This is to certify that the

thesis entitled

The Effect of Altered Serum Glucocorticoid
Concentrations on the Ability of the Newborn
Calf to Absorb Immunoglobulins

presented by

Noel Edgar Johnston

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Dairy Science

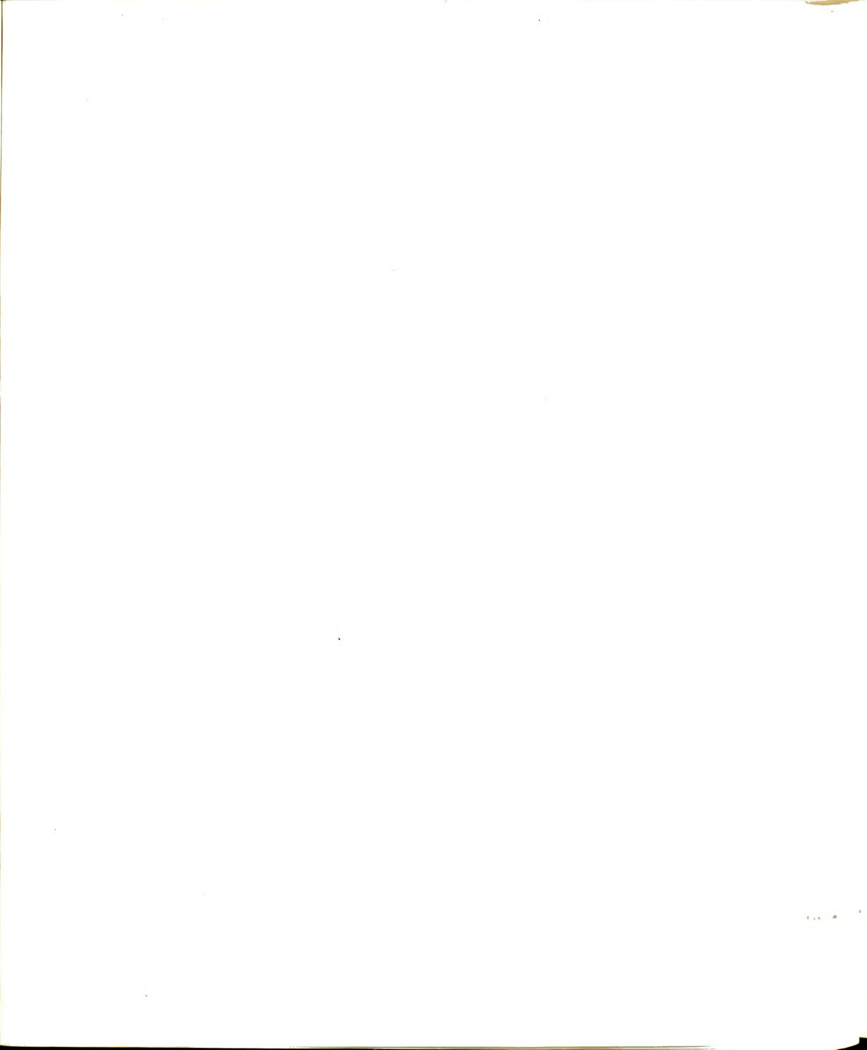
Wayne D. Oxender
Major professor

Date Feb 22, 1978

O-7639

118

007 4-8-93
113



THE EFFECT OF ALTERED SERUM GLUCOCORTICOID CONCENTRATIONS ON THE ABILITY OF THE NEWBORN CALF TO ABSORB IMMUNOGLOBULINS

By

Noel Edgar Johnston

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Dairy Science

1978

ABSTRACT

THE EFFECT OF ALTERED SERUM GLUCOCORTICOID CONCENTRATIONS ON THE ABILITY OF THE NEWBORN CALF TO ABSORB IMMUNOGLOBULINS

By

Noel Edgar Johnston

Many calves fed colostrum were not able to absorb adequate amounts of immunoglobulins. Glucocorticoids have been shown to induce premature cessation of absorption of macromolecules in neonatal rats, mice, and dogs. High serum glucocorticoid concentrations have been shown to be present at the time of birth in calves and they may have some definite role in the termination of immunoglobulin absorption. The objective of this experiment was firstly to increase or decrease serum glucocorticoid concentrations in the newborn calf, and then to determine the effect of altered serum glucocorticoid concentrations on the ability of the newborn calf to absorb colostral immunoglobulin, as shown by the serum concentration of immunoglobulin G (IgG).

Twenty-one male Holstein calves were obtained at birth and allocated to one of three treatment groups. One group received injections of 200 IU of synthetic β^{1-24} adrenocorticotropic hormone (ACTH), the second group received metyrapone (250 mg), and the control group was given only

6108481

the diluent. The injections were given intramuscularly in three milliliters of diluent immediately after the first blood sample was collected, and then at 3, 6, 12, 18, 24, and 30 hours of age. Blood samples were taken from the jugular vein starting at birth and continuing for 48 hours.

Colostrum from first and second milkings from Holstein cows was pooled and frozen. Warm water (.5 liter) was added to one liter of thawed, pooled colostrum and each calf received its first feed from a nipple bottle before it was two hours old. Further feedings were at 6, 12, 18, 24, and 30 hours of age, immediately after blood was collected and the injection administered.

Serum glucocorticoid concentrations in calves receiving ACTH were significantly elevated above those in the control calves from two hours through 36 hours of age. The serum glucocorticoid concentrations of metyrapone-treated calves rapidly declined so that by one hour of age they were significantly lower than the control group, and this difference lasted through 12 hours.

The serum IgG concentrations in the ACTH-treated calves were greater than those of the metyrapone-treated calves, with the IgG concentrations of the control calves intermediate, but differences were not significant, probably because of large variations in IgG concentrations within groups.

Serum glucocorticoid concentrations fell rapidly in the metyrapone-treated calves, and reached basal concentrations within six hours. Five of the seven calves attained

their maximum serum IgG concentration at 24 hours. This is in contrast to the ACTH-treated group in which only one of the seven calves had reached its maximum IgG concentration by that time.

The results of this experiment suggest that metyrapone, through its effect of decreasing serum glucocorticoid concentrations, may have reduced the ability of, or the time available for, the newborn calf to absorb immunoglobulins from colostrum, whereas the increased serum glucocorticoid concentration present in the ACTH-treated calves did not reduce the serum IgG concentration by 48 hours, but may have actually caused an increase.

Dedicated to my dear wife,
Joy,
for her love and support,
and to our three
little Americans,
Miriam,
Rebecca,
Paul

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to the following people who have had an important part in this dissertation:

To Mr. Arthur Nobile of Organon Pharmaceuticals, for the provision of synthetic ACTH for the preliminary work, and to Dr. J. J. Chart and Dr. Claire Engle of Ciba-Geigy Pharmaceuticals, for supplying synthetic ACTH and metyrapone for the main experiment.

To the Victorian Department of Agriculture, for allowing me study leave to complete this Ph.D., and to the Agricultural Experiment Station for funding me on the Calf Mortality Program.

To Dr. Bill Riley, Acting Chairman of the Department of Large Animal Surgery and Medicine, for the use of facilities and laboratory space in the Veterinary Clinical Center, and to Drs. Gabe Conner and Lou Newman for helpful advice and encouragement.

To Dr. Karin Kuersten for her assistance in some of the preliminary experiments.

To Duane and Velmer Green for the use of facilities and calves at Green Meadow Farms.

To Dr. Harold Hafs, Chairman of the Dairy Science Department, for the use of the facilities and laboratory in this department.

To Dr. Jim Schwalm for setting up the glucocorticoid assay and computer program.

To Drs. Roger Neitzel and John Gill for their assistance with the statistical analysis.

To my fellow graduate students, and to the technicians and secretaries, for their assistance in various ways.

To Dr. Allan Tucker for help with the early infusion experiments.

To Dr. Russ Erickson and Doug Lindsay for the provision of facilities and calves at the Dairy Barn.

To Dr. Pat Noden for helpful discussion on improving the assays.

To Dr. Ed Convey for standing in as chairman of my committee while Dr. Oxender was on sabbatical leave.

To Drs. Tal Huber, Gail Riegle, and Jeff Williams for being active members of my committee, and for giving much helpful advice and encouragement.

To my friend and colleague, Dr. Wayne Oxender, for his continual example, advice, and encouragement during both my Master's and Ph.D. programs.

And last, but not least, to my best friend and wife Joy, who has been a real help and encouragement these four years in America, as we have shared together in the good, and the difficult, times.

