increase.

Nightengale and Stott (1981) were the first to report a rise in serum cortisol during the initial 48 hours postpartum. The increase was

elicited between 12 and 24 hours postpartum in calves subjected to delays in feeding of at least twelve hours. Nonfasted calves exhibited gradually declining levels through 48 hours postpartum; no cortisol increase was recorded in these animals.

During the initial 24-48 hours postpartum, the newborn calf undergoes a barrage of stressful factors, many of which would be expected to increase pituitary-adrenal activity (Stott, 1980). Rapid fluctuations in serum cortisol concentrations reflect the adrenal response to a variety of stimuli including feeding (Willett and Erb, 1972). To monitor rapid changes of serum cortisol in the neonate, a more frequent bleeding schedule than those previously reported was incorporated into the experimental design of the present study.

Interaction Between Glucocorticoids and Ig Absorption

In rats adrenocorticol steroids are involved in the ability of the neonate to absorb immunoglobulin from the gut (Halliday, 1959). Unlike the calf, the newborn rat is able to absorb colostral immunoglobulin across the gut epithelium at any age up to 18 days (Appendix D); thereafter this ability declines and is lost by 21 days. Halliday (1959) was unable to advance or postpone the time of cessation of absorption by interfering with the diet or by fostering pups onto mothers at varying stages of lactation. However, administration of large doses of exogenous corticosteroids to pups, ranging in age from 9-16 days, had the initial effect of increasing the amount of antibody absorbed. However, a subsequent reduction in absorption was apparent at 24 hours post-injection and complete cessation after two days. Pups injected with deoxycorticosterone

acetate also exhibited significantly lower antibody absorption than did untreated controls. Injection of other steroids (stilbesterol, testosterone, and progesterone) had no effect on the level of antibody absorption.

Since exogenous glucocorticoids could cause a cessation in the amount of immunoglobulin amassed by the neonate (Halliday, 1959), numerous investigators sought for the processes by which the uptake of antibody could be hormonally controlled. In bovine, porcine, and canine, species which have a relatively short period during which immunoglobulin can be absorbed (24-48 hours), studies have focused on the immediate effects of exogenous hormones typically administered to the neonate at birth.

Gillette and Filkins (1966) investigated the extent to which intestinal antibody absorption in dogs could be influenced by postpartum injections of hydrocortisone, progesterone, or metyrapone (an inhibitor of corticoid synthesis). Colostrum-deprived puppies were treated and then fed a known amount of Salmonella pullorum antisera. No significant differences in the amount of antibody absorbed were found between the treatment groups.

Patt and Eberhart (1976) injected newborn cesarean-deprived pigs with metyrapone, ACTH, or the vehicle to investigate the effects of low, high, or normal plasma cortisol concentrations on immunoglobulin absorption. The newborn pigs were fed pooled bovine colostrum at birth, and serum concentrations of IgG were determined. Serum was collected at 0, 6, 14, 22, 30, and 38 hours postpartum. The concentration of bovine IgG

in the serum of ACTH-treated pigs did not differ significantly from the vehicle-injected control pigs at any of the times tested. However, pigs injected with metyrapone exhibited significantly lower serum concentrations of bovine IgG at the 14, 22, 30, and 38 hour sampling times. The authors suggested that maximal absorption of Ig could not take place unless plasma cortisol levels were adequate. They also stated that increased glucocorticoid concentrations would not affect the duration of intestinal permeability to globular proteins.

In a similar experiment using newborn calves, Johnstone and Oxender (1979) presented evidence in agreement with the findings of Patt and Eberhart (1976). Twenty-one bull calves were divided into three groups and injected with either ACTH, metyrapone, or saline. Before calves were two hours old, each was given one liter of pooled colostrum. Blood samples were taken at 0, 1.5, 3, 6 hours postpartum and again at six hour intervals until 48 hours postpartum. The serum glucocorticoid concentrations of the ACTH-treated calves were significantly greater than those of the control calves within two hours of birth, and the difference lasted for 36 hours. Metyrapone-treated calves had lower glucocorticoid concentrations throughout the duration of the experiment, but they were significantly lower than those of the control calves only during the first twelve hours postpartum. The treatments had no significant effect on serum IgG concentrations, although the 24 and 48 hour values of the control and ACTH-treated calves were greater than those of the metyrapone-treated group. The results implied that increased serum glucocorticoid concentrations in newborn calves would not interfere with the absorptive process of Ig by the newborn calf.

Stott (1980) attempted to increase postpartum serum cortisol concentrations in the bovine neonate. Treatments consisted of one group injected with ACTH immediately after birth, a second group given a highly potent synthetic glucocorticoid called Predef (9-fluoro-prednisolone), and the third group acted as the control with no hormone treatment. All calves were fed one liter of pooled colostrum at 4, 16, and 28 hours postpartum. Blood samples were taken at birth and again at four hour intervals until 40 hours postpartum. Calves treated with ACTH exhibited significantly higher cortisol concentrations than did calves in the other treatment groups. Calves treated with Predef had serum cortisol levels similar to those found in controls. However, it was anticipated that the synthetic steroid would stimulate corticosteroid-dependent activity involved in immunoglobulin absorption and/or precocity of the intestinal epithelium. The hormonal treatments did not significantly alter the serum Ig concentrations at any sampling time. Stott concluded that the absorptive abilities of the intestinal epithelium were not modified by the endocrinological treatments tested.

In a field study by Boyd and Hogg (1981), forty-eight bull calves were injected with ACTH within the first few hours postpartum. Calves were left with their dams for at least 24 hours after birth to allow them to obtain colostrum by natural suckling. Blood samples were collected at 0, 7, 12, and 24 hours postpartum. The ACTH injection caused a temporary delay in the normal decline of serum cortisol in the newborn calf. In addition, serum Ig concentrations at both 12 and 24 hours postpartum were significantly higher in ACTH-treated calves than in untreated calves.

However, contrasting results were obtained when a significant negative correlation was established between serum Ig and cortisol levels in untreated calves soon after birth and at 12 and 24 hour postpartum. The fact that colostral intake was not controlled may have contributed to the conflicting results.

In summary, the immediate or acute effects of exogenous glucocorticoids did not appear to interfere with the absorption of immunoglobulin by the newborn of cattle, swine or dog. In fact, adequate corticosteroid seemed to be required for maximal immunoglobulin absorption.

The plasma corticosteroid patterns in calves subjected to a variety of nutritional or environmental circumstances have provided the opportunity to study the ability of neonates to absorb Ig following endogenous changes in corticoid levels. LaMotte and Eberhart (1976) recorded the corticosteroid concentrations in ten newborn calves that were fed either one liter of colostrum or one liter of unpasteurized whole milk within one hour of birth. The five calves fed milk were considered colostrum deprived. Blood samples were taken at 0, 6, 12, 18, 24, 30, 36, 48, 72, and 144 hours postpartum. No significant difference between the two groups at any sampling time was observed. Peak corticosteroid concentrations were seen in both groups at 0 hour, followed by a significant decrease between 0 and 6 hours postpartum, with a general downward trend thereafter.

An experiment by Stott and Reinhard (1978) investigated the effects of high endogenous concentrations of corticosteroids at birth, related to weather and dystocia, on the capability of the newborn calves

to absorb colostral Ig. The cortisol concentration in serum at birth was less in dystocial than eutocial calves, whereas the steroid levels were correlated negatively with environmental temperature prior to parturition. The absorption of immunoglobulin as indicated by serum concentrations at 16 and 24 hours postpartum was similar in dystocial and eutocial calves. Likewise, the environmental temperature did not influence serum Ig levels. Therefore, it was concluded that hyperadrenalism at birth, due to the birthing process or the environmental temperature, had little or no influence on intestinal absorption of colostral Ig by bovine neonates.

In a study previously outlined, Nightengale and Stott (1981) reported an unexpected finding. Concentrations of serum cortisol related to time of first feeding were increased following ingestion of colostrum. Increases in serum cortisol due to colostrum intake were only observed in calves following a period of inanition of at least twelve hours. Similar increases were not observed following a second colostrum feeding.

The role of endogenous glucocorticoids in the absorption of immunoglobulin by the bovine neonate remains unclear. It is possible that the high serum cortisol levels at birth initiate and control the processes of absorption and closure. However, the finding that serum cortisol concentrations fluctuate in response to colostrum intake (Nightengale and Stott, 1980) substantiates the continued involvement of glucocorticoids during the first 24 hours postpartum. A thorough study of the changes in serum cortisol levels in response to various experimental rations and feeding regimes may elucidate the role of this glucocorticoid in immunoglobulin absorption.

Delayed or chronic effects in large domestic species have been investigated by administration of corticoids or ACTH to the pregnant dam, thus placing the fetus in an environment of elevated corticosteroid levels. Puppies from bitches treated 24 hours prepartum with either ACTH or hydrocortisone absorbed significantly less antibody than did puppies from untreated bitches (Gillette and Filkins, 1966). Since elevated, maternal corticosteroid levels in dogs had an apparent effect on neonatal Ig absorption, Husband et al. (1973) questioned the use of exogenous corticosteroids to induce parturition in cattle. In treated pregnant cows, an intramuscular injection of Opticortenol (dexamethasone trimethylacetate) was slowly released from its injection site and crossed the placenta to enter the fetal circulation. Therefore, the fetus in a treated cow was subjected to evaluate levels of corticosteroid. All calves were fed one liter of pooled colostrum 1-2 hours after birth and another liter four hours later. At 24 hours postpartum, the amount of immunoglobulin absorbed in calves born prematurely from treated cows was half that of calves born from untreated cows with normal gestation periods. However, the published data did not indicate whether corticosteroid administration produced a precocious closure or whether it reduced the efficiency of immunoglobulin absorption. In a similar experiment, Muller et al. (1975) used a more rapid acting glucocorticoid (dexamethasone) to induce premature calving. Blood samples were taken at birth prior to suckling and at 3 days of age. Calves born prematurely and control calves (normal gestation) were allowed to suckle naturally. The steroid administration had no apparent effect on Ig absorption in the premature neonate.

Chronic effects have been studied more closely in the rat where high levels of exogenous corticoids initiate a cessation of the absorptive processes. It should be noted that the period during which rat pups can absorb maternal immunoglobulin differs greatly from bovine (Appendix D). The rat pup is born at a more immature stage of development compared to the newborn of domestic animal species. However, the amount of information generated about the absorptive prccess in rat pups may provide considerable insight into the mechanisms of immunoglobulin absorption in large domestic species.

Daniels et al. (1972) measured corticosterone levels in young rats during the first 28 days after birth, and related the steroid profile to intestinal uptake of polyvinyl pyrrolidone (PVP). Corticosterone concentrations remained consistantly low until day 18. Over the next ten days corticosterone levels quadrupled. This increase correlated closely with a decrease in the absorption of PVP between days 18 and 22. After day 22, PVP uptake was virtually zero. In a later study, Daniels et al. (1973) were able to show that a temporary reduction in PVP uptake by the neonatal rat intestine could be initiated by injections of exogenous corticosteroids. Precocious closure induced by large doses of corticosterone injected five or twelve days after birth reduced PVP uptake during the six days following the injection. However, the reduction was transient and uptake returned to control levels some days after the injection. The temporary reduction in PVP uptake was not associated with any change in the histological appearance of the small intestine at the light microscope level. In contrast, injection of a large dose of cortisone acetate five or twelve days after birth resulted in precocious

closure. Polyvinyl pyrrolidone uptake declined progressively to zero during the 4-6 days following the injection. Histologically, the precocious closure induced by cortisone acetate was comparable to natural closure; a progressive displacement of vacuolated cells occurred from the villi of the distal intestine.

Morris and Morris (1976) presented findings similar to Daniels et al. (1973) but utilized labelled IgG to measure intestinal absorption. They were not able to induce a permanent closure of intestinal epithelium to labelled IgG using corticosterone. Three days after treating twelve-day-old pups there was a marked reduction of IgG transport into the blood. However, four to five days after treatment, some recovery of the IgG transport function was noted. Cortisone injections into a second group of twelve-day-old pups induced precocious cell replacement, a process which took up to four days to complete. It was noted that a marked reduction in the uptake of ^{125}I-labelled PVP occurred during this time.

The combined work of Daniels et al. (1973) and Morris and Morris (1976) suggest that corticoids exert their influence on crypt cells, causing precocious maturation of these cells. As the cells migrate up the villi, the absorptive capability of the gut diminished as vacuolated, absorbing cells were sloughed off and replaced by nonabsorptive cells.

Morris and Morris (1980) presented evidence whereby the effects of exogenous corticosteroids on intestinal cell replacement were distinguished from the action of the steroids on the transmission of Ig to the circulation. Corticoids were shown to influence the absorptive capabilities of vacuolated cells located on the villi. Newborn rat pups

were injected with either hydrocortisone, cortisone acetate, corticosterone, deoxycorticosterone acetate, or the vehicle alone at day 12, 13, and 14 postpartum. The amount of radioactivity in the vascular compartment of 16 and 18 day-old pups was measured two hours after the injection of ^{125}I-labelled IgG into a ligated segment of the proximal small intestine. This value was compared to the radioactivity of the trichloroacetic acid precipitable protein within the same compartment. Low molecular weight fragments resulting from degradation of Ig during the absorptive process would not have been precipitated by trichloroacetic acid, and therefore, were not included in the measurement of intact Ig transported through the proximal epithelial cells. Control animals injected with vehicle only retained the ability to transport intact Ig to the circulation on day 16. However, in some control animals, closure had commenced by day 18. In contrast, pups treated with one of the exogenous steroids exhibited minimal transport capacity or almost complete closure by day 16. The amount of available Ig taken up from the intestinal lumen was also measured. Similar amounts of Ig were internalized by proximal enterocytes in all pups before and after closure, suggesting that the treatments did not affect the rate of pinocytosis. To assess the effects of experimental treatments on cell replacement, a five centimeter terminal portion of the small intestine was fixed for histological study. Treatments were injected on days 14, 15, and 16, and tissue samples were taken on day 18. All steroid treatments, except corticosterone, induced precocious cell replacement in the ileum by day 18. The authors discussed the idea that closure in the rat may not be the result of cell replacement nor cessation of pinocytotic activity.

Their data suggested that a decrease in Ig transport took place following steroid administration while pinocytotic activity was maintained and before full cell replacement occurred. They stated that closure, whether naturally or experimentally induced, was possibly the result of changes in the protein-synthesizing ability of the proximal enterocytes resulting in a cessation of proteins involved in Ig transport and an increase in cellular lysosomal activity. It was concluded that these changes were possibly initiated and controlled by glucocorticoids.

Interaction of Thyroxine and Immunoglobulin Absorption

Recent work in the rat neonate has implicated the thyroid hormone, thyroxine, in at least one aspect of macromolecular absorption: closure (Chan et al., 1973; Moog and Yeh, 1979). A limited number of studies have been published which investigated the involvement of thyroid hormones in immunoglobulin absorption by the newborn of other species. Furthermore, a thorough profile of triiodothyronine or thyroxine concentrations in bovine during the first 48 hours postpartum has yet to be reported.

Hernandez et al. (1972) presented a profile of serum thyroxine (T_4) concentrations in prenatal and neonatal bovine. Blood samples were collected once daily. Serum T_4 levels just prior to and at birth were approximately twice as high as normal adult concentrations, but declined rapidly, approaching adult values at the end of day 6. The authors reported an exponential decline of T_4 over the first four days, and it was suggested that high prenatal concentrations inhibited further T_4 release from the thyroid due to a negative feedback.

Attempting to correlate the acquisition of passive immunity to thyroid activity, Boyd and Hogg (1981) bled forty-eight newborn calves prior to suckling and again 24 hours later. Although they noted a slight decline (16%) of serum T_4 concentrations within the first 24 hours postpartum and significant increase in total serum Ig content, they could not find any statistical relationship between the two parameters. However, amounts and concentrations of ingested colostrum were not known for individual calves. Furthermore, any fluctuations of T_4 levels due to ingestion of colostrum and/or absorption of its constituents during the 24 hour period of Ig absorption were not able to be recorded due to the lack of sampling times.

Several investigators have administered thyroxine to calves during different stages of development and studied the subsequent effects on intestinal absorption of macromolecules. Cabello, Levieux, and Lefaivre (1980) gave intraamniotic injections of T_4 to three prenatal calves. Four control calves were injected with saline. Colostrum was fed as 2.5% of body weight every four hours until 32 hours postpartum. Blood samples were collected at 4 hours postpartum then every four hours until 36 hours postpartum. No significant differences in Ig concentrations were found between the control and treated calves. The authors did report that T_4 injections reduced the duration of IgG absorptive period. Their criteria for termination of IgG absorption was the recorded time of maximum plasma IgG concentrations. Thyroxine treated calves exhibited a maximum IgG concentration at 20.7 hours while control calves peaked at 30.7 hours postpartum. In a second study, Cabello and Levieux (1980) injected six calves at birth and again at 24 hours with

T_4. Six saline injected calves acted as controls. All calves were fed the same colostrum. No difference in Ig concentration due to the treatments could be established.