TABLE OF CONTENTS

Page

INTRODUCTION. 1

REVIEW OF THE LITERATURE. 4

Immunological Capability of the Fetal Calf . . 4
Immunological Capability of the Newborn Calf 6
Prenatal Transfer of Immunoglobulins 7
Postnatal Transfer of Immunoglobulins 8
Immunoglobulins in Serum, Colostrum, and Milk 10
Methods Used to Estimate Immunoglobulin Concentrations in Calves 12
Colostrum and Immunity 13
Absorption of Molecules Across the Intestine . 17
Transfer of Immunoglobulin from Gut Lumen. . . 20
Efficiency of Absorption of Immunoglobulins. . 22
Variation in Serum Immunoglobulin Concentrations in Calves. 25
Duration of the Absorption Mechanism for Immunoglobulins. 27
Factors Promoting Absorption of Immunoglobulins. 28
Factors Involved in the Termination of Absorption of Immunoglobulins. 29
Corticosteroids and the Absorption of Immunoglobulins. 31
Glucocorticoids in the Fetal and Newborn Calf 34
Stress and Absorption of Immunoglobulins . . . 35
Glucocorticoids and the Immune Response in the Newborn Calf 36
Induced Changes in Serum Glucocorticoid Concentrations 37

MATERIALS AND METHODS 41

Animals. 41
Procedure. 41
Experiments 1, 2 and 3. 41
Experiments 4 and 5 43
Experiment 6. 43
Experiment 7. 44
Laboratory Procedures. 47

Page

Glucocorticoid Assay. 47
Immunoglobulin G Assay. 51
Statistical Analysis. 51

RESULTS 52

Experiments 1, 2 and 3 52
Experiment 4 53
Experiment 5 54
Experiment 6 58
Experiment 7 60

DISCUSSION. 72

CONCLUSIONS 83

BIBLIOGRAPHY. 85

APPENDIX. 110

LIST OF TABLES

Table		Page
1	Schedule of times for blood sampling, injections, and feeding of newborn calves for experiment 7	46
2	Glucocorticoid concentrations of two groups of pooled sera with various amounts of 11-deoxycortisol added	50
3	Serum glucocorticoid concentrations (ng/ml) during infusion of corticosterone or cortisol	53
4	Serum glucocorticoid concentrations (ng/ml) after intramuscular injection of 100 IU of pituitary extract ACTH	54
5	Serum glucocorticoid concentrations (ng/ml) after intramuscular injection of 200 IU of pituitary extract ACTH	55
6	Serum glucocorticoid concentrations (ng/ml) during infusion of synthetic β^{1-24} ACTH or diluent	59
7	Comparison of mean serum glucocorticoid concentrations (ng/ml) of the control and ACTH groups, and the control and metyrapone groups	61
8	Mean ± S.E. of serum IgG concentrations (mg/ml)	66
9	Mean ± S.E. of serum IgG concentrations (mg/ml), excluding three calves which died within 24 hours of end of experiment	69
A1	Health history of calves in experiment 7	110
A2	Serum glucocorticoid concentrations in the control calves	111
A3	Serum glucocorticoid concentrations in the ACTH-treated calves	112
A4	Serum glucocorticoid concentrations in the metyrapone-treated calves	113

Table		Page

A5 Serum IgG concentrations (mg/ml) of the control group calves 114

A6 Serum IgG concentrations (mg/ml) of the ACTH group calves. 114

A7 Serum IgG concentrations (mg/ml) of the metyrapone group calves. 115

LIST OF FIGURES

Figure		Page
1	Mean serum glucocorticoid concentrations (ng/ml) after IM injection of 200 IU of pituitary extract ACTH	57
2	Mean serum glucocorticoid concentrations (ng/ml) of control, ACTH, and metyrapone groups	64
3	Mean serum IgG concentrations (mg/ml) of control, ACTH, and metyrapone groups	68
4	Mean serum IgG concentrations (mg/ml) of control, ACTH, and metyrapone groups, excluding three calves which died within 24 hours of the end of the experiment.	71

INTRODUCTION

Calves are born with very low serum immunoglobulin concentrations and thus lack protection against the infectious agents in their environment. To increase their survival rate, calves must ingest colostrum, the first milk of the cow, which is rich in immunoglobulins. These immunoglobulins are absorbed unchanged across the epithelium of the small intestine and reach the blood serum by way of the lymphatic system.

Many workers have shown that calves deficient in serum immunoglobulin have a much reduced survival rate. Some calves which have ingested colostrum may still have inadequate amounts of serum immunoglobulin. In 1971 Fey reported that 175 of 191 calves which died with colisepticemia were hypo- or agammaglobulinemic, even though all had received colostrum in their first day of life.

The absorptive mechanism, whereby the immunoglobulins pass unchanged across the intestinal epithelium, lasts for 24 hours or less after birth. Selman et al. (1971a) suggested that calves should be given their first feeding of colostrum within six hours of birth to insure good absorption of the immunoglobulins. But Gay et al. (1965) reported that some calves appeared to have lost the ability to absorb

colostral immunoglobulins even by four to six hours after birth.

Halliday (1959) demonstrated in mice and rats that adrenal corticosteroids were involved in the cessation of absorption of immunoglobulins by the small intestine. Duodenal alkaline phosphatase increased at the time of normal shutdown of macromolecular transfer. Both the increase in duodenal alkaline phosphatase and the decline in absorptive ability of the gut could be induced prematurely by the administration of large doses of deoxycorticosterone acetate or cortisone acetate. The initial effect of cortisone was to increase the macromolecular uptake, but this was followed by complete cessation of absorption (gut closure) within two days of receiving the hormone. Later work verified these findings and also showed that bilateral adrenalectomy in rats could delay the normal gut closure for several days (Daniels and Hardy, 1972; Daniels et al., 1972).

Serum glucocorticoid concentrations are low in the bovine fetus and begin to increase about 10 days before birth, reaching a peak around the time of parturition. This increase in glucocorticoid concentration is believed to be involved in the induction of parturition (Liggins et al., 1973). High concentrations of glucocorticoids also induce the maturation of various fetal enzyme systems (Moog, 1953; DeLemos et al., 1970; Moscona, 1975), as well as increasing glycogen storage in the liver (Weber et al., 1964; Holt and Oliver, 1968). Simpson-Morgan and Smeaton

(1972) suggested that glucocorticoids may be involved in the termination of absorption in the newborn calf.

The objective of these experiments was, first of all, to increase or decrease serum glucocorticoid concentrations in the newborn calf and then to determine the effect of altered serum glucocorticoid concentrations on the ability of the newborn calf to absorb colostral immunoglobulins, as shown by the serum concentration of immunoglobulin G.

REVIEW OF THE LITERATURE

Immunological Capability of the Fetal Calf

Although calves normally are born with very low serum immunoglobulin concentrations, they are capable of an immune response. While *in utero* their antigenic stimulation is limited since they usually do not come in contact with infectious agents. But some antigenic stimulation does occur in the bovine prenatally, as shown by Miller and Hubbert (1972), who found heteroagglutinins against red blood cells of other species from the fifth month of gestation onwards in a sizable percentage of fetuses, and by Collins et al. (1970), who detected some antibacterial activity in fetal sera in the latter half of gestation. Schimmel (1967) reported *Escherichia coli (E. coli)* O agglutinins in the sera of 59 of 85 newborn calves tested before they had been given colostrum. Using immunofluorescence techniques, Schultz et al. (1973) discovered immunoglobulin M (IgM)-containing cells in bovine fetuses in the first trimester of pregnancy, but immunoglobulin G (IgG)-containing cells were not seen until 140-155 days. The lymphoid tissues developed more rapidly in the presence of bacterial or viral infections, but even without any obvious antigenic stimulation lymphoid development of the thymus occurred at 42 days of gestation and a structural spleen

could be identified at 55 days. Some peripheral lymph nodes were present at 60 days, with mesenteric lymph nodes appearing by 100 days of gestation.

Serum IgM was detected by 130 days and serum IgG by 145 days, even though at very low concentrations (Schultz et al., 1973). However, Rossi et al. (1976) reported finding an IgG concentration of 3.2 mg/ml in the heart-blood serum of a 120-day-old bovine fetus and 5.2 mg/ml in a fetus estimated to be at 180-210 days of gestation. They could find no evidence of viral infection in these two calves.

Many congenital infections stimulate precocious production of immunoglobulins (Sawyer et al., 1973). Osburn et al. (1974) gave a list of infectious agents and the earliest fetal age at which specific immunoglobulin activity against them had been reported. Immunologic maturity varied in the ovine fetus according to the antigen involved (Silverstein et al., 1963; Fahey, 1974), and this may well be the case with the bovine fetus (Osburn, 1973). Fetal calves may also vary in their individual ability to respond to immunologic challenge, as shown by Renshaw et al. (1977) in their evaluation of bovine fetal lymphocytes using phytomitogens. Playfair (1968a,b) conducted experiments on neonatal mice which showed that the rate at which the antibody response to certain antigens developed in the postnatal period was under genetic control. Congenital infections in the human fetus resulted in the production of specific immunoglobulins, and high concentrations of IgM, which did not

cross the placenta, have been found in cord blood at birth (Adinolfi and Billington, 1976). However, human fetuses infected before the sixth month of gestation may not have this response.

Several workers have made use of the fact that the bovine fetus is immunologically competent to many antigens and have vaccinated fetal calves against *E. coli* or reovirus (Gay, 1971, 1975; Conner et al., 1973, 1977; Conner and Carter, 1975; Olson and Waxler, 1977; Wamukoya and Connor, 1976). While this procedure has met with some success, it is not yet in general use and further improvements are required.

Immunological Capability of the Newborn Calf

Even after a calf is born its immunological system is not fully mature. Calves less than two weeks old do not produce immunoglobulins in response to *Salmonella pullorum* vaccination, or to *Klebsiella* polysaccharide if it is administered before the calf is four weeks old (Smith and Ingram, 1965). When Renshaw et al. (1976) tested serum and peripheral blood leukocytes from pre-colostral newborn calves and their dams for bactericidal activity, they found that serum of newborn calves was unable to kill *E. coli* or *Staphylococcus aureus (S. aureus)* organisms. Even so, the leukocytes from the newborn calves were as bactericidal to *E. coli* and *S. aureus* as leukocytes from their dams when both were incubated in the same adult sera. Orlowski et al. (1976) reported a similar finding in the newborn human. The

bactericidal capacity of monocytes from babies was similar to that of monocytes from adults.

Prenatal Transfer of Immunoglobulins

Prenatal transfer of maternal immunoglobulin occurs in many species, such as rat, guinea pig, rabbit, monkey, and man (Brambell, 1958, 1970). But no such transfer has been found in ruminants. Miller (1966) listed the bovine species as having minimal placental transfer, but both references he cited (Mason et al., 1930; Brambell, 1958) reported finding no transfer of immunoglobulins across the placenta in the cow. Grosser (1909, 1927) classified the mammalian placentae into groups depending on the number of layers of fetal and maternal tissues between the blood supply of each. He considered that the placenta acted as an ultrafilter between the mother and the fetus. In 1931 Needham put forward the hypothesis that a reciprocal relationship existed between the number of layers of tissue separating the maternal and fetal circulations and the size of molecules or particles which could pass through this "placental barrier." Although this theory is still held by some today, Brambell et al. (1951) showed convincingly that prenatal transfer of immunoglobulins occurred across the yolk sac instead of, or as well as, across the placenta. Schlamowitz (1976) also felt the ultrafilter theory was unacceptable because the prenatal transfer of immunoglobulins is an active and selective process which is probably mediated by membrane receptors specific for IgG and its F_c fragment.

Since there is no prenatal transfer of immunoglobulins from the cow to its fetus, most calves are born with only trace amounts of immunoglobulin in their sera (Pierce, 1955; Kniazeff et al., 1967; Klaus et al., 1969; Penhale et al., 1970; Merriman, 1971; LaMotte, 1977). However, these minimal amounts are too low to provide the calf with any degree of immunity. To obtain sufficient amounts of maternal immunoglobulin, the newborn calf must ingest colostrum.

Postnatal Transfer of Immunoglobulins

Colostrum is the first milk of the cow secreted after parturition and is very rich in vitamin A, carotene, vitamin E, albumin, and immunoglobulins (Jacobson, 1970). Most of the immunoglobulin in the colostrum and milk of ruminants is not synthesized locally in the mammary gland but is selectively derived from the blood serum (Pierce and Feinstein, 1965; Lascelles, 1969). IgG_1 and IgG_2 are the predominant immunoglobulins in the serum of ruminants. They have similar physical properties and share common antigenic determinants. Although the serum concentrations of IgG_1 and IgG_2 are nearly the same, IgG_1 occurs in bovine colostrum at a much higher concentration than IgG_2. Micusan and Borduas (1976) suggested that this predominance of IgG_1 in the mammary gland secretion was due to the existence of a selective mechanism of transfer which could distinguish between the F_c fragment of IgG_1 and IgG_2. Lascelles (1969, 1971) proposed that the selective transfer of IgG_1 was a function of specific

receptor sites on the basal or intercellular membrane of the glandular epithelial cell. During the formation of ruminant colostrum there was no change in the serum concentration of IgG_2, but the concentration of serum IgG_1 decreased significantly as it was transferred into the mammary secretion (Brandon et al., 1971; Brandon, 1975; Cripps et al., 1976).

IgM and especially IgA were found in higher concentration in colostrum compared to serum. This may have been due to a selective concentrating of the IgM and IgA, but some synthesis very likely occurred within the mammary gland (Lascelles, 1971; Porter, 1973b). Cripps et al. (1976) reported no significant decrease in serum IgM or IgA around the time of parturition, as was found with IgG_1, and suggested that most of the IgM and IgA was produced locally. But Ciupercescu (1977) did find a significant decrease in serum IgM in ewes at the same time as the drop in serum IgG_1, and therefore a selective transfer of IgM from the serum to the colostrum may be of some importance. Plasma cells, which were present in large numbers during the formation of colostrum, probably produced the IgA and IgM (Campbell et al., 1950; Watson and Lascelles, 1973). In support of this, Porter (1972) observed high concentrations of IgA, with activity against *E. coli*, in colostrum and with no relationship to that found in serum. Butler et al. (1967) determined from their research on IgA in nasal secretions that secretory IgA was not assembled from serum IgA but was synthesized *de novo*. This probably applied to the mammary gland too. IgA appeared as secretory IgA in colostrum, and an excess of secretory component in its free

form was present in colostrum and milk (Mach, 1970; Porter, 1971). This "transport piece" was formed in cells different from those responsible for production of the IgA and other immunoglobulins (South et al., 1966).

<u>Immunoglobulins in Serum, Colostrum, and Milk</u>

Various workers have measured serum and colostrum immunoglobulin concentrations in cows at the time of parturition (Klaus et al., 1969; Butler et al., 1971a; Mach and Pahud, 1971; Porter, 1971; Butler, 1971, 1973; Penhale et al., 1973; Brandon and Lascelles, 1975). The reported values varied widely between workers, and this variation may have been due to different times of sampling with respect to parturition (Brandon et al., 1971). Large variations were found in the concentrations of immunoglobulins taken from the same quarter of the same cow on the same day (Kiddy et al., 1971), as well as between different quarter samples from the one cow (Wilson et al., 1972), and this too could explain some of the large differences.

Total IgG values ranged from 12.9 to 26.4 mg/ml in serum, and from 34.1 to 43.3 mg/ml in colostrum. In other cases, IgG_1 was measured, and it was 10.5 to 17.1 mg/ml in serum, but 8.9 to 75.0 mg/ml in colostrum. On the other hand, although serum IgG_2 was 7.9 mg/ml, colostrum IgG_2 was in the range of only 1.9 to 3.67 mg/ml. These figures showed that IgG_1 was concentrated in colostrum, but little IgG_2 was present. IgM was also increased in colostrum from concentrations of 2.5 to 4.57 mg/ml in serum up to 3.2 to 5.79 mg/ml

in colostrum. The most marked increase in concentration occurred with IgA. Serum IgA values varied from 0.08 to 0.78 mg/ml, whereas in colostrum the concentrations reached ranged from 1.46 to 7.14 mg/ml.

The high immunoglobulin concentration found in colostrum fell very rapidly after parturition, and during the first 48 hours there was a six-fold reduction in the total protein content (Porter, 1971, 1972). In the first 24 hours total protein was halved, IgG_1 and IgG_2 were decreased to one-third, IgM to one-tenth, and IgA to one-half. Further reductions occurred so that by three days *post partum* the mammary secretion was approaching the composition of milk. Hoerlein and Jones (1977) stated that colostrum collected 12 hours after calving contained only one-third to one-half of the IgG concentration of colostrum at two hours *post partum.* By 36 hours the colostrum IgG concentration was 10% or less of that at two hours. Total IgG content declined in a similar fashion to the concentration of IgG.

Comparative immunoglobulin concentrations of colostrum and milk have also been measured (Butler, 1971; Butler et al., 1971a; Kiddy et al., 1971; Mach and Pahud, 1971; Wilson et al., 1972; Butler, 1971, 1973; Brandon and Lascelles, 1975). Total IgG has been reported as 17.6 and 35.4 mg/ml in colostrum, but only 0.69 and <2.0 mg/ml in milk. The IgG_1 concentration in colostrum ranged from 8.9 to 75.0 mg/ml, and 0.29 to 1.38 mg/ml in milk. Reported values for IgG_2 were 1.9 to 3.67 mg/ml in colostrum, and 0.02 to 0.06 mg/ml in milk. The concentration of IgM was 3.46 to 5.79 in colostrum, but

only 0.04 to 0.34 in milk. IgA concentrations also decreased from 1.2 to 4.4 mg/ml in colostrum down to 0.05 to 0.47 mg/ml in milk.

Methods Used to Estimate Immunoglobulin Concentrations in Calves

Because calves with low serum immunoglobulin concentrations are more susceptible to disease, it is desirable to identify them early so that remedial treatment can be instituted. Aschaffenburg (1949) modified a serum turbidity test used in humans for the detection of increases in serum immunoglobulins (Kunkel, 1947) and used it in calves to demonstrate the presence of even small amounts of immunoglobulins in their sera. McEwen et al. (1970c) standardized this zinc sulfate turbidity test and showed a high correlation between the turbidity developed in the test and the actual concentrations of IgG and IgM present. McBeath et al. (1971) used a refractometer to estimate plasma immunoglobulin concentrations in newborn calves. This past year a number of researchers have reported on their examinations of various methods for measuring immunoglobulin concentrations quickly and accurately (LaMotte, 1977; Pfeiffer and McGuire, 1977; Pfeiffer et al., 1977; Naylor and Kronfeld, 1977; Naylor et al., 1977). The methods they looked at were single radial immunodiffusion, zinc sulfate turbidity, sodium sulfite precipitation, serum electrophoresis, and plasma or serum total protein measured with a refractometer. The sodium sulfite-precipitation test and total protein measurement appeared to be the most useful methods of estimating immunoglobulins under field conditions.