Chan et al. (1973) examined the effect of T_4 administration on the cessation of macromolecular uptake by the neonate rat intestine. Pups were injected at five days postpartum with T_4 saline and ^{125}I-PVP uptake was measured on a series of animals sacrificed on day 9, 11, 12, or 13 postpartum. Premature closure was shown to occur after a 4-5 day delay (from time of injection). Closure occurred approximately 5-6 days prematurely. The time course of T_4 induced closure was similar to that which occurred in normal pups.

Constituents of Bovine Colostrum Involved in Ig Absorption

General

Bovine colostrum may contain factors which are involved in the absorption of immunoglobulin or in the cessation of absorptive processes. Balfour and Comline (1962) performed a series of experiments which demonstrated that colostral whey contained substances which substantially accelerated the absorption of globulin by the newborn calf. A protein fraction, which was not coagulated or inactivated by heat but was eliminated by trichloroacetic acid precipitation or pepsin digestion, appeared to be involved in globulin absorption. By itself the protein fraction had little effect on the absorptive processes, but if inorganic phosphate and glucose-6-phosphate were added, in concentrations normally present in colostral whey, the globulin was absorbed as fast as from fresh whey. Filtrates prepared from milk whey or adult serum after removal of heat-

coagulable proteins were tested for their ability to enhance globulin absorption. Those from milk whey exhibited considerable activity; those from adult serum did not.

Additional work investigating chemical factors that might be involved in the absorption of macromolecules by the newborn calf was undertaken by Hardy (1969). He substantiated that colostral factors which accelerated absorption reached the terminal ileum via the circulatory system after they themselves had been absorbed from the upper small intestine. When PVP was administered in water, little was absorbed. However, if such an infusion was followed three hours later by a duodenal infusion of colostrum, PVP crossed the ileal epithelium and passed into they lymph almost immediately. This response was too rapid for the colostrum to have reached the absorbing cells in the terminal ileum.

The concentration of histamine has been reported to be higher in bovine colostrum than in milk (Zarkower, 1967). Because of its ability to increase capillary permeability, histamine might be involved in altering intestinal permeability in the calf. However, Patt, Zarkower, and Eberhart (1972) found that neither serum Ig concentrations nor the duration of gammaglobulin absorption were altered by the oral administration of supplemental histamine to newborn calves as compared to control animals.

Certain constituents of bovine colostrum might also serve a protective role to the neonate during the period of macromolecular absorption. Corley et al. (1977) investigated the effect of colostrum on the penetration and transepithelial migration of live Escherichia

coli in the neonatal calf intestine. Colostrum-deprived calves (2 to 6 hours old) were given E. coli in saline, E. coli suspended in colostrum, or E. coli in saline one hour after colostrum. Twenty-four hours after exposure to E. coli, blood, liver, spleen, mesenteric lymph nodes draining jejunal and ileal regions of the small intestine, and segments of the duodenum, jejunum, and ileum were collected. In calves which received colostrum with or prior to E. coli, no attachment or intestinal penetration by bacteria could be detected. However, the apical tubular system was dilated with colostrum. No bacteria were found in the blood, spleen, or liver of these calves, but some E. coli were recovered from the mesenteric lymph nodes of calves given E. coli and colostrum simultaneously. Escherichia coli were not recovered from nodes of calves given colostrum before dosage with the organism. In calves given E. coli in saline, bacteria were recovered from the intestinal cells, the laminal propria, the lymph nodes, and the blood. It was suggested that early exposure to colostrum might have prevented transepithelial migration of microorganisms. Also when invasion had occurred, the interaction of colostral constituents and the reticuloendothelial system of the neonate resulted in the removal of the invading organisms.

Colostrokinin

Guth (1959) and Werle (1959) detected a peptide in bovine colostrum that exhibited smooth muscle stimulating activity resembling bradykinin. It was named "colostrokinin" by Werle. Colostrokinin is found in a precursor form, colostrokininogen. Suckling by the newborn calf causes the activation of the precursor to the biologically active form,

colostrokinin, via the enzymatic action of the salivary enzyme, kallikrein. Kallikrein is found in urine, pancratic juice, salivary glands, saliva, and intestinal epithelium of many animals.

Colostrokinin can be classified into a group of naturally occurring or locally produced humoral substances called "kinins". As a group, kinins cause vasodilation, spasmatic motility of smooth muscle, an increase in capillary permeability, bronchoconstriction, pain, and increased ion transport across endocytes. Currently at least four different naturally occurring kinins are recognized in mammalian systems: bradykinin, lysyl-bradykinin (kallidin), methionyl-lysyl-bradykinin, and colostrokinin (ColK). Kinins have been implicated as humoral substances regulating local tissue homeostasis, especially changes in microcirculation (Altura, 1979). The potential of kinins to regulate gastrointestinal functions suggests that the presence of colostrokinin in colostrum might aid in the transport of immunoglobulin across the intestinal epithelium.

Guth (1959) showed the diminution of colostrokinin in bovine colostrum as it became milk. Lacteal secretions of the dam exhibited graduated decreases with each successive milking and little or no ColK at four days postcalving. The physiological activity of ColK was tested using a series of in vitro assays. He distinguished ColK from substance P, acetylcholine, histamine, angiotensin, and serotonin, all of which stimulate rat duodenal tissue to contract; colostrokinin stimulated relaxation of the rat duodenum.

The isolation and purification of colostrokinin from bovine colostrum was undertaken by Yamazaki and Moriya (1969a). They showed that colostrokininogen belonged to the gammaglobular fraction of colostric proteins following precipitation at 30% saturation with ammonium sulfate. The activity of ColK was tested using the following bioassays: inducing vasodilation in anethetized dogs, stimulating smooth muscle contractions of rat uterus, and increasing capillary permeability in guinea pig. The activity was measured with a calibration curve obtained with bradykinin, which exhibited similar pharmocological properties in the above in vitro assays. The stability of ColK activity in saliva, gastric juice, and intestinal juice was also examined. Colostrokinin maintained its full active potential in saliva and intestinal juice for 30 minutes and then decreased to half of the activity after 60 minutes. When incubated with gastric juice, ColK was stable for 120 minutes.

Cassellato et al. (1977) presented an isolation procedure for colostrokinin which resulted in a more pure form of the peptide. However, the resultant quantity was minute. Therefore, the extraction procedure described by Yamazaki and Moriya (1969a) was modified in order to derive a source of colostrokinin for experimental rations in the present study.

In a second paper, Yamazaki and Moriya (1969b) reported various biological and chemical properties of colostrokinin. The increase in capillary permeability produced by colostrokinin was stronger than that of bradykinin. Colostrokinin activity was completely destroyed by chymotrypsin, while trypsin had little effect following 48 hours of incubation. The peptide was found to be more stable in acid than in

alkaline solution, and its isoelectric point was close to pH 7.4. Phenylalanine and serine were detected as the N-terminal and the C-terminal amino acids, respectively. The molar ratio of amino acid residues of ColK were reported, along with the finding that the ultraviolet absorption spectrum possessed a maximum absorbance at 260 nm. Finally on the basis of the amino acid analysis, the molecular weight was calculated to be 1992.

Beretta et al. (1972) attempted to distinguish between ColK and bradykinin even though the two peptides possessed strikingly similar pharmocological properties. Relaxation of rat duodenal smooth muscle was used to test the respective activities of these two peptides. Normally both peptides caused rat duodenum to relax. However, when muscle preparations were suspended in $NaHCO_3$-free Tyrode solution, ColK elicited a pure contraction while bradykinin proved to be completely inactive. When the pH of the $NaHCO_3$-free Tyrode solution was varied, bradykinin exerted maximal relaxing activity at pH 8.9-9.0 and no effect at pH 6.0 and 5.0. In contrast, ColK studied under the same experimental conditions caused contractions at the lower pH tested (5.0 and 6.0) and relaxations at higher pH values (7.0, 8.0, and 9.0). The effects of ColK were also found to be dependent on the Ca^{++} concentration in the muscle bath. The relaxation effect caused by ColK proportionately decreased as the Ca^{++} content of the incubation media decreased. In Tyrode containing 0.1 mM of Ca^{++}, the pharmocological action due to ColK was nearly absent. This suggested that the actions of ColK involved the Ca^{++} depen-

dent functions of the smooth muscle fibers. ColK did not interfere with adrenergic receptors since its relaxation effects persisted after adrenergic blockade by α- and β-sympatholytic agents.

The presence of colostrokinin in colostrum and not milk, the presence of Ig in colostrum and not milk, the presence of kallikrein in saliva and intestinal epithelial tissue, and the ability of kinins to regulate gastrointestinal function suggest that colostrokinin might be involved in the absorption of Ig by the newborn calf. No published work has yet delininated the role of colostrokinin, if any, in immunoglobulin absorption. The present study attempted to elucidate a role for colostrokinin by feeding a colostral source of immunoglobulin with or without colostrokinin.

It is anticipated that the serum Ig concentrations will reflect the involvement of colostrokinin in the absorption of colostral immunoglobulin. The period, rate, and amount of Ig absorption can be monitored by frequent blood sampling over the first 24 hrs postpartum (Stott et al., 1979a,b,c). The possibility that colostrokinin may have an inhibiting effect on macromolecular absorption was also considered. Corley et al. (1977) showed that colostrum contained constituents which inhibited the transepithelial migration of E. coli; constituents which might also initiate a cessation of macromolecular uptake.

Effects of Inanition of Immunoglobulin Absorption

The physiology and anatomical structure which allow the transfer of large protein molecules across the gut epithelium of the bovine neonate apparently are under strict control since cessation of macro-

molecular uptake occurs spontaneously within 24-36 hours postpartum regardless of dietary regime (Deutsch and Smith, 1957; McCoy et al., 1970; Patt, 1977; Stott et al., 1979a). However, the potentiation of Ig absorption by dietary factors cannot be ruled out (Balfour and Comline, 1962; Hardy, 1969; Burton and Smith, 1977).

The intestinal absorptive cells of the newborn calf are analogous to cells in the intestine of the newborn pig and suckling rat (Staley et al., 1972), but cytological changes during the first day postpartum distinguish them from other species (Appendix D). Payne and Marsh (1962) found that starved piglets took up Ig at 106 hours postpartum suggesting that normal changes in the absorptive ability of the gut did not occur as in piglets fed at birth. The consumption of colostrum or milk stimulated the cellular changes. However, piglets fed only water exhibited extended absorptive capabilities similar to starved newborns. Using PVP as the testing molecule, starved piglets were able to absorb PVP at 86 hours postpartum, and starved lambs maintained their ability to absorb macromolecules through 48 hours postpartum (Lecce and Morgan, 1962).

In contrast, the ability of the bovine neonate to absorb macromolecules is not extended following a period of starvation. Deutsch and Smith (1957) found that the absorptive ability in calves was unsubstantial following a forty-eight hour period of colostral deprivation; the calves were maintained on transfusions of maternal blood. McCoy et al. (1970) studied calves which did not receive colostrum until 24 hours postpartum. During this time the newborns were given either a 1% solution of glucose or nothing. Following colostral feeding at 24 hours, blood samples were taken hourly until 31 hours postpartum. No increase in

serum immunoglobulin concentrations were recorded at any of the seven sampling times. It was suggested that the gut was impermeable to colostral immunoglobulin by 24 hours postpartum. Stott et al. (1979a) studied the effect of delayed feeding on the period of Ig absorption. Treatments consisted of seven periods at which feeding was initiated: 0, 4, 8, 12, 16, 20, and 24 hours postpartum. The initial feeding of colostrum was followed by two more feedings at twelve hour intervals. Blood samples were taken from each calf at birth and at four hour intervals until 40 hours postpartum. Termination of intestinal permeability to colostral immunoglobulin occurred spontaneously with age at a progressively increased rate after 12 hours postpartum. Feeding colostrum did shorten the period from birth to closure by two or three hours. However, spontaneous closure in deprived calves occurred at approximately 24 hours postpartum. As colostrum feeding was delayed, closure also was delayed up to the time of spontaneous closure. In another paper derived from the same field trial, Stott et al. (1979c) showed that the serum Ig concentrations progressively decreased as the initial feeding time was sequentially delayed.

During the first day postpartum, the sequence of events may be so stringent that delayed feeding may disrupt the spontaneous functions of the hormonal, humoral and dietary factors regulating immunoglobulin absorption. Therefore, to further elucidate the roles of cortisol, thyroxine, and colostrokinin over time and changing conditions at the intestinal level, inanition was introduced as an experimental variable.

The absorptive processes affected by cortisol, thyroxine and colostrokinin are under investigation. Whether these factors influence Ig uptake, transport, or degradation is debatable. Although some studies actually measure immunoglobulin absorption across the gut epithelium, a majority of investigators simply quantitate serum immunoglobulin in concentrations. It should be noted that the Ig levels reflect the amount of Ig reaching the circulation, not necessarily that which is actually absorbed. A portion of absorbed Ig may actually be degraded prior to reaching the circulation. Therefore, serum Ig concentrations simply indicate that absorption may be affected by changing experimental conditions, but the concentrations are not a true measurement of total absorption.

In summary the objectives of this study were to:

1) investigate the role of colostrokinin in Ig absorption,
2) determine if colostrokinin is involved in the cessation of Ig uptake,
3) develop a method to control the colostrokinin content in experimental rations.
4) delineate the serum cortisol and thyroxine response following the consumption of various experimental rations prepared to control Ig and colostrokinin intake,
5) investigate the effects that delayed feeding (12 hour inanition) may have on the hormonal and humoral control of Ig absorption.

CHAPTER 2

EXPERIMENTAL PROCEDURE

Experimental Design

Sixty-two newborn unsuckled Holstein-Friesian calves were removed immediately from their dams and randomly assigned to a treatment within a randomized complete block, split plot design with cells of the 4 x 2 factorial design of each block as main plots and bleeding time as subplots. The eight treatment combinations were replicated twice per block within four blocks in the experiment. Treatments consisted of four rations: pooled first-milking colostrum (I), whole milk (II), milk plus a colostrokinin-free immunoglobulin extract (III), and milk plus immunoglobulin extract plus colostrokinin extract (IV). One-half of the calves were fed one liter of their assigned ration within the first hour following birth (0 hour postpartum), while the second half were fed after twelve hours inanition (Table 1). All calves, regardless of their initially assigned ration, were fed one liter of pooled colostrum at 24 hours postpartum.

Preparation of Rations

Pooled colostrum was collected prior to the start of the trial. Each collection (approximately 24 liters) was equally divided for the preparation of three different rations (Figure 1).

The first portion remained unmodified and was frozen at -20 C to

TABLE 1. CONSTITUENTS OF THE EXPERIMENTAL RATIONS AND FEEDING REGIME FOR SIXTY-TWO PRECOLOSTRAL BOVINE NEONATES

RATION	ITEM	VOLUME (ml) PER FEEDING	TOTAL Ig CONCENTRATION (mg/ml)	FEEDING REGIME[a]			
				Fed at 0hr		Fasted 12hr	
				Symbol	N	Symbol	N
I	Colostrum (C)	1,000	65	PC-0	8	PC-12	8
II	Milk (M)	1,000	0	M-0	7	M-12	8
III	Milk IgEx[b] ColK[c]	650 350 -	75	IgEx-0	7	IgEx-12	8
IV	Milk IgEx ColK	400 350 250	75	ColK-0	9	ColK-12	7

[a]Calves fed at 0hr were not fed again until 24hrs. All calves were fed one liter of pooled colostrum at 24 hrs postpartum. Rations were suckled from a nursing bottle.

[b]Colostrokinin-free immunoglobulin extract.

[c]Colostrokinin extract.