Colostrum and Immunity

Ehrlich (1892) was the first to show that the ingestion of colostrum and milk provided newborn animals with immunity from their dam. He reported that immunity could be transferred in the milk from an immune lactating mouse to neonatal mice, either her own or fostered. Famulener (1912) found that the newborn goat obtained its passive immunity from the antibodies (immunoglobulins) contained in colostrum. He postulated that these colostral antibodies came from the maternal serum. When the kid ingested colostrum, the antibodies were absorbed unchanged, but this absorption could only occur in the first few days of life.

In the 1920s a series of experiments were carried out by Smith and his co-workers (Howe, 1921, 1922, 1924; Little and Orcutt, 1922; Orcutt and Howe, 1922; Smith and Little, 1922a,b, 1923; Smith, 1930). They demonstrated that calves did not receive any immunity across the placenta but were born without detectable globulins (immunoglobulins) or agglutinins in their serum. Instead the calf received its immunity from colostrum which contained immunoglobulins derived from the maternal serum. Smith and Little (1922b) discovered that feeding serum from normal lactating cows to newborn calves provided as good a protection against *E. coli* septicemia as did the feeding of colostrum. A direct association was shown to exist between the early ingestion of colostrum by the newborn calf and its resistance to *E. coli* (Smith and Little, 1922a, 1923; Smith and Orcutt, 1925).

Aschaffenburg and co-workers (1949) demonstrated that the active component of colostrum was found in the whey fraction, and that just 80 ml of whey would provide sufficient immunoglobulin to protect a newborn calf. Logan et al. (1974b) found that, even under adverse conditions, 1500 ml of pooled colostral whey fed at birth would protect 80% of calves from death due to colibacillosis. The protection colostrum provided against the diarrhea associated with colibacillosis was not as marked as that provided against septicemia (Smith and Little, 1922a,b; Gay et al., 1964; Fey, 1971; Gay, 1971; Logan and Penhale, 1971a; Logan et al., 1974b; Johnston et al., 1977).

A number of workers have looked at the concentrations of serum immunoglobulins and their effect in preventing colibacillosis (Gay et al., 1965; Penhale, 1965; McEwan et al., 1970b; Penhale et al., 1970; Penhale et al., 1973; Irwin, 1974) or diarrhea associated with rotovirus infection (Woode et al., 1975; McNulty et al., 1976). Calves which had high serum concentrations of immunoglobulin survived the infection, but those with low concentrations died (Gay et al., 1965; McEwan et al., 1970b; Penhale et al., 1970; Irwin, 1974). Calves with intermediate concentrations were susceptible to diarrhea, but those which died usually did not have septicemia (Penhale et al., 1970). Woode et al. (1975) with McNulty et al. (1976) reported that calves with high immunoglobulin concentrations did not have diarrhea following oral challenge with rotovirus, whereas calves with lower concentrations did.

In a survey in Michigan conducted by Oxender et al. (1973), significant effects were found between calf mortality and time of first feeding of colostrum after birth, and duration of colostrum feeding. Management of the dams and calves from before birth and onwards was also significant in the health of the calves (Dam, 1968; Speicher and Hepp, 1973; Ferris and Thomas, 1974). Calves in hygienic conditions could survive with lower serum immunoglobulin concentrations than were required for calves raised in contaminated areas (Dam, 1968; Ferris and Thomas, 1974).

Both systemic and local immunoglobulins were necessary for complete protection of the newborn calf (Logan and Penhale, 1971b; Logan, 1974). Systemic protection was mediated primarily by IgM, which could prevent septicemia but not enteric disease (Logan and Penhale, 1971a,b,c, 1972; Penhale et al., 1971; Logan et al., 1974b,c). However, when hyperimmune serum was given to colostrum-deprived calves, IgG appeared to provide the protection against septicemia (McBeath, 1977). IgM and IgG given orally were found to be more effective than IgA in the prevention of diarrhea, but colostrum was much better than each individually (Logan et al., 1974b). Each class of immunoglobulin has been reported to be the dominant one in the intestine by different groups of workers: Cripps et al. (1974) determined IgA to be the major immunoglobulin; Porter et al. (1972) found IgM to be the most important immunoglobulin in the secretions of the majority of calves they investigated, although IgG_2 predominated in some individuals; and others concluded that

IgG_1 was present in higher concentrations than IgG_2 in the intestinal secretions, and in fact was the major immunoglobulin, with IgA present in smaller amounts (Curtain et al., 1971; Mach and Pahud, 1971; Newby and Bourne, 1976a).

Colostrum was found to be prophylactic in its action and had little influence once diarrhea had commenced (Logan and Penhale, 1971a; Johnston et al., 1977; Corley et al., 1977). This led to the recommendation that calves ingest colostrum as soon as possible, and Reisenger (1965) suggested they should be given colostrum within 15 minutes of birth.

Enteric infections were reported to be the most important diseases in young calves (Oxender et al., 1973), and the infections usually commenced with the attachment of bacteria to the intestinal epithelium (Smith and Linggood, 1972). A primary role of colostral immunoglobulin in protection of the neonate was the interference with local attachment of enteropathogenic organisms (Corley et al., 1977). However, the calf ingested substantial amounts of maternal immunoglobulins only for the first two or three days, since by then the mammary secretions were low in immunoglobulins; and besides this, many dairy calves were weaned to milk-substitute feeds.

Since absorption in the newborn calf was nonselective, the post-colostral calf had large amounts of secretory IgA in its serum (Porter, 1972, 1973a). This unusual situation did not last long because of the short half-life of secretory IgA. Serum secretory IgA was passed by a transudate process into various external secretions, including those of the alimentary tract, and this continued for approximately

10 days, providing the basis of a short-term, passive barrier to infection (Porter, 1976). By early in the second week of life, active synthesis and secretion of IgA was occurring in the intestinal mucosa to act as an effective host defense mechanism (Porter et al., 1972).

Butler et al. (1972) confirmed that a state of IgA deficiency existed in the bovine mammary gland, as shown by the low IgA concentrations in the milk. This situation was found in other ruminants, but herbivores, such as the rabbit and horse, had comparatively high concentrations of IgA in their milk (Vaerman, 1970). An IgA deficiency in the ruminant mammary gland may be necessary for the normal development of rumen function in the young calf, since the potent anti-bacterial activity of IgA might inhibit the microflora which promote rumen development (Porter, 1973a).

Absorption of Molecules Across the Intestine

After ingestion by the newborn calf, the colostral immunoglobulins were absorbed across the small intestine and carried in the lymphatics to the blood stream (Comline et al., 1951a). Minimal immunoglobulin was transported in the portal blood. During absorption there was no detectable change in the immunoglobulins (Jameson et al., 1942; Smith and Holm, 1948; Johnson and Pierce, 1959; Pierce and Feinstein, 1965). They were taken into the epithelial cells of the small intestine by a mechanism called pinocytosis, whereby the surface membrane of the cell folds and invaginates, engulfing the immunoglobulins (Lecce, 1966b; El-Nageh, 1967a). Vacuoles

were formed this way and apparently transported the immunoglobulins across the cell (Comline et al., 1951b; Clark, 1959; Kraehenbuhl et al., 1967; Kraehenbuhl and Campiche, 1969). A positive correlation existed between the presence of vacuoles in the epithelial cells and uptake of immunoglobulins (Clarke and Hardy, 1971a,b). This process was very rapid and immunoglobulin could be detected in the thoracic duct lymph within 80-120 minutes of its being introduced into the duodenum (Balfour and Comline, 1962).

Munn and Smith (1974) used everted sacs of small intestine of newborn piglets, which had been removed from the sow at birth, to examine the *in vitro* uptake of albumin by the epithelial cells. Small, empty vesicles present at the beginning of incubation swelled up and filled with an electron-dense material they believed to be albumin. Within 60 minutes one or more large vesicles were present in the cells, often displacing the nucleus to one side. When sections of mucosa were taken from normal suckling piglets, similar changes in the epithelial cells were seen. Identical results were obtained using bovine immunoglobulin instead of albumin (Smith et al., 1975).

Other workers have examined the transport of proteins in everted gut sac preparations from rats (Walker et al., 1972, 1973; Walker and Isselbacher, 1974). They reported that the active uptake was energy dependent. They found that most of the internalized protein was digested by lysosomal enzymes, but small quantities were transported through the cell to reach the lymph.

Absorption across the small intestine of the newborn calf is non-specific, and this appeared to be the case in other animals, such as lamb and piglet, where absorption occurred for only a short period of time (Lecce, 1966b). Calves were able to absorb, apparently with equal facility, such substances as bovine serum proteins (Deutsch and Smith, 1957; Bangham et al., 1958; Pierce, 1959, 1961), human IgG_2 and egg-white proteins (Deutsch and Smith, 1957), insulin with its activity still present (Pierce et al., 1964), dextran (Balfour and Comline, 1959b), and a synthetic macromolecule, polyvinylpyrrolidone (PVP) (Hardy, 1969). In addition, enzymes have been fed to pigs and have been detected in the piglet serum in their active form (Balconi and Lecce, 1966). Absorbed immunoglobulin still retained its specific activity in the neonate (Olsson, 1959a,b), had the same electrophoretic and sedimentation constants (Johnson and Pierce, 1959), and the same immunoelectrophoretic and antigenic determinants as that fed (Lecce et al., 1961; Pierce and Feinstein, 1965).