POOLED COLOSTRUM (1st milking)

↓

↓	↓	↓
1/3 of total volume; CONTROL RATION Frozen -20 C.	1/3 of total volume; IMMUNOGLOBULIN EXTRACT (colostrokinin-free)	1/3 of total volume COLOSTROKININ EXTRACT (immunoglobulin-free)
	2.5 mg rennet/ml colostrum; incubate at 37 C for 1 hr; centrifuge; decant off whey.	2.5 mg rennet/ml colostrum; incubate at 37 C for 1 hr; centrifuge; decant off whey.
	pH whey to 7.6-7.8 with 5N NaOH.	pH whey to 3.0 with 5N HCl; incubate at 37 C for 5 min.
	Add 50 BAEE units kallikrein/1 whey; stir 15 min at 25 C; incubate at 37 C for 1 hr.	pH to 5.5 with 5N NaOH.
	Dialyze in Amicon Hollow Fiber (100,000 daltons) dialyzer for 24 hrs at 4 C against 0.85% saline.	Dialyze at 4 C for 24 hr in cellulose tubing against running tap water
	Concentrate retained fluid 5X.	pH to 7.6-7.8 with 5N NaOH.
		Add 50 BAEE units kallikrein/1 eq of original whey; stir 15 min at 25 C; incubate at 37 C for 1 hour.
		pH to 4.5 with 5N acetic acid.
		Add two volumes of 95% EtOH; reflux for 10 min.
		Centrifuge for 15 min at 4 C.
		Evaporate supernatant in a vacuum flask warmed by a 40 C water bath to 1/3 of original starting volume of whey.
		Test for presence of EtOH. (Appendix B)

Figure 1. Allocation of pooled colostrum. Preparation of extracts to be used in experimental rations. Colostrokinin procedure modified in part from that of Yamazaki and Moriya, 1969a.

be later used as the control ration (whole colostrum). Immediately prior to the start of the trial, all colostrum fractions were mixed so that each control calf was fed identically.

The second portion of pooled colostrum was used to prepare a colostral source of colostrokinin-free immunoglobulin as outlined in Figure 1. Colostrokinin (2,000 daltons) was not retained by the hollow fibers of the dialyzer, and it was eliminated in the ultrafiltrate. The remaining concentrated solution was called immunoglobulin extract (IgEx). The concentrate was frozen at -20 C to be later used as a colostral source of Ig. Prior to the start of the trial, all IgEx fractions were pooled.

The third portion of pooled colostrum was used to prepare an immunoglobulin-free colostrokinin extract (ColK). The procedure of Yamazaki and Moriya (1969a) was followed in preparing "crude colostrokinin" (Figure 1). Modifications of the published procedure included the use of fifty BAEE units of kallikrein per liter of whey. One BAEE unit of kallikrein will hydrolyze 1.0 μmole of Nα-benzoyl-L-arginine ethyl ester (BAEE) to Nα-benzoyl-L-arginine and ethanol per minute at pH 8.7 at 25 C (Sigma Chemical Company). Other modifications included evaporation of the refluxed solution to one-third the original starting volume of whey, and an alcohol determination test. The final volume containing the active colostrokinin was frozen at -20 C to be later used as a source of colostrokinin, free of Ig. Prior to the start of the trial, all ColK fractions were pooled.

Fresh whole milk was acquired as needed from the holding tank of the University dairy. Whole milk was used as a negative control

ration and as a vehicle since it contained neither Ig nor ColK but contained additional constituents similar to those found in colostrum.

The rations were prepared as outlined in Table 1. After addition of the other constituents, the volume of IgEx included in rations III and IV furnished an immunoglobulin concentration approximating that of the whole colostrum ration. Following the trial, radial immunodiffusion analysis revealed the total Ig concentration of the whole pooled colostrum to be 65 mg/ml. Immunoglobulin content of rations III and IV was 75 mg/ml. The constituents of rations III and IV were mixed just prior to feeding in order to avoid any degradation of the ColK by kininases contained in milk. The colostrokinin content in the rations was tested as in Appendix A. All calves, regardless of their initially assigned treatment, were fed one liter of pooled colostrum at 24 hours postpartum.

Calf Collection

The trial was run in late September, and all calves were collected within a two week period. The weather was comparatively constant during this period; the days were warm but mild (27 C), and the nights remained comfortable (16 C). Therefore, the calves were under little or no stress due to the environment. Calves were collected 24 hours per day. Time of birth was assumed to be random, thereby not biassing the random assignment of calves to treatment groups.

Blood samples were collected via jugular puncture at 0, 1, 2, 3, 4, 6, 8, 12, 13, 14, 15, 16, 18, 20, 24, 28, and 32 hours postpartum. The initial bleeding (0 hour) was taken within 30 minutes following birth. Blood samples were always taken prior to feeding. Blood was immediately stored at 4 C, and serum was separated by centrifugation

within twelve hours after sampling time. Serum samples were frozen at -20 C until needed for analysis following the termination of the trial.

Serum Analysis

Serum samples were analyzed for total cortisol concentration by a radioimmunoassay adapted from that of Abraham, Buster and Teller (1972). Within assay variability was 4.7% for duplicate determinations. Between assay variability was 10.9%.

Blood serum IgG concentrations were quantitated by a modification of the single radial immunodiffusion (sRID) procedure of Fahey and McKelvery (1965). Polyethylene glycol 4,000 (0.5% w/v) was added to the agarose (1% w/v) to enhance precipitin rings. Innoculated plates were allowed to incubate twelve hours at 4 C in a humidified chamber. Antisera to bovine IgG was produced in goat following methods similar to those described by Campbell et al. (1970). Specificity of the antisera was determined by Ouchterlony plates and immunoelectrophoresis. Standards for immunodiffusion analysis were quantitated by a Bio-Rad protein determination kit using purified bovine gammaglobulin. Standard serum was serially diluted with unimmunized goat serum to generate a standard curve.

Total thyroxine concentrations in serum were determined using a competitive radioimmunoassay diagnostic kit (Abbott Laboratories). Within assay variability was 3.9% for duplicate determination.

Statistical Analysis

Statistical analysis of the resultant data was by analysis of variance (Steele and Torrie, 1960). For each parameter studied

(cortisol, thyroxine, and IgG) sources of variation were treatment, block, collection time, and a treatment by collection time interaction (Appendix C).

For cortisol and thyroxine means, where the treatment x collection time interaction was significant, Student's t-test was used to test for significant differences between means at selected collection times within a treatment group. Every mean in the same treatment group was tested against all means in a succeeding four hour period. Additional testing was not performed. Student's t-test was also used to test differences between means at the same collection time of calves fed the same ration but at different times (0 or 12 hours postpartum).

Differences among treatments for the IgG means were tested for significance using the Student-Newman-Keuls' multiple range test (α=0.05). Curves for the IgG means of selected groups were plotted using orthogonal polynomial regressions. The highest degree polynomial with a significant increase in the R^2 value was utilized in the graph (Table 11).

CHAPTER 3

RESULTS AND DISCUSSION

Serum Cortisol Profiles

The average serum cortisol concentration of all calves at birth was 88±13 ng/ml (Tables 2-5; Figures 2-4). This is in agreement with the findings of Hudson et al. (1976), Johnstone and Oxender (1976), and Nightengale and Stott (1981). Over the next two hours, the serum cortisol levels fell significantly ($P<.05$) for all treatment groups except M-12 and IgEx-12. Although a significant decrease was not recorded for these two groups, the concentrations did show a progressive decline during the first two hours postpartum similar to that exhibited by calves from the other treatments. Johnstone and Oxender (1976) were the only group to report a similar rapid decrease in cortisol concentrations postpartum in bovine because of their sampling frequency. In the present study calves reached an average serum cortisol concentration of 51±7 ng/ml by 2 hours postpartum.

The consensus of the literature is that serum cortisol concentrations continually decrease over the first 8-12 hours postpartum and then plateau, maintaining baseline levels for a period of days (LaMotte and Eberhart, 1976; Johnstone and Oxender, 1976). Nightengale and Stott (1981), however, showed an increase in serum cortisol due to colostrum intake following a period of inanition of at least twelve hours. One objective of the present study was to delineate neonatal cortisol

TABLE 2. Serum cortisol concentrations (ng/ml) of calves at birth and after feeding pooled colostrum (PC) at either 0 or 12 hrs postpartum.

	TREATMENT			
	PC-0		PC-12	
HOURS POSTPARTUM	$\bar{X}^{\dagger}$	SEM	$\bar{X}^{\dagger}$	SEM
0	77.1^{a}	9.3	82.0^{a}	7.8
1	65.4	11.2	64.3	8.9
2	53.3^{ab}	5.3	55.1^{a}	7.7
3	74.6^{b*}	7.3	51.6^{*}	8.1
4	68.4^{*c}	8.3	46.9^{*}	6.0
6	58.8^{*}	5.8	41.5^{*}	5.3
8	45.5^{c}	4.4	37.9	3.8
12	45.7	7.9	42.6	4.6
13	41.5	4.9	38.8	4.2
14	47.8	5.4	41.4^{q}	4.7
15	39.2^{**}	7.0	76.1^{q**}	9.9
16	42.5^{**}	5.3	74.2^{**}	9.3
18	40.7^{*}	4.9	58.4^{*}	6.4
20	41.7	6.4	56.5	6.6
24	41.6	6.0	49.4	4.5
28	57.5	9.9	46.4	7.1
32	56.0	9.3	49.3	10.4

†Every mean in the same column was tested against all means in a suceeding four hour period. Additional testing was not performed.

^{abc}Means in the same column bearing the same superscript differ significantly ($P<.05$).

^{q}Means in the same column bearing the same superscript differ significantly ($P<.01$).

*Means in the same row differ significantly ($P<.05$).

**Means in the same row differ significantly ($P<.01$).

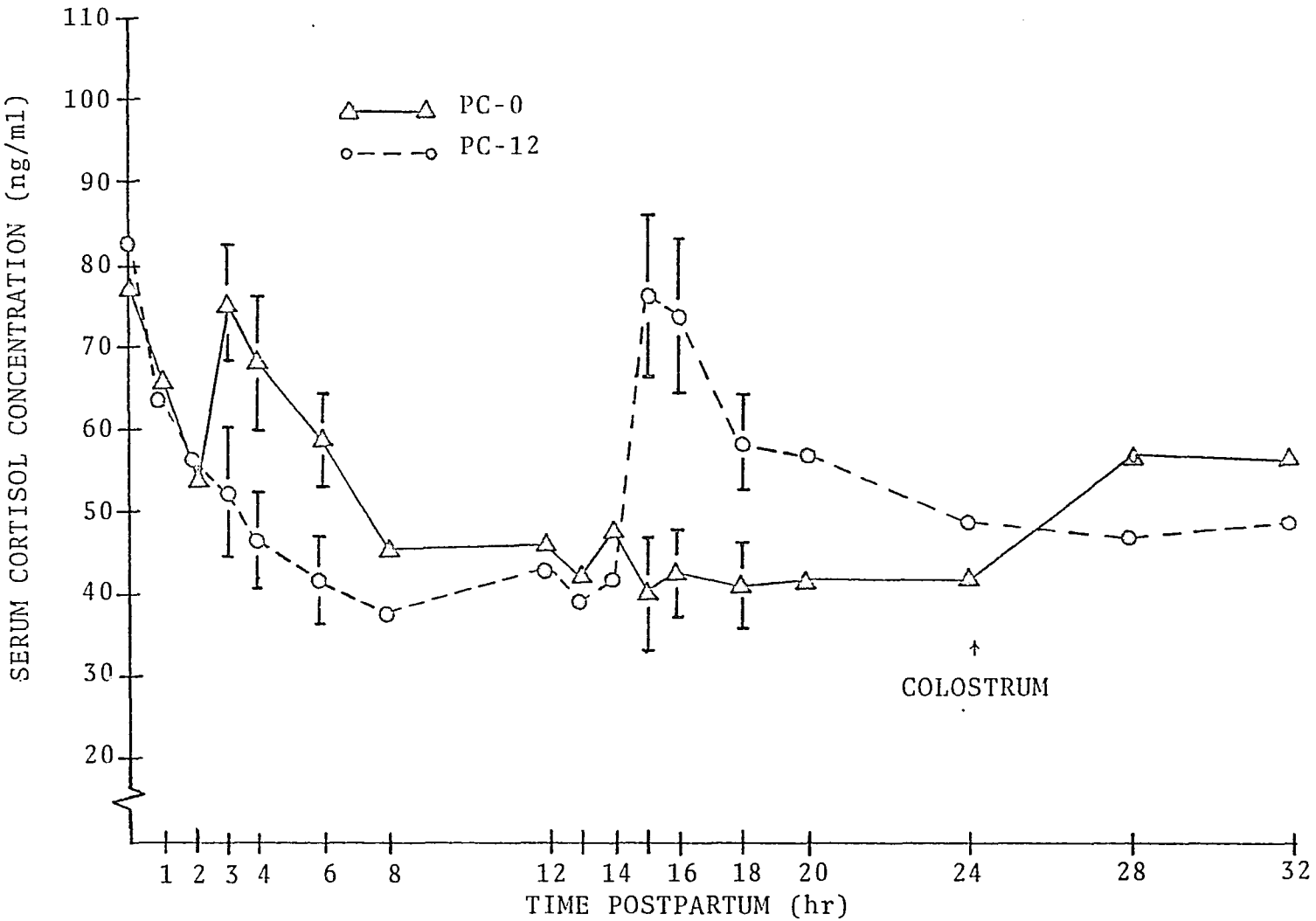

Figure 2. Serum cortisol concentrations of calves at birth and after feeding pooled colostrum at either 0 or 12 hrs postpartum.

TABLE 3. Serum cortisol concentrations (ng/ml) of calves at birth and after feeding milk plus immunoglobulin extract (IgEx) without colostrokinin extract at either 0 or 12 hrs postpartum.

	TREATMENT			
	IgEx-0		IgEx-12	
HOURS POSTPARTUM	$\bar{X}$[†]	SEM	$\bar{X}$[†]	SEM
0	85.8[a]	15.2	83.7[a]	10.5
1	52.9	12.8	65.8	9.4
2	37.7[abcd]	9.5	57.5	7.9
3	78.3[b]	14.4	57.7	6.0
4	83.9[c*]	13.2	52.8[a*]	7.3
6	69.7[d]	11.9	55.6	8.1
8	59.1	13.4	50.2	6.8
12	51.8[c]	7.3	59.1[b]	4.8
13	56.5	5.8	49.2[c]	5.8
14	57.7	5.0	52.6[d]	6.2
15	51.6[*]	7.6	78.4[bcd*]	7.0
16	49.4	7.3	69.1	10.0
18	45.8	6.9	66.8	13.7
20	41.7[**]	6.2	72.6[**]	8.6
24	45.2[*]	8.4	73.7[*]	9.4
28	49.7	12.9	74.1	8.8
32	45.6	11.4	64.2	8.5

[†]Every mean in the same column was tested against all means in a succeeding four hour period. Additional testing was not performed.

[abcd]Means in the same column bearing the same superscript differ significantly ($P<.01$).

[*]Means in the same row differ significantly ($P<.05$).

[**]Means in the same row differ significantly ($P<.01$).

TABLE 4. Serum cortisol concentrations (ng/ml) of calves at birth and after feeding milk plus immunoglobulin extract with colostrokinin extract (ColK) at either 0 or 12 hrs postpartum.

	TREATMENT			
	ColK-0		ColK-12	
HOURS POSTPARTUM	$\bar{X}^{\dagger}$	SEM	$\bar{X}^{\dagger}$	SEM
0	94.3	12.1	111.5	17.3
1	59.9^{z}	7.7	89.5^{ab}	13.5
2	55.3^{az}	7.5	59.3^{az}	9.6
3	76.8	9.0	59.4^{z}	11.3
4	80.4^{a}	10.3	53.0^{bz}	8.6
6	70.2^{*}	7.8	48.5^{*}	3.7
8	65.4^{*}	8.7	42.8^{*}	4.3
12	64.4	8.6	51.8^{cy}	7.2
13	61.2^{*}	8.0	36.3^{cy*}	2.7
14	59.2	7.8	58.5^{y}	6.3
15	58.6^{*}	7.6	91.8^{*}	10.7
16	52.5^{**}	6.4	80.5^{**}	4.4
18	56.4^{**}	6.6	87.9^{**}	8.7
20	53.8	5.5	76.6	11.5
24	55.4	11.0	86.1	14.2
28	52.6	15.6	80.4	16.0
32	47.9	10.8	64.3	9.5

†Every mean in the same column was tested against all means in a succeeding four hour period. Additional testing was not performed.

^{abc}Means in the same column bearing the same superscript differ significantly ($P<.05$).

^{y}Mean differs significantly from 15 hr mean in the same column ($P<.05$).

^{z}Mean differs significantly from 0 hr mean in the same column ($P<.05$).

*Means in the same row differ significantly ($P<.05$).