Smaller molecules were also readily absorbed, and some of the smaller proteins were excreted by the kidneys, causing a proteinuria for several days (Deutsch and Smith, 1957; Pierce et al., 1964).

This non-specific absorption is in contrast to mice and rats where selective absorption had been regarded as being present (Brambell, 1970; Rodewald, 1970). In fact, the rat and mouse were selective in what was passed on into the blood, but Lecce (1972) showed, both *in vivo* and *in vitro*, that at

the level of the intestinal epithelium the neonatal mouse was no more selective than the piglet. Proteins from several species were detected within the epithelial cells, even though they could not be found in the circulation. He suggested that the theory of Brambell et al. (1964), which stated that the selectivity barrier may be within the epithelial cell, may prove to be correct.

Using horseradish peroxidase (HRP)-conjugated IgG and conjugated Fc fragment in the neonatal rat, Rodewald (1975) discovered that the transport of Fc-HRP and IgG-HRP was similar, but Fab-HRP was very poorly absorbed. This suggested an active uptake with receptors for the antigenic part of the IgG molecule. Transport appeared to be partially pH dependent, with reduced binding and uptake when the lumen pH was raised from pH 6.5 to 7.4. The pH of the lumen tended to be acid in the neonatal rat, and the extracellular fluids had a slightly alkaline pH. This pH difference could be involved in the normal uptake and release of IgG from receptor sites. Waldeman and Jones (1975) also reported similar findings and conclusions, using radioiodinated IgG in neonatal rats.

Transfer of Immunoglobulin from Gut Lumen

There are three basic hypotheses for the uptake and transmission of immunoglobulins either *in utero* by the fetus, or postnatally by the newborn. Brambell (1966, 1970) put forward a hypothesis to explain the mechanism of selective transport in the rabbit yolk sac and the young rat intestine.

Primary uptake of substances into the cell was by pinocytosis and therefore nonselective. On the walls of the pinocytotic vesicles were receptors, specific for the homologous IgG-Fc piece, but still capable of some cross reaction with various heterologous IgGs. These specific receptors bound the entering protein and protected it from breakdown by cathepsins released when lysosomes fused with the vesicle. Thus the vesicle soon contained only attached protein which was then discharged by reverse pinocytosis through the laterobasal membrane.

Wild (1975) modified Brambell's hypothesis and suggested two types of vesicles were involved. Large macropinocytotic vesicles were supposedly involved in nonselective uptake of protein all of which was digested after fusion of the vesicles with lysosomes. Small micropinocytotic vesicles, coated with glycocalyx, were presumed to be exclusively involved in taking up transferable proteins, specifically binding them and, after passing through the cell, releasing them by exocytosis at the basal or basolateral cell surface.

In the third mechanism Hemmings and Williams (1975) postulated that the major route of entry of proteins into the cell was by way of nonselective pinocytosis. They proposed that there were no specific receptors for homologous immunoglobulins on the surface of the cell, but that the general stickiness of the glycocalyx attached equally well all protein presented to it. Within the cell some of the protein escaped into the cytoplasm either by rupture of the vesicles or by diffusion out of them. This free material

was then transported through the cell and selectively released across the basolateral membrane of the cell.

Morris (1975) combined the hypotheses of Brambell (1966) and Wild (1975) with the work done by Rodewald (1973), which showed that the capacities for selective and nonselective uptake of proteins in the rat were segregated in different regions of the small intestine, to form a new hypothesis.

Schlamowitz (1976) discussed these hypotheses and concluded that no mechanism yet proposed fully answered all the questions regarding specificity of transport combined with the moving of large amounts of protein from mother to fetus or to neonate over a relatively short period of time.

Efficiency of Absorption of Immunoglobulins

The absorption of colostral immunoglobulin has been regarded as nonselective, with the immunoglobulin profile of post-colostral calf serum resembling that of the colostrum (Pierce and Feinstein, 1965; Klaus et al., 1969; Brandon and Lascelles, 1971; Porter, 1971, 1973b). However, Hammer et al. (1968) reported that IgG was absorbed more efficiently than IgM. Penhale et al. (1973) agreed with this, saying that approximately 90% of the IgG was absorbed, whereas only 59% of the IgM and 48% of the IgA appeared in the calf serum. In contrast to this, Klaus et al. (1969) found a significant correlation between the absorption of IgG and IgM, even when three of 10 calves had low serum IgG and IgM concentrations. They considered too that there may be a relationship between the amount of IgG uptake and that of IgM. Husband et al. (1972) regarded IgM as having been absorbed with an

efficiency of almost 100%, and that the lower efficiencies of absorption for IgG_1 and IgG_2 were due to the loss of these lower molecular weight molecules into the interstitial fluid. These findings agreed with those of Brandon and Lascelles (1971), who calculated the relative efficiencies of uptake for IgG_1, IgG_2, IgM and IgA to be very similar, even though their molecular weights ranged from 160,000 to 1,000,000. It is interesting to note here that ferritin, although similar in molecular weight to the immunoglobulins, is not absorbed (unless bound to IgG) (Staley et al., 1972; Rodewald, 1973).

McEwan et al. (1970a) determined the average efficiency of absorption to be about 65%, after allowing for the casein in colostrum, the extravascular pool of immunoglobulins, and plasma expansion. In another experiment, 45% of the immunoglobulin ingested at birth and 12 hours was present in the serum at 24 hours of age (Bush et al., 1971). Baumwart et al. (1977) reported an efficiency of 35.7% for IgG absorption by 24 hours. The differing opinions on selectivity of absorption in the newborn calf may be explained, in part, by the large variation that occurs in immunoglobulin concentration in colostrum samples (Kiddy et al., 1971; Wilson et al., 1972).

The serum immunoglobulin concentrations in newborn calves at 24 hours of age ranged from 6.4 to 16.32 mg/ml for IgG_1, 1.14 to 2.09 mg/ml for IgG_2, and 14.4 to 22.3 mg/ml for total IgG (Klaus et al., 1969; Husband et al., 1972,

1973; Logan et al., 1972). Concentrations for IgM and IgA were 1.22 to 6.01 mg/ml, and 0.58 to 2.42 mg/ml, respectively. By 48 hours of age, the serum values were 14.43 to 43.16 mg/ml for IgG_1, 1.52 to 1.70 mg/ml for IgG_2, 22.6 mg/ml for total IgG, 1.16 to 4.65 mg/ml for IgM, and 2.0 mg/ml for IgA (Klaus et al., 1969; Husband et al., 1972; Porter, 1972). There appeared to be a lack of correlation between individual calf serum and colostral immunoglobulin concentrations (Klaus et al., 1969; Logan et al., 1972; Hoerlein and Jones, 1977; Cunningham, 1977), although Selman et al. (1971b) were able to obtain fairly uniform 48-hour serum concentrations of absorbed immunoglobulins after feeding fixed amounts of a pooled colostrum at set times after birth to newborn calves. Bush et al. (1971) estimated that approximately 68% of the variation in blood serum immunoglobulin concentration in calves at 24 hours could be attributed to differences in immunoglobulin consumed per unit of body weight.

Concentrations of immunoglobulins usually peaked in newborn calf serum between 24 and 48 hours of age, but Husband et al. (1972) found that both IgM and IgA reached maximum concentrations at 12 hours after birth. The half-lives of the immunoglobulins have been estimated as 21.0, 4.0 and 2.8 days for IgG, IgM, and IgA, respectively (Logan et al., 1972), and 16-32 days for IgG_1 and IgG_2, 4 days for IgM, and 2.5 days for secretory IgA (Husband et al., 1972).

Variation in Serum Immunoglobulin Concentrations in Calves

Unfortunately, not all calves possess adequate concentrations of immunoglobulins in their serum even after receiving colostrum. Fey and Margadant (1962) reported that five of 46 "normal" calves they examined were hypogammaglobulinemic, while 21 of 22 colisepticemic calves were severely hypogammaglobulinemic or even agammaglobulinemic. All these calves had been given colostrum on the first day. Smith (1962) found that six of 52 calves left with their dams for the first two days of life were deficient in serum immunoglobulin. Other workers have detected immunoglobulin-deficient calves which were supposedly fed colostrum (Gay, 1965; Smith et al., 1967; Klaus et al., 1969; Fey, 1971; McBeath et al., 1971; Irwin, 1974; Logan et al., 1974a). Even 25% of beef calves sampled, which were nursing their dams at pasture, were found to have inadequate concentrations of immunoglobulin (Logan and Gibson, 1975). Selman et al. (1970a,b) observed the behavior of beef and dairy cows and heifers and their calves *post partum*. Beef cows were better in their mothering, so that their calves stood earlier and suckled sooner than the dairy calves. Body conformation was also seen to be important, since it took much longer for calves to locate the teats on pendulous udders. Calves that remained with their dams to be mothered attained significantly higher concentrations of serum immunoglobulin at 48 hours of age than the control calves which were removed and fed in individual pens even though both were given the same amounts of pooled

colostrum (Selman et al., 1971a,b). Suckling usually resulted in higher serum immunoglobulin concentrations than bucket feeding (Smith et al., 1967; Selman et al., 1971c), and this was also the case when calves were born out-of-doors, and thus allowed to suckle, rather than being born indoors, removed from their dams, and bucket fed (Selman et al., 1971a,b). Roy (1970) stated that the amount of colostrum taken by calves left with their dams was usually much greater than that given by bucket feeding.

The time of first feeding of colostrum was found to be a significant factor, since calves fed within six hours of birth had much greater immunoglobulin concentrations than those fed after this time (Selman et al., 1971c). Penhale et al. (1973) reported that there was a progressive closure of the absorption mechanism, and plasma concentrations were inversely related to the delay interval from birth to first feeding. Kruse (1970) calculated that the absorption coefficient, expressing the absorbed fraction of a given amount of immunoglobulin, was primarily determined by the age of the calf at first feeding, and it decreased linearly to half by 20 hours after birth. Selman (1973) listed a number of factors which affect the absorption of colostral immunoglobulins by newborn calves: the age at which colostrum was first ingested, the amount of immunoglobulin ingested, the method by which the colostrum was obtained, the presence of the dam, the breed of the calf, and the season (winter-born calves had lower concentrations, and this was usually due to being removed from the dam at birth and then being bucket-fed

later). The inability of some calves to absorb sufficient immunoglobulins may be genetic in origin (Stormont, 1972), but is more likely due to other factors. The great variations in colostrum yield and immunoglobulin concentration would also be of importance, especially if the amount of colostrum was small (Logan, 1977).

Failure of colostral IgG transfer was found to have occurred partially or completely in 21 of 87 Thoroughbred foals, and in only one case was the failure attributable to nursing problems (McGuire et al., 1977). Six of these failures were due to low colostral IgG content, even though the foals were not born prematurely or late with regard to the expected date of parturition. This inability to obtain adequate colostral immunoglobulins was regarded as the single most important factor predisposing these otherwise normal foals to infection and death.

Duration of the Absorption Mechanism for Immunoglobulins

The absorptive period in the newborn calf has been estimated at 36 hours (Brambell, 1958), 24-30 hours (Smith et al., 1964; Dam, 1968), or 24 hours (Deutsch and Smith, 1957). Selman et al. (1970c) suggested the cut-off point may be less than 24 hours, and could be as low as eight hours after birth. Gay et al. (1965) reported that even by four to six hours of age some calves may not be able to absorb colostral immunoglobulins. Kruse (1970) measured total immunoglobulin concentrations in calves and revealed that within six hours *post partum* absorption was already diminished. He observed that efficiency of absorption was

not influenced by the concentration of immunoglobulin fed to the calf, but rather by the age of the calf at first feeding and the total immunoglobulin fed. Penhale et al. (1973) examined the absorption of each immunoglobulin class and showed there was marked individual variation in the duration of absorption. They proposed that, whereas IgG was absorbed for 27 hours *post partum* and IgA for 22 hours, IgM was only absorbed for 16 hours.

Factors Promoting Absorption of Immunoglobulins

Colostrum has been found to contain substances which accelerate the absorption of globulins and other macromolecules by the small intestine of the calf (Balfour and Comline, 1959a, 1962; Hardy, 1968, 1969, 1970). Fresh colostral whey promoted rapid absorption of immunoglobulins (Balfour and Comline, 1962), but sodium lactate and sodium pyruvate were similar to whey in their facilitation of absorption of IgG and PVP, an inert macromolecule with a molecular weight of 160,000 (Hardy, 1969). Potassium isobutyrate gave even better results, but these chemicals had to be used at much higher than physiological concentrations (Hardy, 1969). Baumwart et al. (1977) disagreed with Hardy in their results, since in their experiment potassium isobutyrate significantly depressed IgG absorption efficiency.

Balfour and Comline (1962) suggested that a low molecular weight protein fraction isolated from colostral whey may be the active factor in promoting absorption. For its effect this protein required inorganic phosphate and glucose-6-

phosphate at physiological concentrations. McEwan et al. (1970a) recognized there was a tendency for the amount of immunoglobulin absorbed to increase with the amount of colostrum fed, and Halliday and Williams (1976) reported similar results in lambs. The presence of the dam, in some unknown way, also stimulated an increased absorption of immunoglobulin (Selman et al., 1971b).

Factors Involved in the Termination of Absorption of Immunoglobulins

This shutting down of the absorption mechanism has been called "gut closure." Hill (1956) put forward a theory that the development of gastric activity over the first few days of life resulted in destruction of the immunoglobulins by proteolysis. However, work by Smith and Erwin (1959) showed that gastrointestinal enzyme development was not the primary reason for gut closure. Fey (1971) further examined the effect of pepsin on the immunoglobulin molecule and found that absorption of the pepsinized immunoglobulin still occurred in the newborn calf. Besides this, bovine colostrum contained a trypsin inhibitor, and IgG_1 and IgA had some resistance to proteolysis (Hardy, 1970; Newby and Bourne, 1976b), although in sufficient amounts trypsin did destroy the bactericidal activity of IgG_1 (Brock et al., 1977a,b).

Payne and Marsh (1962a,b) regarded gut closure as an "all-or-none" phenomenon. Their work in piglets had shown that, once an intestinal epithelial cell had been exposed to protein and absorbed it, further absorption by that cell ceased. A similar theory stated that absorption ceased when

all the pinocytotic activity of the intestinal cell membrane was "used up" (Lecce, 1966b, 1973; Broughton and Lecce, 1970; Rundell and Lecce, 1972). Thus closure would be expected to start in the duodenum and end with the ileum, and this has been reported (Lecce, 1966a, 1973; Clarke and Hardy, 1971a,b).

Micromolecules, such as glucose, can cause gut closure, independent of colostrum or protein (Lecce, 1966a). Time did not appear to be an important factor in some species, since starved piglets were able to absorb PVP at 86 hours of age (Lecce and Morgan, 1962) or IgG at 106 hours (Payne and Marsh, 1962a). But Deutsch and Smith (1957) could not maintain intestinal permeability in newborn calves beyond the first 24 hours after birth.

El-Nageh (1967b) suggested that gut closure occurred due to the loss of absorptive epithelial cells from the intestinal villi and their replacement with mature, non-absorptive cells from the crypts of Lieberkühn. Clarke and Hardy (1969) and Smeaton (1969) have described similar phenomena in rats and lambs, respectively. However, Sunshine et al. (1971), Rundell and Lecce (1972), and Moon and Joel (1975) have discounted this theory, since the turnover time for intestinal epithelial cells was much slower than would be required to influence the occurrence of gut closure. Closure appeared to happen in two stages (Clarke and Hardy, 1971a,b). First of all, cells lost their ability to release macromolecules into the lacteal circulation, but could absorb them from the intestinal lumen. The second stage of closure occurred with progressive loss of the ability to take

up macromolecules. These processes have been seen in the terminal ileum, and can be present for up to 18 days in the piglet (Clarke and Hardy, 1971a; Martinsson and Jönsson, 1976), and goat (Clarke and Hardy, 1971b).

Corticosteroids and the Absorption of Immunoglobulins

The concentration of intestinal alkaline phosphatase increased to a peak at the time of gut closure in the mouse and rat (Moog and Thomas, 1955; Halliday, 1959; Moog, 1962), and a two-fold increase in endogenous glucocorticoids also occurred at this time (Daniels et al., 1972). Administration of deoxycorticosterone or cortisone acetate to young mice or rats caused an increase in intestinal alkaline phosphatase and brought about premature closure, even up to nine days earlier than usual (Clark, 1959; Halliday, 1959; Moog, 1962). Gut closure normally occurred between 14 and 17 days of age in the mouse, and at 18 to 20 days in the rat. Conversely, bilateral adrenalectomy of rats at 15 to 18 days of age caused a delay in gut closure by up to four days (Daniels and Hardy, 1972).

There are species differences in relation to glucocorticoid administration and gut closure. While cortisone treatment shortened the absorptive period in neonatal rats, mice, starved piglets (Payne and Marsh, 1962b) and, if given prenatally, in dogs (Gillette and Filkins, 1966), it did not appear to have an effect in hedgehogs (Morris and Steel, 1964) or calves (Deutsch and Smith, 1957). Cortisol may also be involved in gut closure in guinea pigs and rabbits, since cortisol concentrations were increased around that time

(Malinowska et al., 1972). High glucocorticoid concentrations in cesarian-derived piglets had no effect on their absorption of IgG from bovine colostrum, but those piglets with metyrapone-induced low glucocorticoid concentrations were found to have significantly reduced amounts of IgG in their serum (Patt and Eberhart, 1976).

In studies on the fetal lamb, Smeaton (1969) found no sign of cessation of absorption, even after prolonged infusions of concentrated immunoglobulin solution. However, after birth, closure did occur in the lambs. Calves delivered by hysterotomy up to 19 days before term were able to absorb IgG, but closure occurred at the normal time after delivery (Smith et al., 1964). One cesarian-derived calf was not fed colostrum until 38 hours after delivery, and no absorption of IgG occurred. Unfortunately, this calf was maintained on fluids intravenously and mature milk orally before it was fed the colostrum, and the milk probably induced the gut closure.