**Means in the same row differ significantly ($P<.01$).

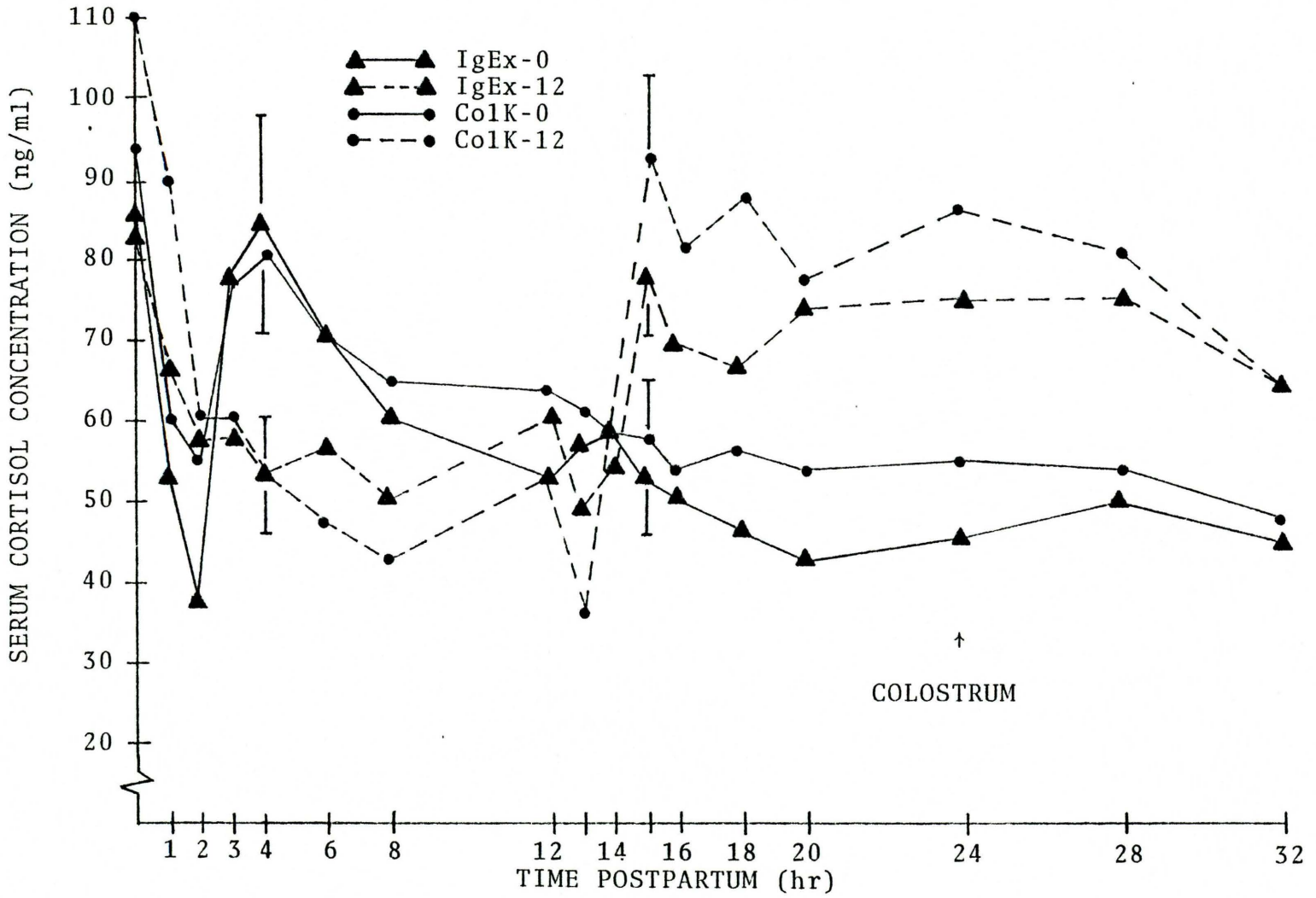

Figure 3. Serum cortisol concentrations of calves at birth and after feeding milk plus immunoglobulin extract with and without colostrokinin extract at either 0 or 12 hrs postpartum.

TABLE 5. Serum cortisol concentrations (ng/ml) of calves at birth and after feeding whole milk (M) at either 0 or 12 hrs postpartum.

	TREATMENT			
	M-0		M-12	
HOURS POSTPARTUM	$\bar{X}$[†]	SEM	$\bar{X}$[†]	SEM
0	83.6	12.3	84.8[a]	17.4
1	55.3	7.8	58.8	10.7
2	37.4[yz]	5.0	53.2	7.2
3	30.3[yz*]	3.4	59.3[*]	9.6
4	24.3[yz*]	2.3	48.4[a*]	8.8
6	36.4	5.6	48.1	7.5
8	36.7	4.7	48.3	9.0
12	35.1	5.0	45.1[b]	5.9
13	40.0	4.0	35.7	5.4
14	41.1[*]	4.8	27.8[bc*]	3.4
15	39.4	6.2	38.9	7.6
16	44.7	3.8	42.0	6.2
18	40.3	3.9	49.8[c]	7.2
20	45.5	6.0	57.1	8.8
24	64.5	7.6	49.3	9.9
28	64.1	8.3	67.6	13.2
32	53.8	8.6	70.0	17.1

[†]Every mean in the same column was tested against all means in a succeeding four hour period. Additional testing was not performed.

[abc]Means in the same column bearing the same superscript differ significantly ($P<.05$).

[y]Mean differs significantly from 1 hr mean in the same column ($P<.05$).

[z]Mean differs significantly from 0 hr mean in the same column ($P<.05$).

[*]Means in the same row differ significantly ($P<.05$).

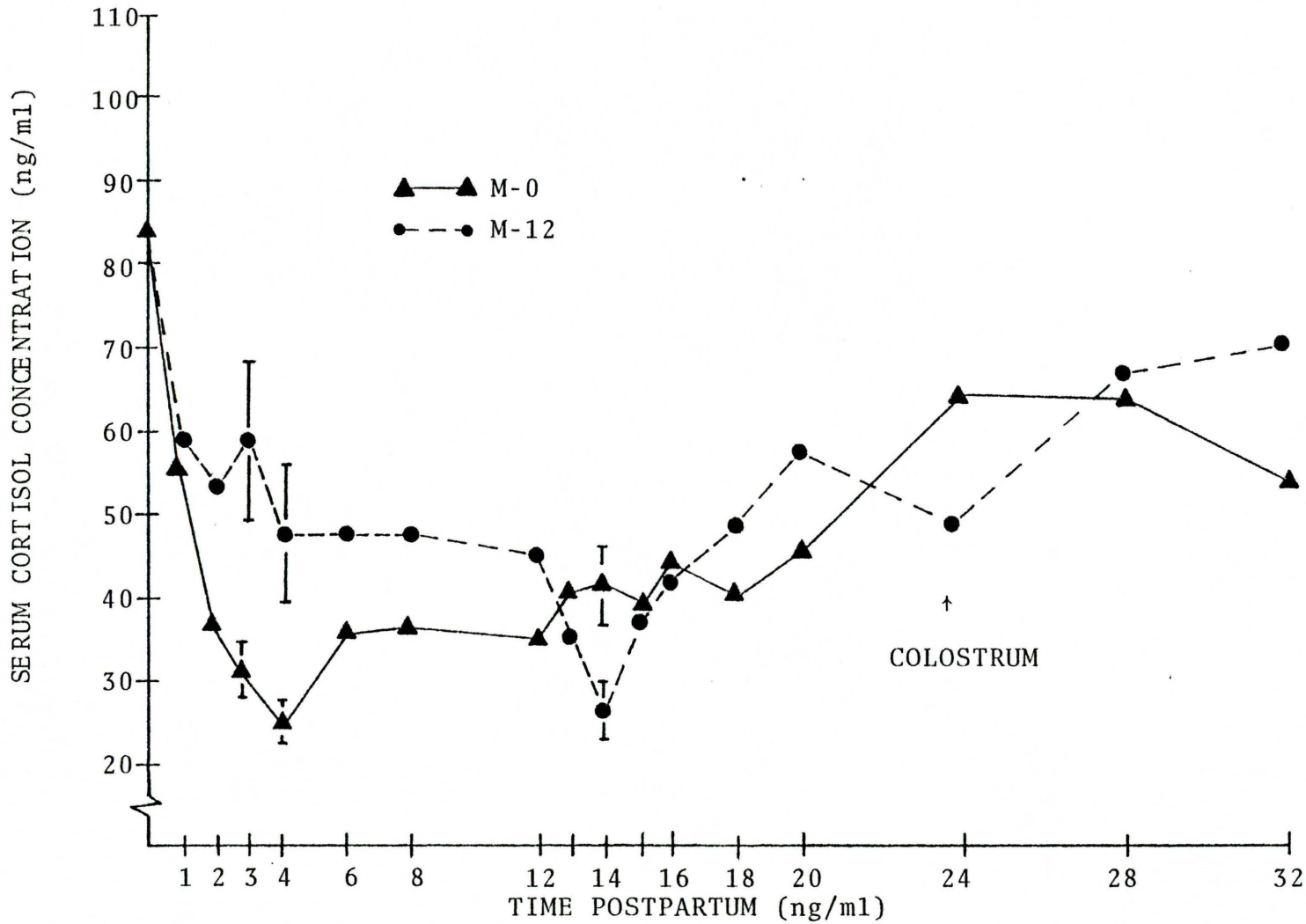

Figure 4. Serum cortisol concentrations of calves at birth and after feeding whole milk at either 0 or 12 hrs postpartum.

fluctuations in response to colostrum consumption at birth or following a period of inanition.

In calves fed either ration I, III, or IV (whole colostrum, milk plus IgEx, and milk plus IgEx plus ColK, respectively) at birth, serum cortisol concentrations increased ($P<.05$) between 2 and 3 hours postfeeding and peaked by 4 hours postfeeding. The maximum concentrations attained postfeeding were comparable to cortisol levels at birth. After peaking, the serum cortisol levels again declined and by 12 hours postpartum reached concentrations similar to the baseline levels of fasted calves (Tables 2, 3, and 4; Figures 2 and 3). The animals in these treatment groups had no significant fluctuations throughout the remainder of the sampling period.

Calves fed ration II (milk) at birth did not show any increase in serum cortisol concentrations between 2 and 4 hours postfeeding; they exhibited a profile similar to fasted neonates (Table 5; Figure 4). Serum cortisol levels increased slightly between 4 and 6 hours postfeeding, but the change was not significant.

Fasted calves maintained baseline levels of serum cortisol between 2 and 12 hours postpartum (Tables 2-5; Figures 2-4). Animals fed ration I, III, or IV at 12 hours postpartum had a rapid increase ($P<.05$) in serum cortisol concentrations between 2 and 3 hours postfeeding (14-15 hours postpartum). The average peak value for these three treatment groups at 3 hours postfeeding (15 hours postpartum) was 82±9 ng/ml, approximating cortisol concentrations at birth. The cortisol level in the PC-12 fed calves returned to a baseline concentration by twelve hours postfeeding. The cortisol concentrations of IgEx-12 and

ColK-12 treated calves remained elevated for the duration of the sampling time.

Calves fed milk (ration II) at 12 hours postpartum displayed no increase in serum cortisol between 2 and 3 hours postfeeding. In fact, the cortisol levels decreased ($P<.05$) immediately following feeding. The serum cortisol levels of PC-12, IgEx-12, and ColK-12 calves also had a tendency to decrease within one hour postfeeding. This fall in serum cortisol concentration may have been a neurally mediated action in response to deglutition or some other digestive reflex. Since this study was not designed to control for such a response, it is not possible to draw any conclusions from these results.

Calves in the M-12 group did show a progressive increase in serum cortisol between 2 and 8 hours postfeeding. However, the peak of 57 ng/ml at 8 hours postfeeding was no higher than baseline concentrations of PC-12, IgEx-12, or ColK-12 calves at this same sampling time.

All animals fed at birth exhibited baseline concentrations of serum cortisol between 12 and 20 hours postpartum. These calves were not fed at 12 hours postpartum.

Statistical comparisons were made at each collection time between treatment groups fed the same ration (Tables 2-5). The mean cortisol concentrations of PC-0 calves at 3, 4, and 5 hours postpartum were greater ($P<.05$) than those of PC-12 neonates at the same times, respectively. The cortisol means of PC-12 calves at 15 and 16 hours were higher ($P<.01$) than their PC-0 counterparts at the identical collection times (Table 2). Calves fed ration III (IgEx) at birth had a higher ($P<.05$) cortisol concentration than IgEx-12 animals at 4 hours

postpartum. The cortisol level at 15 hours postpartum was greater ($P<.05$) in IgEx-12 calves than IgEx-0 animals (Table 3). ColK-0 treated calves had an increased cortisol level at 4 hours postpartum when compared to ColK-12 animals at the same time. Significantly higher ($P<.05$) cortisol concentrations were recorded for ColK-12 calves at 15, 16, and 17 hours postpartum compared to the levels found in ColK-0 calves at respective times (Table 4). The M-0 and M-12 treatment groups did exhibit significant ($P<.05$) differences in cortisol concentrations at 3, 4, and 14 hours postpartum, but these were due to immediate decreases resulting from the consumption of a ration (as previously discussed) rather than increases as in calves fed ration I, III, and IV (Table 5).

The cortisol profiles from 20 hours postpartum throughout the duration of the sampling period did not significantly change. However, the patterns displayed by calves fed ration II (M-0 and M-12) appeared much more erratic during this time than those of calves within the other treatment groups. Neither inanition nor the presence or absence of colostrokinin in the rations appeared to have any effect on the cortisol profiles.

These results demonstrate that the immunoglobulin fraction of colostrum is responsible for initiating an increase in cortisol secretion by the adrenal cortex. In rations containing colostral immunoglobulin (I, III, and IV), serum cortisol concentrations significantly increased between 2 and 4 hours postfeeding, independent of the initial time of feeding. The ingestion of whole milk (ration II), which contained no immunoglobulin, did not stimulate an increase in serum cortisol. However, the addition of a colostral immunoglobulin extract to whole milk

stimulated a substantial rise in serum cortisol concentrations. Nightengale and Stott (1981) found that serum cortisol in newborn calves increased due to colostrum intake following a period of at least twelve hours inanition, but the present study gives a much clearer picture of the events that occur following ingestion of colostrum or milk. The evocation of an endocrine response in the neonate by maternal immunoglobulin suggests that this class of protein may have acted as a "primary messenger" or signal initiating a cortisol surge.

Johnstone and Oxender (1979) and Stott (1980) suggested that increased serum glucocorticoid concentrations did not interfere with the absorptive processes of immunoglobulin by the newborn calf. In and earlier study using newborn pigs, Patt and Eberhart (1976) went one step further and suggested that maximal absorption of immunoglobulin could not take place unless plasma cortisol levels were adequate. In contrast, Husband et al. (1973) stated that glucocorticoids had an inhibitory effect on immunoglobulin absorption, while Boyd and Hogg (1981) presented evidence showing calves injected with ACTH within the first few hours postpartum had significantly higher serum Ig concentrations after 24 hours of suckling than untreated calves.

Glucocorticoids are involved in the cessation of macromolecular uptake by the intestinal epithelium of rat (Halliday, 1959; Daniels et al., 1973a; Morris and Morris, 1976), although the mechanism by which these steroids exert their effects is still being debated. Precocious closure caused by glucocorticoid administration appeared due to a progressive displacement of vacuolated, absorptive cells by more mature, nonabsorptive endocytes. In addition, the precocious closure

usually required three to four days to manifest itself following the glucocorticoid administration. These findings are difficult to apply to bovine since closure occurs within 24 hours postpartum in the newborn calf. However, Morris and Morris (1980) presented evidence whereby the effects of exogenous corticosteroids on intestinal cell replacement were distinguished from the action of the steroid on the transmission of Ig to the circulation. They were also able to show that the corticosteroids did not affect the rate of pinocytosis. Their data suggested that a decrease in Ig transport took place while pinocytotic activity was maintained and before full cell replacement occurred. It was suggested that glucocorticoids may initiate and control the protein-synthesizing ability of the intestinal epithelium, resulting in a cessation of proteins involved in Ig transport and an increase in cellular lysosomal activity.

The cortisol surge in the bovine neonate following Ig absorption may regulate macromolecular uptake at the intestinal level. Therefore, the present study is consistant with the hypothesis of Morris and Morris (1980).

Serum Thyroxine Profiles

Calves were born with a mean thyroxine (T_4) concentration of 12±2 µg/dL. The thyroxine level significantly increased ($P<.05$) by 4 hours postpartum in all treatment groups regardless of whether the calves were fed or fasted (Tables 6-9; Figures 5-7). The average increase was 6.7 µg/dL. Thyroxine concentrations reached a peak at 4 hours postpartum then declined gradually throughout the duration of the sampling period.

TABLE 6. Serum thyroxine concentrations (μg/dL) of calves at birth and after feeding pooled colostrum (PC) at either 0 or 12 hrs postpartum.