Gut closure appears to be related to birth or the start of independent existence in the large domestic animals (Simpson-Morgan and Smeaton, 1972). Glucocorticoids are involved in the birth process of lambs and calves (Liggins, 1968; Comline et al., 1974), and at least are increased as a result of it in other species (Dvorak, 1972; Murphy, 1973). So glucocorticoids may have some definite role in the termination of immunoglobulin absorption in ruminants.

The induction of parturition in cows with synthetic glucocorticoids is becoming more common as the technique is

improved. When long-acting glucocorticoids were used, the induced calves had a significantly higher incidence of hypogammaglobulinemia than the controls (Bailey et al., 1973; Husband et al., 1973). There was no significant difference between the colostrum of treated or control cows (Bailey et al., 1973). When calves were fed two liters of a pooled colostrum in the first six hours after birth, the induced calves had an efficiency of absorption which was only half that for the control calves (Husband et al., 1973). The glucocorticoid was implicated as causing premature gut closure.

However, when a short-acting synthetic glucocorticoid was used, no difference was seen in the serum immunoglobulin concentrations of control and treatment calves (Beardsley et al., 1973b; Muller et al., 1975; Hoerlein and Jones, 1977). Again, no difference in colostral immunoglobulin content was present between normal and induced cows (Beardsley et al., 1973a; Beardsley et al., 1976). Halliday (1959) reported in his work with neonatal rats that the initial effect of administered cortisone was to increase the absorption of immunoglobulins. The decrease in absorption followed two days later. Perhaps something similar occurred here in that the short-acting glucocorticoids, which induced parturition about 40 hours after injection, did not significantly reduce immunoglobulin uptake by the newborn calves. On the other hand, the long-acting glucocorticoids, which took about 12 days to induce parturition, had the necessary time to prematurely activate the mechanism of gut closure. In fact,

the serum immunoglobulin concentrations of the induced calves were negatively related to the response time, as was the colostral immunoglobulin concentration of their dams, and there was a significant correlation between the immunoglobulin concentrations of treated cows and their calves (Bailey et al., 1973).

The concentration of biologically active corticosteroid to which the induced calf has been exposed *in utero* may be extremely high, since Comline et al. (1974) measured serum cortisol concentrations of up to 10,000 ng/ml in a calf in which they were inducing parturition by cortisol infusion.

Glucocorticoids in the Fetal and Newborn Calf

The adrenal cortex has been regarded as mature in many species late in gestation, and evidence for this was seen as a decrease in ACTH concentrations in the fetal serum (Sakamoto et al., 1977). The adrenal glands from mid-trimester fetal calves could synthesize cortisol and corticosterone *in vitro* (Chouraqui and Weniger, 1970), even though serum corticosteroids were still low at this time (Lin et al., 1978). Glucocorticoid concentrations increased in fetal serum prior to parturition in the lamb (Bassett and Thorburn, 1969; Liggins, 1969), the human (Murphy, 1973), and the calf (Comline et al., 1974). This increase in glucocorticoids has been shown to be involved in the maturation of various fetal enzyme systems (Moog, 1953; DeLemos et al., 1970; Moscona, 1975; Sugimoto et al., 1976), increased production of gluconeogenic enzymes and the deposition of glycogen in the fetal liver (Weber et al., 1964; Holt and Oliver, 1968), and the induction of parturition

(Liggins et al., 1973). In the lamb (Madill and Bassett, 1973) and the calf (Lin et al., 1978), cortisol became the major glucocorticoid hormone with increasing maturity of the fetus, reaching concentrations 10 times that of corticosterone at 260 days of gestation in the calf.

At about 10 days prior to parturition the fetal serum glucocorticoid concentrations started to increase from 10 ng/ml to reach a peak of approximately 70-100 ng/ml around the time of birth (Comline et al., 1974; Hunter et al., 1977). If the birth was delayed, as with an oversized fetus, the glucocorticoid concentrations at time of birth were decreased (Plog, unpublished data). This high concentration decreased rapidly over the first 24 hours to less than 30 ng/ml, and by two to three weeks was at basal concentrations of approximately 3 ng/ml (Hudson et al., 1976).

Various factors may delay this decrease in glucocorticoid concentrations, and heat stress (Stott et al., 1976), diarrhea (Hudson et al., 1976), or cold stress (Khan et al., 1970) have been examples of this. Removal of four-day-old calves from their dams did not delay the decrease in plasma glucocorticoid concentrations, and so did not appear to be stressful to these calves (Hudson et al., 1976).

Stress and Absorption of Immunoglobulins

Stott et al. (1976) examined the effect of heat stress on newborn calves. Calves exposed to the hotter environment responded by having a higher serum glucocorticoid concentration, lower serum IgG_1 at two and 10 days after birth, and a higher mortality rate. Hudson et al. (1976) obtained

jugular blood plasma from 21 calves at birth and then every morning for the following 20 days. Plasma glucocorticoid concentrations in calves which remained healthy decreased rapidly during the first five days and then more gradually to reach a steady concentration over the next 10 days. However, calves which later developed diarrhea (from day five onwards) had significantly higher glucocorticoid concentrations during the first four days of life than those of the healthy calves. These concentrations also decreased with age, except during periods of diarrhea when there was some increase. It would be very interesting to know the immunoglobulin concentrations of these two groups of calves, and to see if there was any difference bewteen them, and any relationship between the early glucocorticoid concentrations and those of the immunoglobulins. An increase in plasma glucocorticoid concentration usually occurred before diarrhea was seen, and perhaps the stress of bacterial infection in those calves which later developed diarrhea caused the higher glucocorticoid concentrations during the first four days.

Glucocorticoids and the Immune Response in the Newborn Calf

The high concentration of glucocorticoids at birth depressed the functional activity of both lymph node lymphocytes and peripheral lymphocytes, as determined by their responsiveness to phytohemagglutinin (Osburn and Stabenfeldt, 1973). Lymphopenia has been observed in newborn calves, and was probably due to the high glucocorticoid concentrations

present at birth (Osborn et al., 1974). The lymphopenia and depressed function of the lymphocytes appeared to cause an increased susceptibility to infection in neonatal calves. LaMotte and Eberhart (1976) examined newborn calves for neutrophil numbers and neutrophil phagocytosis of killed *E. coli in vitro*. Ingestion of one liter of colostrum within one hour of birth resulted in a marked increase in neutrophil numbers, and this did not occur in colostrum-deprived calves. Although phagocytosis was inactive at birth, it rapidly increased and was more efficient in the colostrum-fed calves, even when measured at six days of age. This work demonstrated the importance of the early feeding of colostrum to the newborn calf, and especially in contaminated environments.

Induced Changes in Serum Glucocorticoid Concentrations

A very small amount of ACTH elicited a large response from the adrenal cortex, and Papaikonomou (1977) calculated from his work in rats that one ACTH molecule activated synthesis and release of 1.3 million corticosterone molecules (corticosterone is the main glucocorticoid hormone in the rat).

ACTH is released by corticotrophin releasing factor (CRF). Saffran and Schally (1955) discovered CRF as the first hypothalamic releasing factor to be recognized. It has been difficult to isolate and a number of other CRFs have been postulated (Brodish, 1977). However, Schally et al. (1977) reported that they were able to isolate CRF from porcine hypothalami, and so far have been able to show that

it was a basic peptide with a molecular weight of about 4,800.

In 1963, Schwyzer and Sieber reported that they were able to totally synthesize porcine ACTH. They also produced β^{1-24}ACTH in pure form. Ciba Laboratories market this synthetic ACTH under the trade name of Synacthen (corticotrophin-(1-24)-tetracosapeptide) (β^{1-24}ACTH) (Baker et al., 1976). In rats this synthetic β^{1-24}ACTH was almost equipotent with natural ACTH *in vivo* (McMartin and Peters, 1975). When synthetic β^{1-24}ACTH was labeled with I^{131} and intravenously administered to normal human subjects, the plasma half-life was seven minutes (Wolf et al., 1965), whereas ^{125}I-ACTH had a half-life of only one minute in the circulation of pregnant and fetal sheep (Jones et al., 1975). No evidence of placental transfer of immunologically reactive ACTH was seen by these workers. The biological half-life of synthetic β^{1-24}ACTH was found to range between 19.4 and 38.7 minutes in young men (Moncloa et al., 1966).

Edwards et al. (1974, 1975) examined the effects of infusions of synthetic β^{1-24}ACTH in conscious, unrestrained calves. Infusions of this ACTH increased the blood flow to the adrenal glands, and this reached a peak between 10 and 30 minutes. A significant increase in cortisol, the main glucocorticoid hormone in calves, and corticosterone occurred within five minutes, and peaked within 10 to 20 minutes.

High and very high doses (50 $ng.kg^{-1}$ min^{-1} and 500 $ng.kg^{-1}$ min^{-1}) gave similar maximal cortisol outputs, but the larger dose significantly prolonged the glucocorticoid

secretion and maintained it beyond two hours after the infusion had stopped.