	TREATMENT			
	PC-0		PC-12	
HOURS POSTPARTUM	$\bar{X}$ †	SEM	$\bar{X}$ †	SEM
0	13.1	0.7	10.8	1.4
1	16.5^{z}	0.9	14.8	1.9
2	19.3^{z}	0.9	17.2^{y}	2.3
3	20.9^{z}	1.1	19.1^{z}	1.5
4	21.5^{az}	1.9	18.6	2.2
6	18.4	1.5	18.1	1.9
8	15.7^{a}	2.0	16.3	1.5
12	13.9	1.8	15.3	2.1
13	13.5	1.9	16.6	2.1
14	13.3	1.7	16.8	1.9
15	12.2	1.5	17.4	2.4
16	12.8	1.6	16.3	2.2
18	11.5	1.5	15.5	2.1
20	11.1	1.8	13.7	1.8
24	9.9	1.7	12.3	1.8
28	10.9	1.6	11.7	1.6
32	9.8	1.3	11.1	1.6

† Starting with the peak value after birth, every mean in the same column was tested against all means in a succeeding four hour period. Additional testing was not performed.

[a] Means in the same column bearing the same superscript differ significantly ($P<.05$).

[y] Mean differs significantly from 0 hr mean in the same column ($P<.05$).

[z] Mean differs significantly from 0 hr mean in the same column ($P<.01$).

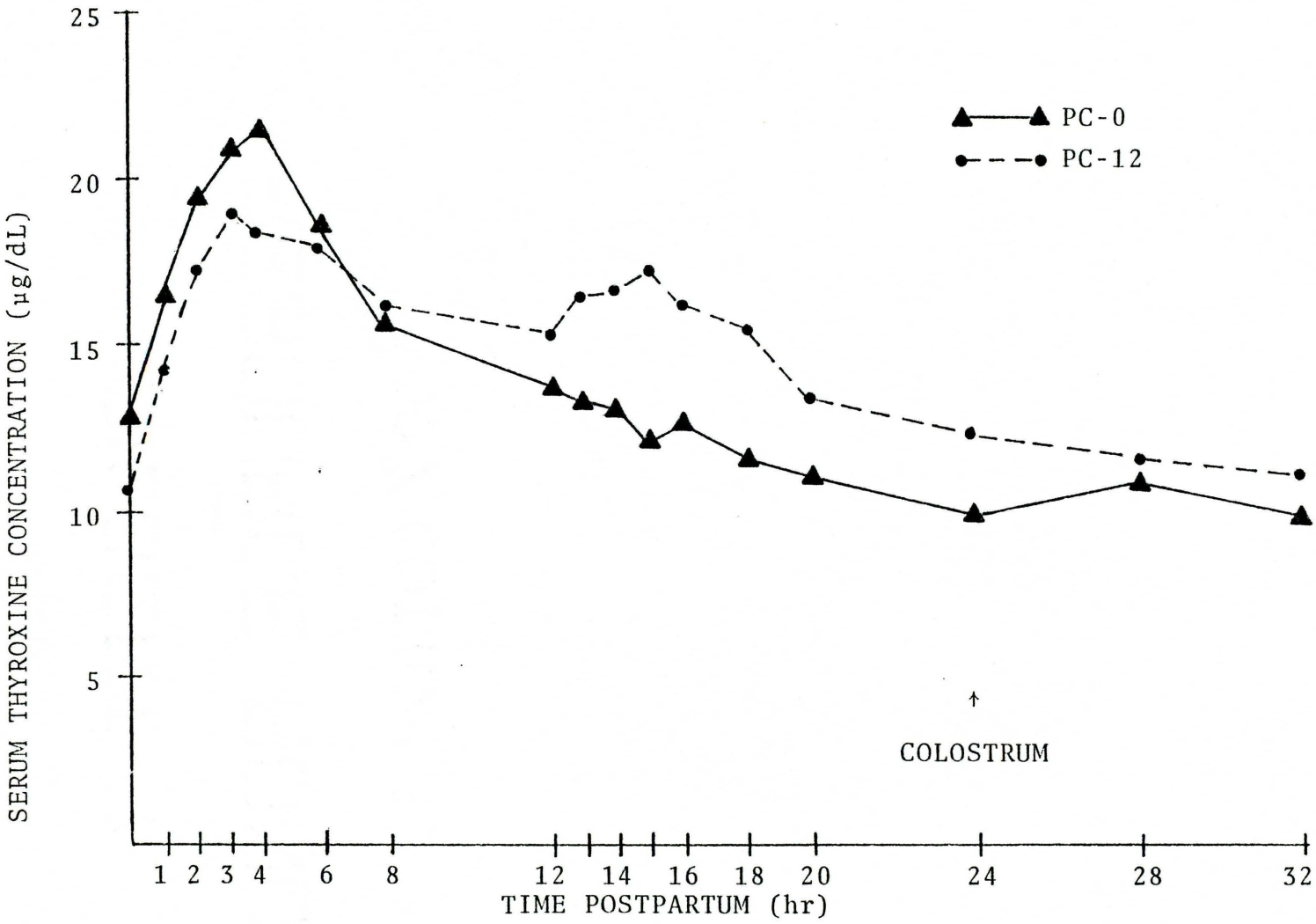

Figure 5. Serum thryroxine concentrations of calves at birth and after feeding pooled colostrum at either 0 or 12 hrs postpartum.

TABLE 7. Serum thyroxine concentrations (μg/dL) of calves at birth and after feeding milk plus immunoglobulin extract (IgEx) without colostrokinin at either 0 or 12 hrs postpartum.

HOURS POSTPARTUM	TREATMENT: IgEx-0 $\bar{X}$†	IgEx-0 SEM	IgEx-12 $\bar{X}$†	IgEx-12 SEM
0	12.0	1.3	12.1	1.4
1	14.7	1.4	13.4	1.2
2	18.7^{az}	1.3	16.9^{z}	1.1
3	18.6^{bz}	1.4	17.8^{z}	1.0
4	17.5^{cz}	1.3	18.3^{z}	1.1
6	14.6^{ab}	1.3	16.2	1.3
8	11.6^{c*}	1.9	15.9*	0.9
12	9.9	1.1	13.2	1.3
13	9.8*	1.2	14.8*	1.6
14	8.6**	1.0	15.3**	1.8
15	9.0**	1.1	15.4**	1.3
16	8.2**	1.4	14.2**	1.3
18	10.1	2.4	13.3	1.1
20	7.5**	0.8	12.1**	1.2
24	7.5	1.2	9.8	0.8
28	8.3	0.9	9.5	0.9
32	8.0	0.8	9.3	0.7

†Starting with the peak value after birth, every mean in the same column was tested against all means in a succeeding four hour period. Additional testing was not performed.

^{abc}Means in the same column bearing the same superscript differ significantly ($P<.05$).

^{z}Mean differs significantly from 0 hr mean in the same column ($P<.01$).

*Means in the same row differ significantly ($P<.05$).

**Means in the same row differ significantly ($P<.01$).

TABLE 8. Serum thyroxine concentrations (µg/dL) of calves at birth and after feeding milk plus immunoglobulin extract with colostrokinin extract (ColK) at either 0 or 12 hrs postpartum.

	TREATMENT			
	ColK-0		ColK-12	
HOURS POSTPARTUM	$\bar{X}$ †	SEM	$\bar{X}$ †	SEM
0	13.5	2.1	11.6[a]	1.5
1	14.2	2.0	13.1	1.7
2	17.7	2.3	14.5	0.9
3	18.2	3.0	16.4[a]	1.2
4	18.7	2.6	15.8	1.2
6	15.6	2.3	16.5	1.8
8	14.3	2.1	16.0	0.9
12	10.9	1.6	14.8	1.0
13	10.4*	1.6	15.5*	1.0
14	10.1**	1.7	17.1**	1.1
15	9.2**	1.8	17.0**	1.3
16	8.9**	1.7	16.1**	1.1
18	9.0**	1.6	14.6**	0.9
20	8.7	1.7	12.9	1.0
24	7.8	1.4	11.0	1.0
28	6.8	1.0	9.7	0.9
32	7.6	1.2	9.4	1.2

†Every mean in the same column was tested against all means in a succeeding four hour period. Additional testing was not performed.

[a]Means in the same column bearing the same superscript differ significantly (P<.05).

*Means in the same row differ significantly (P<.05).

**Means in the same row differ significantly (P<.01).

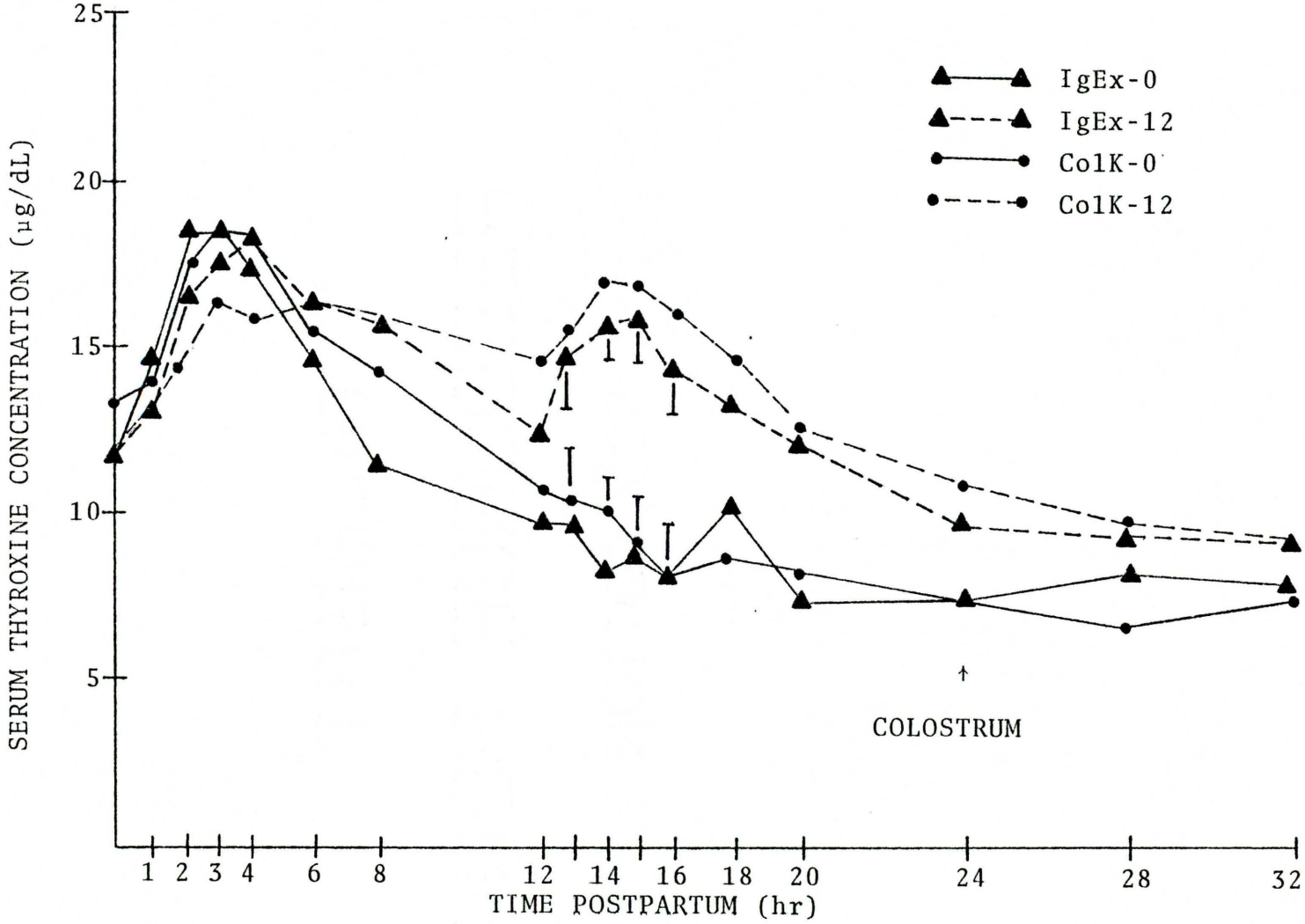

Figure 6. Serum thyroxine concentrations of calves at birth and after feeding milk plus immunoglobulin extract with and without colostrokinin extract at either 0 or 12 hrs postpartum.

TABLE 9. Serum thyroxine concentrations (µg/dL) of calves at birth and after feeding whole milk (M) at either 0 or 12 hrs postpartum.

	TREATMENT			
	M-0		M-12	
HOURS POSTPARTUM	$\bar{X}^{\dagger}$	SEM	$\bar{X}^{\dagger}$	SEM
0	14.7*	2.5	7.4*	1.2
1	16.9	2.9	11.6^{y}	1.2
2	19.3	2.5	14.7^{z}	1.4
3	22.7^{y*}	3.4	15.0^{z*}	1.5
4	21.7	2.4	16.1^{z}	1.8
6	21.3*	2.6	14.5*	1.3
8	19.2*	2.0	13.2*	1.5
12	17.7*	2.3	10.9*	1.2
13	13.7	1.7	12.1	1.3
14	15.2	4.0	11.6	3.7
15	14.8	2.0	11.8	1.1
16	15.8	2.1	12.0	1.0
18	15.1	1.8	11.6	0.9
20	13.8	2.3	9.5	1.2
24	14.5	1.8	10.1	1.3
28	14.8*	2.2	9.4*	0.8
32	13.0*	2.0	7.7*	1.1

†Starting with the peak value after birth, every mean in the same column was tested against all means in a succeeding four hour period. Additional testing was not performed.

^{y}Mean differs significantly from 0 hr mean in the same column ($P<.05$).

^{z}Mean differs significantly from 0 hr mean in the same column ($P<.01$).

*Means in the same row differ significantly ($P<.05$).

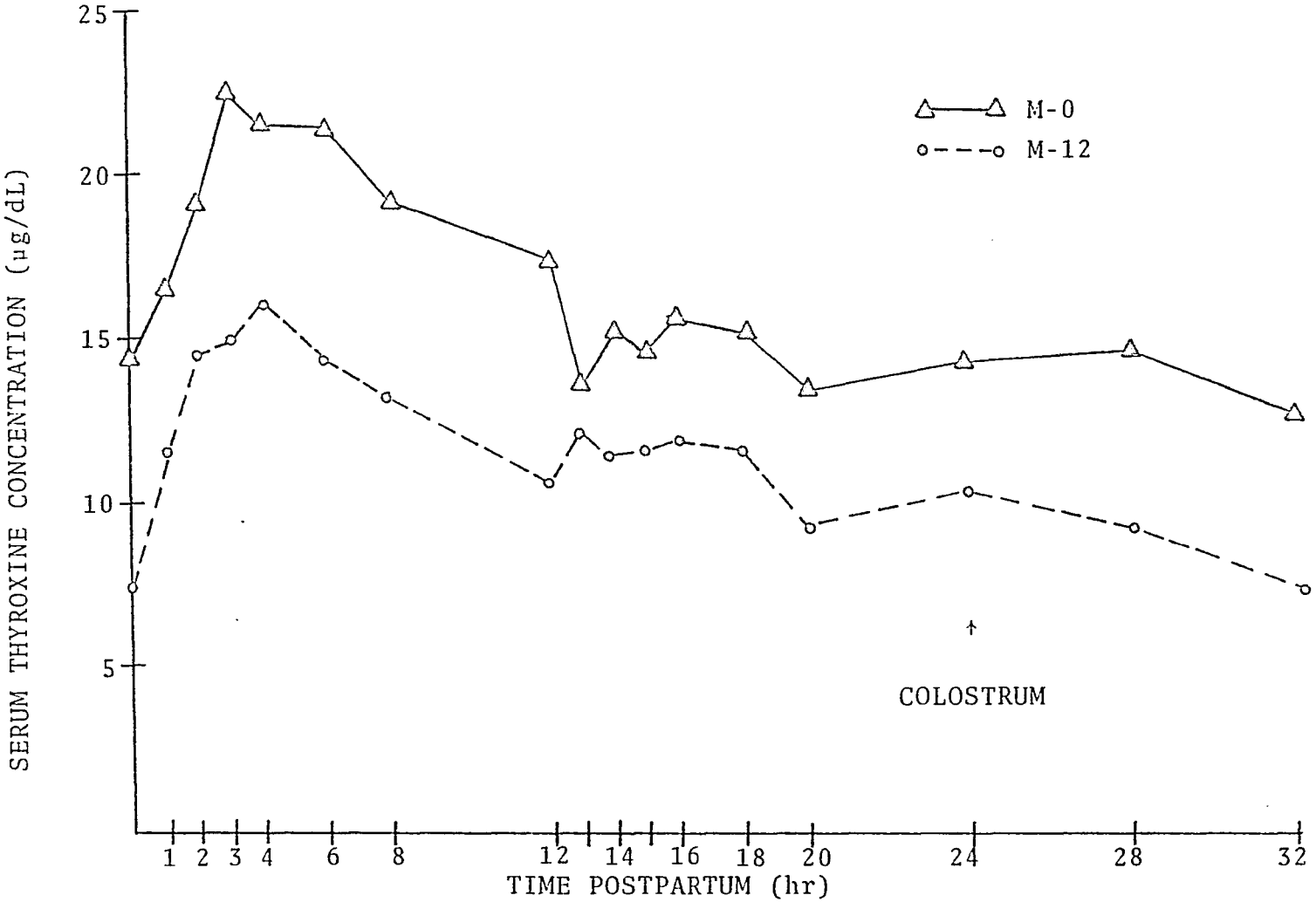

Figure 7. Serum thyroxine concentrations of calves at birth and after feeding whole milk at either 0 or 12 hrs postpartum.

This data differs from that reported by Hernandez et al. (1972), who found high thyroxine concentrations at birth followed by a rapid decline over the next four days. They suggested that high prenatal concentrations inhibited further T_4 release from the thyroid due to a negative feedback. In the present study, thyroxine levels increased by at least 50% immediately after birth.

The consumption of an assigned ration by fasted calves at 12 hours postpartum appeared to elicit an increase in thyroxine within one hour postfeeding. Serum IgG levels in IgEx-12 and ColK-12 calves were higher ($P<.05$) at 13-16 hours than their 0 hour counterparts. The possibility that the rise in serum thyroxine was in response to a particular constituent of colostrum, as found for cortisol, seems unlikely since calves fed milk at 12 hours also exhibited a slight increase.

Animals fed ration II at 0 hour postpartum (M-0) had significantly ($P<.05$) higher serum thyroxine concentrations at birth than did M-12 calves (Table 9; Figure 7). The thyroxine levels of M-0 calves remained higher at successive collection times for the duration of the sampling period. The standard error of the means for M-0 and M-12 calves at 0 hour were relatively low, thus eliminating the possibility of one or two calves causing a skew of the data. The sequence in which the serum was analyzed was by block; the serum of all calves in block 1 was analyzed prior to starting block 2. Therefore analytical error can be eliminated.

These results do allow for an interesting observation. The increase in serum thyroxine during the four hours immediately postpartum was nearly the same for M-0 and M-12 calves, 8.0 µg/dL and 8.7 µg/dL

respectively. The thyroid function in calves born with low T_4 levels did not appear to be suppressed or less sensitive compared to calves born with high T_4 concentrations.

Interaction of Colostrokinin and Ig Absorption

Calves were born with little or no IgG in serum, but animals fed ration I, III, or IV at birth attained an average serum IgG concentration of 23 mg/ml by 12 hours postfeeding (Table 10; Figure 8). Calves fed whole colostrum (ration I) at birth had lower ($P<.05$) IgG concentrations at 12-14 hours postfeeding than newborns given either of the artificially constituted rations (III and IV). This may have been due to the slightly lower Ig concentration of ration I versus rations III and IV (Table 1). However by 15 hours postfeeding, there was no significant difference in serum IgG concentrations of calves in these three treatment groups.

The lack of colostrokinin in ration III did not affect the absorptive abilities of the calves during a fifteen hour period after feeding. From 0 to 15 hours postpartum there were no differences in the amount of Ig agsorbed or the rate of absorption between IgEx-0 and ColK-0 fed calves (Table 10; Figure 8). After 15 hours postfeeding, colostrokinin appeared to exhibit a regulatory effect. Calves fed ration IV at birth (ColK-0) maintained serum IgG concentrations at an average peak value of 27.5 mg/ml. Following a second feeding of pooled colostrum at 24 hours postpartum, ColK-0 treated calves had an additional increase in serum IgG concentrations (Table 10). In contrast, IgEx-0 calves had a slight decrease in serum IgG after 15 hours postfeeding, and did not have any

TABLE 10. Serum IgG concentrations (mg/ml) of calves at birth and after feeding either pooled colostrum (PC), whole milk (M), or immunoglobulin extract with (ColK) and without (IgEx) colostrokinin extract at either 0 or 12 hrs postpartum.

HOURS POSTPARTUM	TREATMENT							
	PC-0	PC-12	M-0	M-12	IgEx-0	IgEx-12	ColK-0	ColK-12
0	0.1	0.1	0.1	0.2	0.1	0.1	0.3	0.0
1	0.7	0.1	0.1	0.3	0.1	0.1	3.1	0.0
2	5.9^{a}	0.1^{b}	0.1^{b}	0.3^{b}	8.0^{a}	0.1^{b}	8.5^{a}	0.0^{b}
3	10.5^{a}	0.1^{b}	0.1^{b}	0.3^{b}	14.1^{a}	0.1^{b}	13.4^{a}	0.0^{b}
4	12.7^{a}	0.1^{b}	0.1^{b}	0.3^{b}	17.0^{a}	0.1^{b}	16.4^{a}	0.0^{b}
6	15.8^{a}	0.1^{b}	0.1^{b}	0.3^{b}	18.8^{a}	0.1^{b}	19.7^{a}	0.0^{b}
8	18.0^{a}	0.1^{b}	0.1^{b}	0.3^{b}	22.7^{ac}	0.1^{b}	23.6^{c}	0.0^{b}
12	18.7^{a}	0.1^{b}	0.1^{b}	0.3^{b}	24.1^{c}	0.1^{b}	27.1^{c}	0.0^{b}
13	19.9^{a}	0.2^{b}	0.1^{b}	0.4^{b}	25.3^{c}	0.1^{b}	27.6^{c}	0.0^{b}
14	19.6^{a}	4.6^{b}	0.1^{b}	0.4^{b}	25.3^{c}	3.5^{b}	26.7^{c}	3.3^{b}
15	22.2^{a}	6.0^{b}	0.1^{b}	0.4^{b}	24.3^{a}	4.6^{b}	25.8^{a}	5.3^{b}
16	21.3^{a}	6.7^{b}	0.1^{b}	0.4^{b}	24.3^{a}	6.1^{b}	26.7^{a}	7.0^{b}
18	20.5^{a}	8.0^{b}	0.1^{c}	0.4^{c}	23.0^{a}	7.3^{b}	28.1^{d}	7.7^{b}
20	19.6^{a}	10.1^{b}	0.2^{c}	0.5^{c}	22.2^{a}	10.3^{b}	27.8^{d}	9.8^{b}
24	19.0^{a}	11.7^{b}	0.2^{c}	0.5^{c}	23.2^{ad}	13.2^{b}	27.8^{d}	11.8^{b}
28	20.7^{a}	13.4^{b}	2.1^{c}	1.9^{c}	22.7^{a}	19.1^{a}	30.8^{d}	12.0^{b}
32	24.3^{a}	15.9^{b}	2.9^{c}	2.0^{c}	22.8^{a}	22.0^{a}	30.7^{d}	12.2^{b}
AVE. SEM	1.6	1.7	1.0	1.0	2.6	2.6	3.0	1.6

^{a,b,c,d}Means in the same row bearing different superscripts differ significantly ($P<.05$).

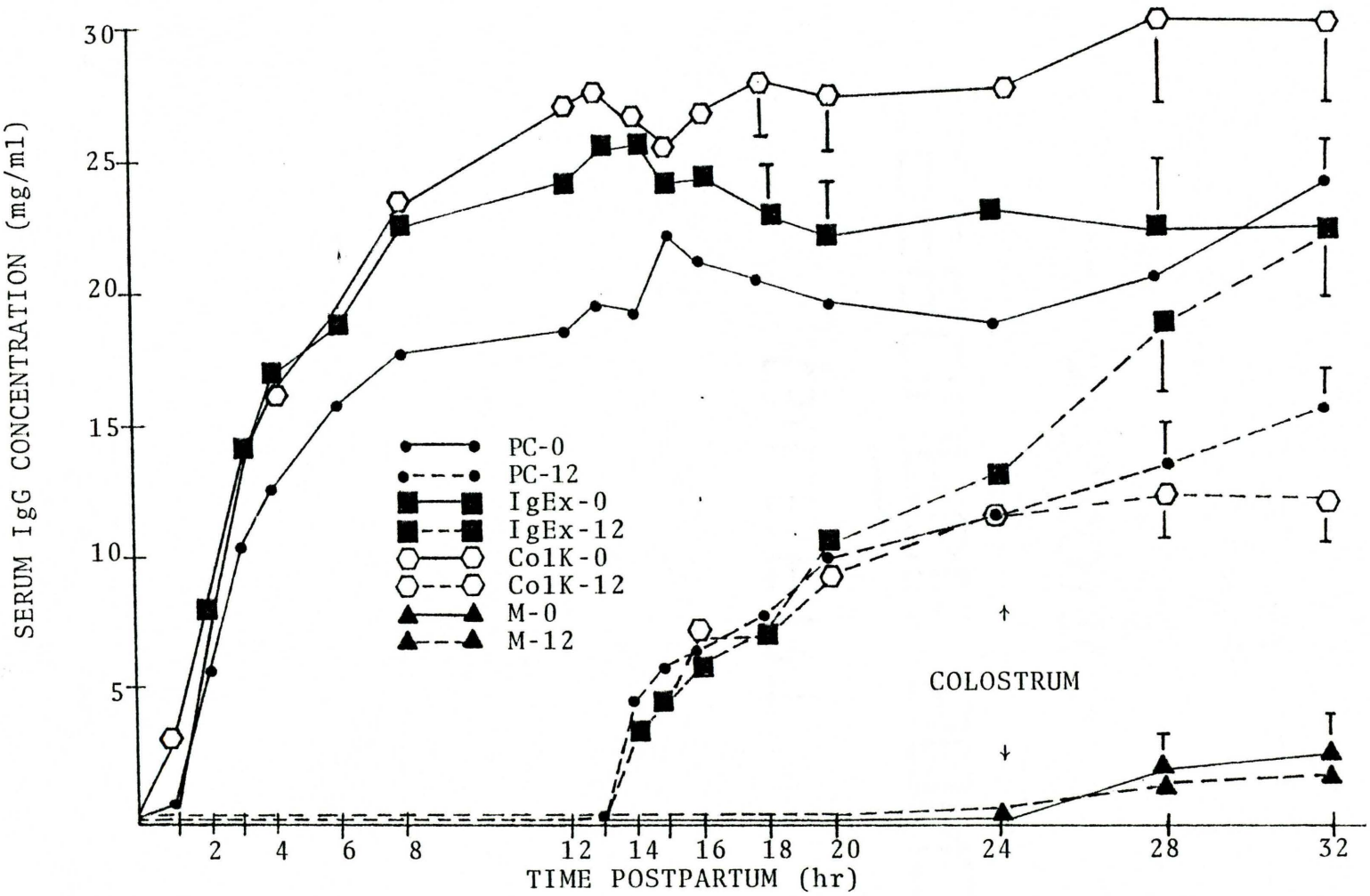

Figure 8. Serum IgG concentrations of calves at birth and after feeding pooled colostrum (PC), whole milk (M), or Ig extract with (Col K) or without (IgEx) colostrokinin extract at either 0 or 12 hours postpartum.

additional increase in serum IgG after the second, 24 hour feeding of colostrum. Serum IgG concentrations in IgEx-0 calves were lower ($P<.05$) than ColK-0 calves at all sampling times after 16 hours postpartum, except the 24 hour collection time (Table 10).

Apparently colostrokinin maintained an intestinal environment conducive to continued absorption of immunoglobulin or to reduced Ig degradation. Colostrokinin has been shown to increase capillary permeability and cause vasodilation, therefore it may have facilitated a continual movement of Ig into the circulation. However, Yamazaki and Moriya (1969a) have shown that the full active potential of colostrokinin in intestinal juice was maintained for a maximum of 30 minutes and then decreased to half of that activity after 60 minutes. In this study, a similar half-life for colostrokinin was also determined in in vitro tests. Therefore it seems improbable that colostrokinin would exert a direct regulatory effect 15-20 hours postfeeding. Other possibilities, however, do exist.

Altura (1979) presented evidence suggesting that the tissue hormone, bradykinin, may owe some of its dilator activity to its capability to stimulate the synthesis and release of prostaglandin-like compounds and that a great deal of bradykinin's microcirculatory properties may be due to mediation by prostaglandin-like compounds. It is possible that colostrokinin might also initiate and/or regulate other biologically active compounds which would exert regulatory effects at the intestinal level for a longer duration.

Evidence is accumulating to suggest that circulating steroids, such as glucocorticoids, can interact with the physiological actions of

kinins (Altura and Altura, 1974). Potentiation of the vasoconstrictor actions of catecholamines by glucocorticoids attenuate the vasodilatory actions of kinins during acute inflammation of tissue. This in turn modifies the movement of leukocytes and plasma across the capillary and venule walls. In the case of Ig absorption, glucocorticoids might be antagonistic to the action of colostrokinin. Although the present study showed that colostrokinin did not have an effect on the cortisol surge following Ig absorption, it does not rule out the possibility of an interaction between cortisol and colostrokinin at the intestinal level.

Calves that were fasted for twelve hours and then fed ration I, III, or IV absorbed colostral IgG at a slower rate than neonates fed at birth. By 12 hours postsuckling, fasted calves had not attained the magnitude of serum IgG concentrations as did their 0 hour counterparts twelve hours after suckling. The average serum IgG concentration in fasted calves of 13 mg/ml at 12 hours postfeeding (24 hours postpartum) was 43% less than the 12 hour postfeeding IgG level of calves fed at birth (Table 10; Figure 8). Stott et al. (1979b) found that the amount of absorbed immunoglobulin progressively decreased as the initial feeding time was sequentially delayed. At 24 hours postpartum, fasted calves fed ration I, III, or IV had approximately the same serum IgG content. In each case it was lower ($P<.05$) than the IgG concentrations of animals fed the same rations at birth (Table 10).

As in calves fed at birth, the lack of colostrokinin in ration III did not affect the absorptive abilities of the calves during at least a twelve hour period after feeding. From 12 to 24 hours postpartum there were no differences in the amount of immunoglobulin absorbed or the

rate of absorption between PC-12, IgEx-12, and ColK-12 calves (Figure 8). However, the presence of colostrokinin in the 12 hour feeding had a definite regulatory effect on subsequent serum IgG concentrations following the second feeding of whole colostrum at 24 hours postpartum. The response was the exact opposite of that exhibited by calves fed a source of colostrokinin at birth. Following the 24 hour feeding, IgEx-12 calves exhibited a two-fold increase in serum IgG concentrations over the next eight hours. By 32 hours postpartum, serum IgG concentrations in IgEx-12 calves were equal to IgG levels of PC-0 and IgEx-0 animals, attaining a peak concentration of 22 mg/ml. There is no reported evidence to date which demonstrates such a dramatic increase in serum immunoglobulin concentrations after a feeding of colostrum at 24 hours postpartum, especially following a period of inanition. In a natural system, calves that do not suckle by 12 hours postpartum remain hypogammaglobulinanemic (<10 mg/ml), and feeding colostrum after this time will not significantly increase the serum Ig concentration. These calves would exhibit a higher incidence of morbidity and mortality. However, by altering the ColK content in rations fed to fasted calves, the period of inanition did not affect the ability of the IgEx-12 calves to acquire relatively high levels of serum Ig.

In comparison, PC-12 and ColK-12 calves, groups in which colostrokinin was present in the 12 hour feeding, exhibited little or no increase in serum IgG concentrations following the 24 hour colostrum feeding. Serum IgG levels in PC-12 and ColK-12 calves were significantly lower ($P<.05$) than concentrations in IgEx-12 neonates at 28 and 32 hours postpartum (Table 10).

Colostrokinin exhibited a biphasic effect on Ig absorption which was dependent on the initial time of feeding. When colostrokinin was absent in a ration fed at birth (IgEx-0) no increase in serum IgG was observed after a 24 hour feeding. In contrast, the absence of colostrokinin in a ration fed to calves fasted for twelve hours (IgEx-12) resulted in a substantial increase in serum IgG levels after the 24 hour feeding. The regression curves in Figure 9 clearly show this biphasic phenomenon in calves fed rations which were identical except for the colostrokinin content. The findings also suggest that the regulatory function of colostrokinin apparently requires an approximate twelve hour period to manifest itself.

Additional studies are needed to explain the biphasic role of colostrokinin, but two points shoud be emphasized. First, the time duration between the first and second feeding for calves fed at birth was twenty-four hours. The interval for fasted calves was 12 hours, therefore exposing the intestinal tissue to additional ColK within a much shorter period. This difference may have had an influencing role on the intestinal response to colostrokinin. Second, changes in the absorptive function of the intestinal epithelium during the twelve hour period of inanition (possibly due to high serum cortisol levels at birth) could have been a predominate factor in determining the role of colostrokinin.

Calves fed milk at either 0 or 12 hours postpartum exhibited very low IgG concentrations through 24 hours postpartum due to the absence of immunoglobulin in milk. They absorbed little IgG following the 24 hour colostrum feeding as evidenced by their low serum IgG

TABLE 11. Orthogonal polynomial regression equations and R^2 values for serum IgG concentrations of calves at birth and after feeding milk plus immunoglobulin extract with and without colostrokinin extract at either 0 or 12 hours postpartum.

TREATMENT	REGRESSION EQUATION[a]	R^2
IgEx-0	$Y = -10.6 + 8.2(x) - 0.62(x^2) + 0.015(x^3)$	0.54
IgEx-12	$Y = 0.19 + 0.29(x) - 0.06(x^2) + 0.008(x^3)$	0.59
ColK-0	$Y = -9.0 + 7.8(x) - 0.57(x^2) + 0.01(x^3)$	0.51
ColK-12	$Y = -1.3 + 1.6(x) - 0.55(x^2) + 0.06(x^3) - 0.002(x^4)$	0.70

[a]Y equals the IgG concentration at a selected collection time (x); x equals 1 through 17.

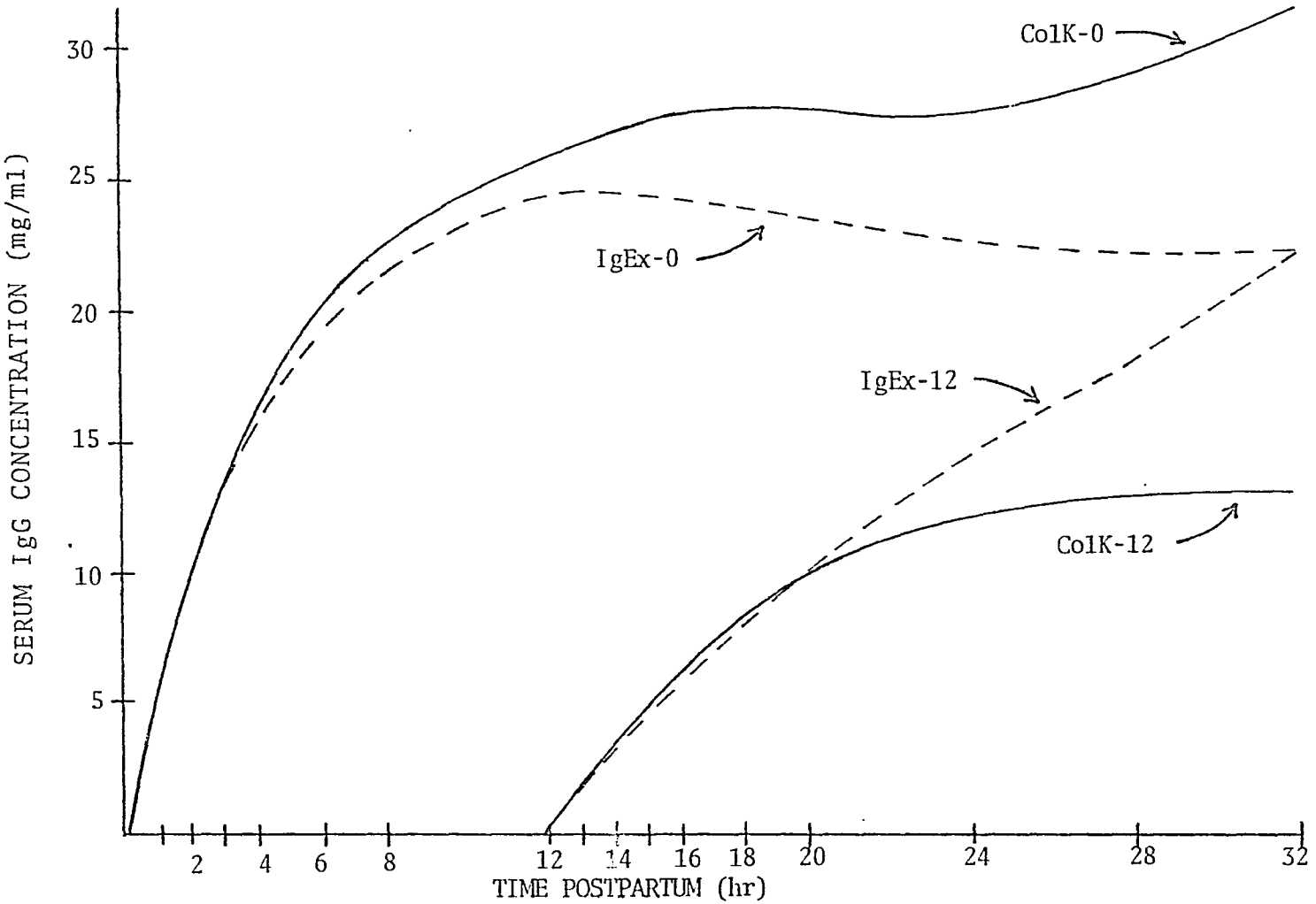

Figure 9. Regression curves for serum IgG concentrations of calves at birth and after feeding milk plus immunoglobulin extract with and without colostrokinin extract at either 0 or 12 hours postpartum.

concentrations at 28 and 32 hours postpartum (Table 10; Figure 8). Their serum IgG levels were significantly lower ($P<.05$) than all other treatment groups at the final two collection times.

CHAPTER 4

SUMMARY AND CONCLUSIONS

Sixty-two, newborn Holstein-Friesian calves were used to study the role of colostrokinin, serum cortisol, and serum thyroxine in the absorption of maternal immunoglobulin. Calves were removed from their dams prior to suckling and assigned one of four rations: colostrum (I), whole milk (II), milk plus colostral Ig (III), and milk plus Ig plus ColK (IV). Calves were fed their assigned ration either at birth or after twelve hours inanition. All calves were fed pooled colostrum at 24 hours postpartum. Blood samples were collected at seventeen times during the first 32 hours postpartum.

Calves were born with high cortisol concentrations (88 ng/ml) which decreased ($P<.05$) within two hours postpartum. Serum cortisol levels increased ($P<.05$) between two and three hours after calves ingested a colostral source of immunoglobulin. Time of initial feeding had no effect on the cortisol surge. No such increase was observed in calves consuming an immunoglobulin-free milk ration.

The evocation of an endocrine response in the neonate by maternal Ig suggests that this class of protein acted as a "primary messenger" and initiated an increase in cortisol secretion by the adrenal cortex. The cortisol surge may have a pivotal role inducing a cessation of macromolecular uptake by the intestinal epithelium. The increased cortisol concentrations might also have an immunosuppressive function in

the neonate. Even though maternal Ig is vital for adequate immunological protection, the calf might recognize these large proteins as foreign. A response by the endogenous immune system of the calf would seem contradictory to its acquisition of passive immune protection. Therefore the cortisol surge may have a dual purpose: cessation of Ig uptake and suppression of the endogenous immune system.

Within 4 hours postpartum, serum thyroxine concentrations increased ($P<.05$) at least 50% in all treatment groups regardless of whether the calves were fed or fasted. After peaking at 18 µg/dL, the serum thyroxine concentrations fell gradually throughout the duration of the collection period.

Colostrokinin exhibited a biphasic effect on serum IgG concentrations which was dependent on the initial time of feeding. Serum IgG concentrations in calves fed ration IV at birth were higher ($P<.05$) after 16 hours postpartum than neonates given ration III at birth. Calves initially fed ration IV exhibited an additional increase in serum IgG following a 24 hour feeding of colostrum. No increase in serum IgG was observed after the second feeding in calves initially fed ration III. The effect of ColK in fasted calves was the exact opposite of that in calves fed ColK at birth. Following a 24 hour feeding of colostum, calves initially fed ration III at 12 hours postpartum exhibited a two-fold increase in serum IgG concentration over the next eight hours. Calves fed ration IV at 12 hours postpartum had no increase in serum IgG concentration following the 24 hour colostrum feeding. Fasted calves fed ration III had higher ($P<.05$) serum IgG concentrations at 28 and 32 hours postpartum than fasted calves fed ration IV. Colostrokinin did

not have an immediate effect on Ig absorption. The regulatory function of ColK required an approximate twelve hour period to manifest itself. The presence or absence of ColK in the experimental rations did not have any effect on the cortisol or thyroxine profiles.

Colostrokinin appeared to have a definite role in the absorptive processes of the intestine. Its ability to increase capillary permeability suggests its involvement in the transfer of Ig to the circulation which was evidenced by calves fed immediately postpartum. However, this study has clearly shown that ColK actually prevented an increase in serum IgG concentrations if calves were fasted. Therefore, ColK seems to be involved in the cessation of Ig uptake, and may even be considered detrimental to the calf that does not immediately stand up and suckle. ColK may have more than one physiological role, and further research is needed to elucidate its multiple function.

APPENDIX A

BIOASSAY FOR COLOSTROKININ

The presence of ColK following the extraction procedure was verified by using an in vitro bioassay utilizing an isotonic muscle suspension. Sprague-Dawley rats were fasted for twenty-four hours prior to the start of the assay. Three to four centimeters of rat duodenum were suspended in 100 ml Tyrode muscle bath solution, oxygenated with 95% O_2, 5% CO_2 at 37 C. Changes in the intrinsic contraction/relaxation patterns were recorded electromanometrically on an ink writing physiograph (Beretta et al., 1972). The addition of ColK to the muscle bath stimulated a relaxation of the duodenal smooth muscle.

A primary consideration of this study was to semi-quantitate the activity of the ColK extract. It was intended that ration IV, containing a supplemental volume of ColK extract, would exhibit similar activity as that found in one liter of whole colostrum. In preliminary studies, the ColK activity of colostrum and the ColK extract were referenced to bradykinin. Bradykinin was used as a reference standard since it exhibits strikingly similar properties to colostrokinin especially in its ability to cause rat duodenum to relax. By using an identical bioassay as that used in this study, Beretta et al. (1972) have clearly shown that the two peptides are different entities.

Pooled colostrum was incubated with 0.05 BAEE units of kallikrein per milliliter of colostrum for ten minutes at 37 C. One-half milliliter of this mixture was then added to the muscle bath. An

immediate relaxation of the rat duodenum occurred. This relaxation pattern was similar to that exhibited by 25 ng of bradykinin per 100 ml Tyrode solution (Figure 10a,b). The volume of ColK extract constituting a portion of ration IV was adjusted in order that 0.5 ml of that ration caused a similar relaxation pattern as the bradykinin standard (Figure 10a). The colostrokinin extract comprised one-fourth the total volume of ration IV (Table 1). This amount was calculated to be derived from 1.5 liters of colostrum.

The presence or absence of colostrokinin in the other rations was also verified (Figure 10a,b). Kallikrein was incubated with rations II and III to confirm that the inactive molecule, colostrokininogen, was not present.

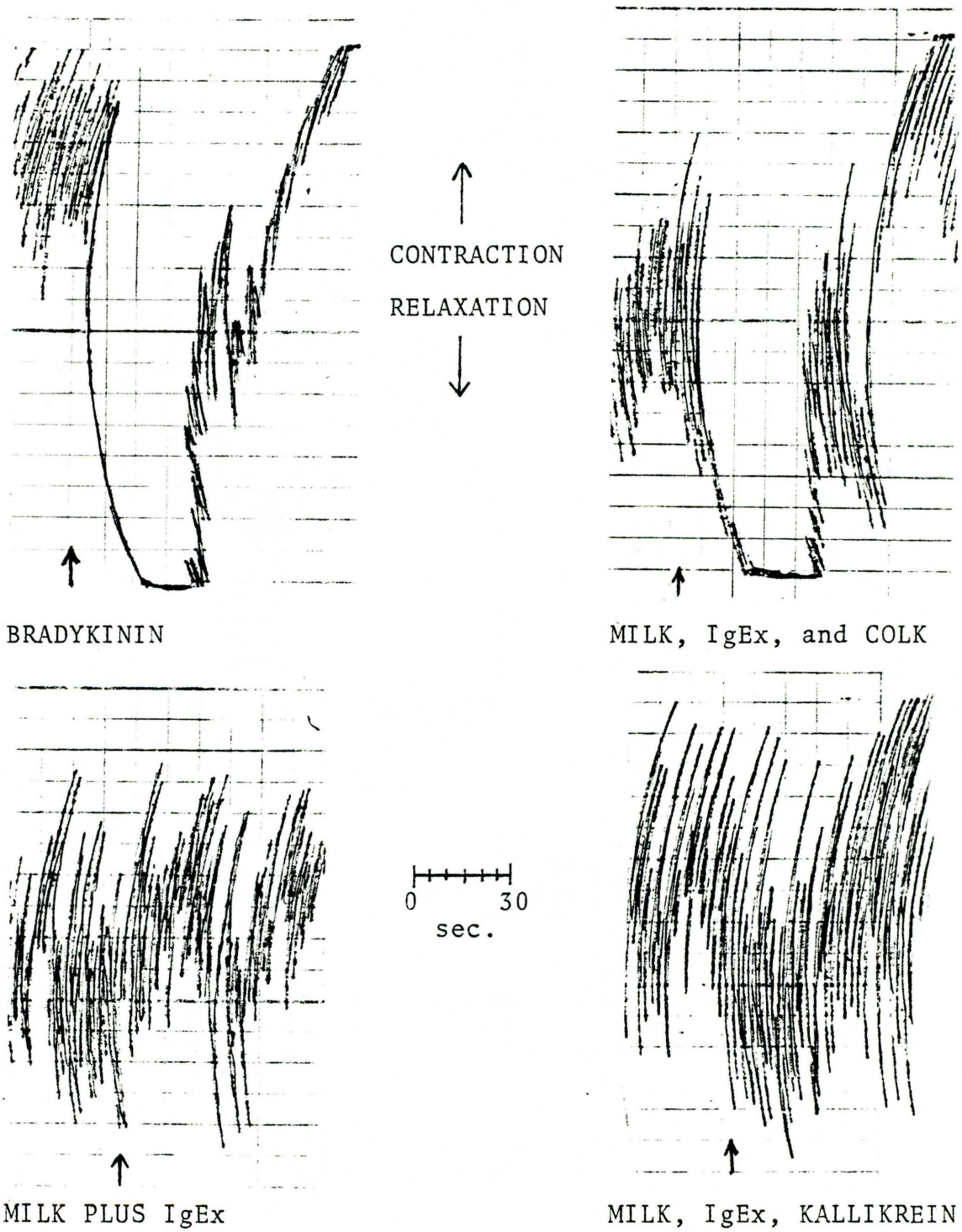

Figure 10a. The effects of the experimental rations on the intrinsic contraction patterns of rat duodenum due to colostrokinin. One-half milliliter added (↑) to 100ml Tyrode muscle bath solution. Bradykinin acted as reference standard.

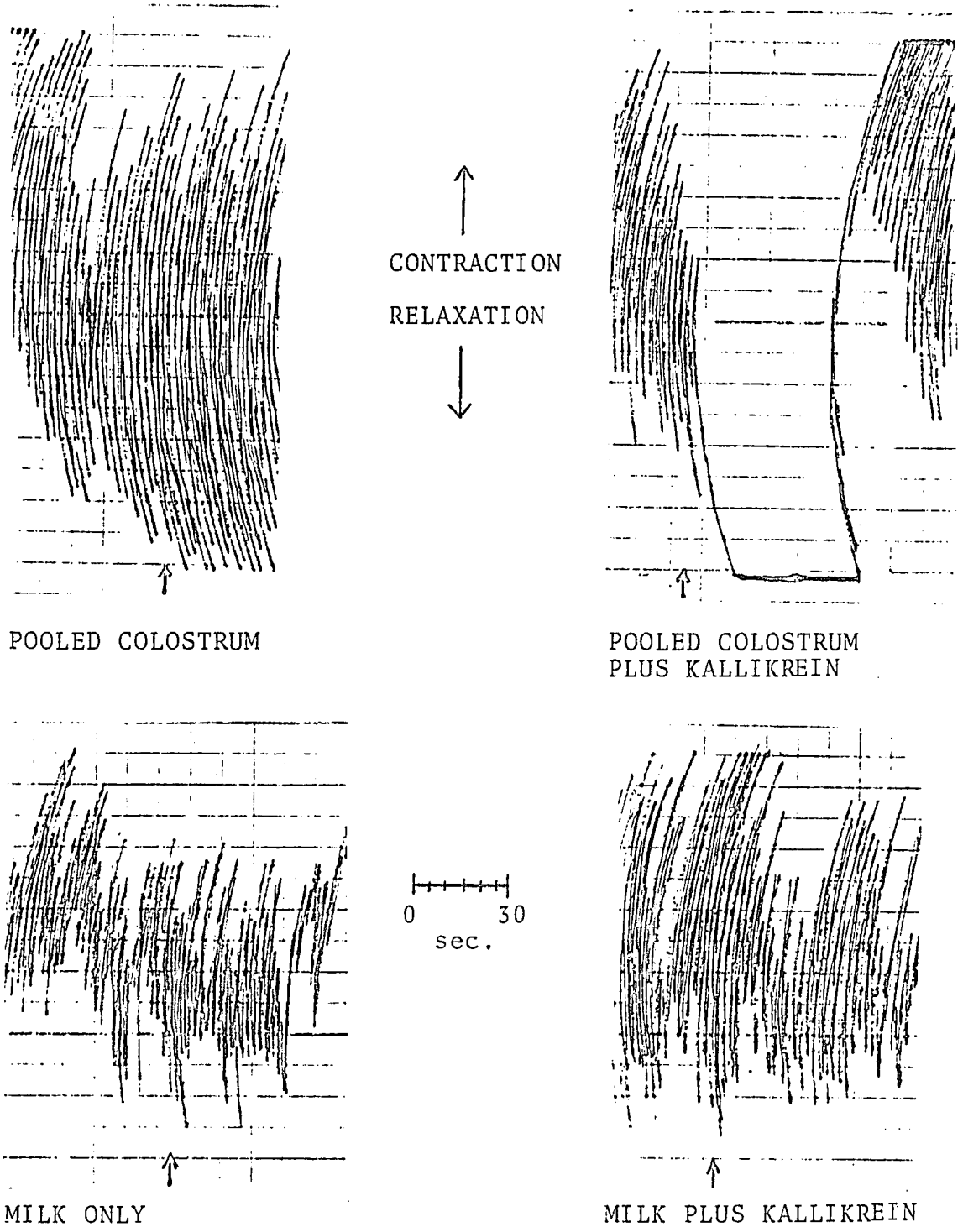

Figure 10b. The effects of the experimental rations on the intrinsic contraction patterns of rat duodenum due to colostrokinin. One-half milliliter added (↑) to 100ml Tyrode muscle bath solution.

APPENDIX B

ETHANOL DETERMINATION ASSAY

It is possible that the use of large quantities of ethanol (EtOH) in the ColK extraction procedure had a deleterious or contradictory effect on the bioassay used in this study. Therefore the pharmacological effect of ethanol on the spasmatic motility patterns of the rat duodenum were investigated.

A serial diultion of 95% EtOH was prepared as in Table 12. Each concentration was separately tested for its effects on the isotonic muscle suspension. An ethanol concentration of 475 µl/dL Tyrode solution caused the rat duodenum to relax in a manner similar to that produced by the presence of ColK or bradykinin standard. Lower concentrations had little or no measureable effect on the intestinal motility.

Following evaporation and volume reduction in the final step of the ColK extraction procedure, the preparation was tested for the presence of ethanol. An ethanol determination kit (Sigma Chemical Co.) was utilized for the measurement. The concentration of ethanol in the extracted ColK solution was found to be no greater than 0.95 µl/dL.

This test verified that any ethanol remaining in the ColK extract would not cause the rat duodenal suspension to relax. Therefore any relaxation that occurred could be attributed to the presence of ColK.

TABLE 12. The effects of ethanol (EtOH) on the relaxation/contraction patterns of rat duodenal smooth muscle.

% EtOH in physiological saline	Volume of diluted EtOH added to 100ml Tyrode muscle bath	Concentration of EtOH in 100ml Tyrode solution	Response of duodenal smooth muscle[a]
	ml	µl/dL	
95.0	0.5	475.0	+ + +
9.5	0.5	47.5	+
0.95	0.5	4.75	-
0.475	0.5	2.37	-
0.19[b]	0.5	0.95	-
Bradykinin	(25ng / 100ml Tyrode solution)		+ + +

[a] + signifies degree of relaxation.
- signifies no change in intrinsic motility pattern.

[b] Concentration of EtOH in colostrokinin extract.

APPENDIX C

TABLE 13. Analysis of variance for individual parameters after quantification of serum cortisol, thyroxine, and IgG concentrations.

Source of Variation	DF	MS	F	Significance of F[a]	
		CORTISOL			
Within Cells	507	534.0			
Collection Time	16	5,493.1	1.8	.000	*
Treatment x Collection Time	112	969.4	1.8	.000	*
Error	21	5,227.5			
Treatment	7	7,441.2	1.4	.248	NS
Block	3	13,302.3	2.5	.084	NS
		THYROXINE			
Within Cells	507	22.2			
Collection Time	16	493.1	22.2	.000	*
Treatment x Collection Time	112	19.5	0.9	.792	NS
Error	21	246.4			
Treatment	7	457.1	1.9	.129	NS
Block	3	140.8	0.6	.640	NS
		IgG			
Within Cells	507	22.6			
Collection Time	16	1,504.7	66.7	.000	*
Treatment x Collection Time	112	153.5	6.8	.000	*
Error	21	477.5			
Treatment	7	9,566.0	20.0	.000	*
Block	3	584.5	1.2	.326	NS

[a]NS represents nonsignificant F-test

* represents significant F-test

APPENDIX D

TABLE 14a. COMPARATIVE ASPECTS OF PASSIVELY ACQUIRED IMMUNITY IN VARIOUS SPECIES

SPECIES	MATERNAL PLACENTAL CLASSIFICATION	TRANSFER OF Ig FROM MATERNAL SERUM TO FETUS	ABSORPTION OF COLOSTRAL Ig	DURATION OF Ig ABSORPTION IF FED (TIME POSTPARTUM)	DURATION OF Ig ABSORPTION IF STARVED (TIME POSTPARTUM)
Bovine	Epitheliochorial	-	+	24 hr (1)	24 hr (1)
Ovine	Epitheliochorial	-	+	24-36 hr (2)	48 hr (2)
Porcine	Epitheliochorial	-	+	36 hr (3)	106 hr (3)
Canine	Endotheliochorial	-	+	24 hr (4)	N.A.*
Rat	Hemochorial	+	+	18-21 days (5)	18-21 days (5)

*N.A. - no reference available

(1) McCoy et al. (1970); (2) Lecce and Morgan (1962); (3) Payne and Marsh (1962); (4) Gillette and Filkins (1966); (5) Halliday (1959)

TABLE 14b. COMPARATIVE ASPECTS OF PASSIVELY ACQUIRED IMMUNITY IN VARIOUS SPECIES - continued

SPECIES	EFFECTS OF EXOGENOUS GLUCOCORTICOIDS ON ABSORPTIVE PROCESSES	EFFECTS OF EXOGENOUS THYROXINE ON ABSORPTIVE PROCESSES	PRESENCE OF COLOSTROKININ IN COLOSTRUM
Bovine	No inhibitory effect (1) Potentiates absorption (2)	No established effect (8)	+ (10)
Porcine	Potentiates absorption (3) Initiates closure (4)	N.A.*	N.A.
Canine	No established effect (5)	N.A.	N.A.
Rat	Causes precocious maturation of endocytes (6) Causes a cessation of Ig transport (7)	Causes precocious closure (9)	N.A.

*N.A. - no reference available

(1) Stott (1980); (2) Johnstone and Oxender (1979); (3) Patt and Eberhart (1976); (4) Payne and Marsh (1962); (5) Gillette and Filkins (1966); (6) Daniels et al. (1973); (7) Morris and Morris (1980); (8) Cabello et al. (1980); (9) Chan et al. (1973); (10) Guth (1959)

LITERATURE CITED

Abraham, G.E., J.E. Buster and R.C. Teller. 1972. Radioimmunoassay of cortisol. Anal. Let. 5:757.

Altura, B.M. 1979. Interactions of kinins with amines and peptides in the microcirculation. Advances in the Biosciences 17:47. G.L. Haverland and U. Hamburg (ed.). Pergamon Press, N.Y.

Altura, B.M. and B.T. Altura. 1974. Effects of local anesthetics, antihistamines, and glucocorticoids on peripheral blood flow and vascular smooth muscle. Anesthesiology 41:197.

Balfour, W.E. and R.S. Comline. 1962. Acceleration of the absorption of unchanged globulin in the newborn calf by factors in colostrum. J. Physiol. 160:234.

Beretta, C., G. Gallina, P.O. Ormas, and R. Faustini. 1972. Effects of a crude preparation of colostrokinin compared with those of bradykinin on isolated rat duodenal segments. Pharm. Res. Comm. 4:87.

Boyd, J.W. and R.A. Hogg. 1981. Field investigations on colostrum composition and serum thyroxine, cortisol and immunoglobulin in naturally suckled dairy calves. J. Comp. Path. 91:193.

Burton, K.A. and M.W. Smith. 1977. Endocytosis and immunoglobulin transport across the small intestine in the newborn pig. J. Physiol. 270:473.

Cabello, G. and D. Levieux. 1980. Comparative absorption of colostral immunoglobulin G1 and immunoglobulin M in the newborn calf: Effects of thyroxine, cortisol, and environmental factors. Annales de Recherches Veterinaires 11:1.

Cabello, G., D. Levieux and J. Lefaivre. 1980. The effect of intra-amniotic injections of thyroxine on the absorption of colostral IgG1 by the newborn calf. Brit. Vet. J. 136:193.

Campbell, D.H., G.S. Justine, C.E. Natalie and H.S. Dieter. 1970. Methods in immunology. 2nd Ed. W.A. Benjamin Inc., N.Y.

Casellato, M.M., G. Lugaro, P. Pasta, E. Manera, P. Ormas, and C. Beretta. 1977. A new approach to preparation and purification of colostrokinin from bovine colostrum. Experientia 33:485.

Chan, W.S., V.G. Daniels and A.L. Thomas. 1973. Premature cessation of macromolecule uptake by the young rat intestine following thyroxine administration. J. Physiol. 231:112P.

Corley, L.D., T.E. Staley, L.J. Bush and E.W. Jones. 1977. Influence of colostrum on transepithelial movement of Escherichia coli 055. J. Dairy Sci. 60:1416.

Daniels, V.G., R.N. Hardy, K.W. Malinowska and P.W. Nathanielsz. 1972. Adrenocortical hormones and absorption of macromolecules by the small intestine of the young rat. J. Endocr. 52:405.

Daniels, V.G., R.N. Hardy, K.W. Malinowska and P.W. Nathanielsz. 1973. The influence of exogenous steroids on macomolecular uptake by the small intestine on the newborn rat. J. Physiol. 229:681.

Deutsch H.F. and V.R. Smith. 1957. Intestinal permeability to proteins in the newborn herbivore. Am. J. Phys. 191:271.

Fahey, J.L. and E.M. McKelvey. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immunol. 94:84.

Ganong, W.F. 1981. Review of Medical Physiology. 10th Ed. Lange Medical Publications, California.

Gillette, D.D. and M. Filkins. 1966. Factors affecting antibody transfer in the newborn puppy. Am. J. Phys. 210:419.

Guth, P.S. 1959. Kinins produced from bovine colostrum by kallikrein and saliva. Brit. J. Pharmacol. 14:549.

Halliday, R. 1959. The effect of steroid hormones on the absorption of antibody of the young rat. J. Endocrinol. 18:56.

Hardy, R.N. 1969. The influence of specific chemical factors in the solvent on the absorption of macromolecular substances from the small intestine of the newborn calf. J. Physiol. 204:607.

Hernandez, M.V., K.M. Etta, E.P. Reinkee, W.D. Oxender and H.D. Hafs. 1972. Thyroid function in the prenatal and neonatal bovine. J. Anim. Sci. 34:780.

Hudson S., M. Mullford, W.G. Whittlestone and E. Payne. 1976. Bovine plasma corticoids during parturition. J. Dairy Sci. 59:745.

Husband, A.J., M.R. Brandon and A.K. Lascelles. 1973. The effect of corticosteroid on absorption and endogenous production of immunoglobulins in calves. Aust. J. Exp. Biol. Med. 51:707.

Johnstone, N.E. and W.D. Oxender. 1979. Effect of altered serum glucocorticoid concentrations on the ability of the newborn calf to absorb colostral immunoglobulin. Am. J. Vet. Res. 40:32.

Khan, M.A., W.M. Dickson and K.M. Meyers. 1970. The effect of low environmental temperature on plasma corticosteroid and glucose concentrations in the newborn calf. J. Endocrin. 48:355.

LaMotte G.B. and R.J. Eberhart. 1976. Blood leukocytes, neutophil phagocytosis, and plasma corticosteroids in colostrum-fed and colostrum-deprived calves. Am. J. Vet. Res. 37:1189.

Lecce, J.G. and D.O. Morgan. 1962. Effect of dietary regimen on cessation of intestinal absorption of large molecules (closure) in the neonatal pig and lamb. J. Nutrition 78:263.

Mancini, G., A.O. Carbonara and J.F. Heremans. 1965. Immunochemical quantitation on antigens by single radial immunodiffusion. Immunochemistry 2:235.

McCoy, G.C., J.K. Renneau, A.G. Hunter, and J.B. Williams. 1970. Effects of diet and time on blood serum proteins in the newborn calf. J. Dairy Sci. 53:358.

Moog, F. and K. Yeh. 1979. Pinocytosis persists in the ileum of hypophysectomized rats unless closure is induced by thyroxine or cortisone. Develop. Biol. 69:159.

Morris, B. and R. Morris. 1976. The effects of corticosterone and cortisone on the uptake of polyvinyl pyrrolidone and the transmission of immunoglobulin G by the small intestine in young rats. J. Physiol. 254:389.

Morris, B. and R. Morris. 1980. The effect of exogenous steroids and steroid inhibitors on IgG transmission in young rats. Biol. Neonate 38:169.

Muller, L.D., G.L. Beardsley, R.P. Ellis, D.E. Reed and M.J. Owens. 1975. Calf response to the initiation of parturition in dairy cows with dexamethasone or dexamethasone with estradiol benzoate. J. Anim. Sci. 41:1711.

Nightengale, G.T. and G.H. Stott. 1981. Adrenal response of the newborn calf to acute inanition and colostral feeding. J. Dairy Sci. 64:236.

Patt, J.A. 1977. Factors affecting the duration of intestinal permeability to macromolecules in newborn animals. Biol. Rev. 52:411.

Patt, J.A. and R.J. Eberhart. 1976. Effects of metyrapone and ACTH on intestinal absorption of immunoreactive bovine IgG in cesarean-derived pigs. Am. J. Vet. Res. 37:1409.

Patt, J.A., A. Zarkower, and R.J. Eberhart. 1972. Effect of histamine on intestinal absorption of gammaglobulin in newborn calves. J. Dairy Sci. 55:645.

Payne, L.C. and C.L. Marsh. 1962. Gamma globulin absorption in the baby pig: the nonselective absorption of heterologous globulins and factors influencing absorption time. J. Nutrition 76:151.

Staley, T.E., L.D. Corley, L.J. Bush and E.W. Jones. 1972. The ultra-structure of neonatal calf intestine and absorption of heterologous proteins. Anat. Rec. 172:559.

Steele, R.G.D. and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., N.Y.

Stott, G.H. 1980. Immunoglobulin absorption in calf neonates with special considerations of stress. J. Dairy Sci. 63:681.

Stott, G.H., D.B. Marx, B.E. Menefee, and G.T. Nightengale. 1979a. Colostral immunoglobulin transfer in calves. I. Period of absorption. J. Dairy Sci. 62:1632.

Stott, G.H., D.B. Marx, B.E. Meneffee, and G.T. Nightengale. 1979b. Colostral immunoglobulin transfer in calves. II. The rate of absorption. J. Dairy Sci. 62:1766.

Stott, G.H., D.B. Marx, B.E. Menefee, and G.T. Nightengale. 1979c. Colostral immunoglobulin transfer in calves. III. Amount of absorption. J. Dairy Sci. 62:1902.

Stott, G.H. and E.J. Reinhard. 1978. Adrenal function and passive immunity in the dystocial calf. J. Dairy Sci. 61:1457.

Werle, E. 1959. Polypeptides which affect smooth muscle and blood vessels. M. Schachter, ed. Pergamon Press, London.

Willett, L.B. and R.E. Erb. 1972. Short term changes in plasma corticoids in dairy cattle. J. Anim. Sci. 34:103

Yamazaki, K. and H. Moriya. 1969a. Isolation and purification of colostrokinin from bovine colostrum. Biochem. Pharm. 18:2303.

Yamazaki, K. and H. Moriya. 1969b. Properties of colostrokinin from bovine colostrum. Biochem. Pharm. 18:2313.

Zarkower, A. 1967. Histamine in the cow: pre- and postparturition histamine concentrations in plasma, milk and tissue. Am. J. Vet. Res. 28:1751.