Paape et al. (1977) reported that, as was expected, increasing the ACTH dose from one IU up to 200 IU resulted in a greater response from the adrenal cortex of lactating cows. Plasma glucocorticoid concentrations were still significantly elevated at six hours after injection with both 100 IU and 200 IU of ACTH.

In 1958 Chart et al. found that 2-methyl-1,2-bis-(3-pyridyl)-1-propanone (SU-4885) inhibited the secretion of 17-hydroxycorticoids by the adrenal glands of dogs, and Liddle et al. (1958) postulated from their work with this drug in humans that SU-4885 was a specific inhibitor of 11β-hydroxylation of steroids by the adrenal cortex. SU-4885 is now manufactured as "Metopirone" (Metyrapone) by Ciba Pharmaceuticals.

Besides the shift from cortisol production to deoxycortisol secretion that metyrapone induced, Carballeira et al. (1976) accounted for the decline that occurred in total glucocorticoid output by showing that metyrapone inhibited not only 11β-hydroxylation but also pregnenolone synthesis from cholesterol. Other researchers have demonstrated that metyrapone shortened the cortisol disappearance rate (Bruno et al., 1971; Blichert-Toft et al., 1972), promoted hyperglycemia (Bruno et al., 1972), and stimulated the secretion of growth hormone (Takahara et al., 1972). It has also been shown to have a direct effect on pituitary ACTH secretion (Ganong and Gold, 1960). Human subjects have reported

various central nervous system side effects, such as sweating, malaise, dizziness, vertigo, and confusion (Bruno et al., 1972).

When metyrapone was administered, the 11-deoxycorticosteroids (11-deoxycortisol and 11-deoxycorticosterone) increased in plasma, the glucocorticoids (cortisol and corticosterone) decreased, and this led to an increase in the ACTH plasma concentration (Strott et al., 1969).

MATERIALS AND METHODS

Animals

Newborn Holstein calves were used in all experiments. In experiments 1 through 6 both male and female calves were used, but, since experiment 7 was conducted on a commercial dairy farm, only male calves were allocated to this experiment.

Procedure

Experiments 1, 2 and 3. All five calves in these three experiments were less than 24 hours old when removed from their dams for about six hours for the infusion studies. Both jugular veins were cannulated[a,b] aseptically, one cannula being connected to an infusion pump[c] and the other being used for obtaining blood samples of approximately 10 ml in volume.

---

[a]The three calves in experiment 1 were cannulated with polyvinyl cannulae of V10 tubing, Bolab Inc., Derry, NH, using a 12 gauge thin-walled needle to insert the cannulae.

[b]The two calves in experiments 2 and 3 were cannulated with a Venocath Radiopaque I.V. Catheter with needle, Venocath 14-Surgical, Abbot Laboratories, North Chicago, IL 60064.

[c]Multi-Speed Transmission, Harvard Apparatus Co., Millis, MA.

In experiments 1 and 2 the calves were infused for four hours with corticosterone[d] dissolved in 50% ethanol[e] at the rate of 0.34 mg and 2.0 mg of corticosterone per hour, respectively. The calf in experiment 3 received 7 mg per hour of cortisol,[f] dissolved in absolute ethanol, with the infusion lasting four hours also.

A blood sample was taken before the infusion began, and sampling continued at 15 to 30 minute intervals until 30 minutes after completing the infusion (Table 3). A sterile 3.5% solution of sodium citrate[g] was flushed through the cannula after each blood sample was removed to prevent clotting of blood in the sampling cannula. In some cases dextrose[h] was added to the 3.5% citrate solution to make a 50% dextrose solution for flushing cannulae. At the end of the experiments each calf was given oxytetracycline (1 g) intravenously, the cannulae were removed, and the calves were returned to the boxstalls with their dams.

---

[d]Corticosterone, Sigma Chemical Company, St. Louis, MO 63178.

[e]Gold Shield Alcohol, 200 Proof, IMC Chemical Group, Inc., Terre Haute, IN.

[f]Hydrocortisone-21-Acetate, Sigma Chemical Company, 3500 DeKalb Street, St. Louis, MO 63178.

[g]Sodium Citrate, Mallinckrodt, Inc., St. Louis, MO 63147.

[h]D-Glucose Anhydrous (Granular), Mallinckrodt, Inc., St. Louis, MO 63147.

Experiments 4 and 5. In these two experiments the calves remained with their dams in boxstalls and blood samples were collected with Vacutainer[j] tubes. Immediately at birth, or as soon as possible thereafter, a blood sample was taken from the jugular vein and adrenocorticotropic hormone[k] (ACTH) was given intramuscularly. Two calves in experiment 4 were injected with ACTH (100 IU) after the first blood sample, and again at about 24 hours of age. The control calf did not receive any injections (Table 4).

In experiment 5 all calves were sampled in their first hour of life. Five calves received two injections of ACTH (200 IU), the first immediately after the first blood sample and the second after the 24-hour blood sample. A sixth calf received ACTH (200 IU) only at birth, and one calf did not receive any injections and served as a control (Table 5). Blood sampling continued until 48 hours after birth.

Experiment 6. Four calves were obtained within one hour of birth and removed from their dams before they had suckled. An initial blood sample was taken, and then a synthetic adrenocorticotropic hormone (β^{1-24} ACTH),[l] 100 or

---

[j]Vacutainer Evacuated Glass Tubes, Becton-Dickinson, Division of Becton, Dickinson and Company, Rutherford, NJ 07070.

[k]Adrenomone (Repository Corticotropin Injection), Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Inc., Okland, CA 94621.

[l]Cosyntropin Synthetic ACTH, Organon Pharmaceuticals, West Orange, NJ 07052.

200 IU dissolved in 3 ml of diluent (0.1% gelatin[m] in 0.85% saline[n]), was injected intramuscularly. The two control calves received only the diluent (3 ml intramuscularly). All calves were cannulated[o] aseptically in both jugular veins using a 14 gauge thin-walled needle[p] and connected to an infusion pump. The infusion was run for 36 hours. The two treatment calves received 600 IU or 1200 IU, respectively, of synthetic ACTH in 156 ml of diluent for the 36 hours, and the control calves received 156 ml of the diluent during the 36-hour infusion period. Blood samples were obtained at 0, 0.5, 1, 1.5, 2, 3 and 6 hours, and then at 6-hour intervals through 48 hours (Table 6). Cannulae were flushed with a 3.5% sodium citrate solution to prevent blood clotting as in previous experiments.

Experiment 7. Twenty-three newborn bull calves were placed on experiment at birth, or within one hour following birth. The initial blood sample was taken, and the calves were given an intramuscular injection of synthetic

---

[m]Knox Unflavored Gelatine, Knox Gelatine Inc., Johnstown, NY.

[n]Sodium Chloride, Mallinckrodt, Inc., St. Louis, MO 63147.

[o]IntraMedic Polyethylene Tubing, PE 190. O.D. 0.067", I.D. 0.047" Clay Adams, Division of Becton, Dickinson and Company, Parsippany, NJ.

[p]Kindly supplied by Abbot Laboratories, North Chicago, IL 60064.

adrenocorticotropic hormone[q] (200 IU), metyrapone[r] (250 mg), or diluent, depending on the treatment group to which the calf had been allotted. Each injection was 3 ml in volume, with both the synthetic ACTH and the metyrapone being dissolved, or at least suspended, in the diluent (0.1% gelatin in 0.85% saline).

One jugular vein was cannulated aseptically using a technique similar to that used in experiment 6. Blood samples were obtained at various intervals from birth through 48 hours (Table 1). A pool of colostrum had been prepared from the first and second milkings of a number of Holstein cows, with good mixing to guarantee uniformity, and was stored at -20 C. As this first pool of colostrum was not large enough to feed 23 calves, a second pool of colostrum was made in a similar fashion. Frozen, pooled colostrum was thawed and one liter, with 0.5 liter of warm water added, was fed within two hours of birth, at six hours, and then every six hours through 30 hours. The blood sample was taken, the injection given, and then the colostrum was fed using a nipple bottle (Table 1). If calves would not drink from the nipple bottle, a stomach

---

[q]Synacthen, (Synthetic β^{1-24} ACTH), Ciba Pharmaceutical Company, Summit, NJ 07901.

[r]Metopirone (metyrapone - Ciba), Ciba Pharmaceutical Company, Summit, NJ 07901.

tube[s] was used to put colostrum in the stomach (see Appendix, Table A-1).

Table 1. Schedule of times for blood sampling, injections, and feeding of newborn calves for experiment 7

Age (hours)	Blood Sample*	Injection (intramuscular)	Feeding Colostrum
0	+	+**	
0.25	+		
0.5	+		
1	+		
1.5	+		+
2	+		
2.5	+		
3	+	+	
6	+	+	+
12	+	+	+
18	+	+	+
24	+	+	+
30	+	+	+
36	+		
42	+		
48	+		

*First blood sample was taken immediately after birth in 11 calves, at 0.25 hours in seven calves, at 0.5 hours in two calves, and at one hour in one calf.

**First injection was usually given after the first blood sample, and before the second sample.

The details regarding assistance at birth, age of dam, and comments on the health of each calf used in this experiment are shown in Appendix Table A-1. Calves were randomly assigned to treatment groups in order to balance animal variation.

[s]Bovine Esophageal Feeder, Diamond Laboratories, 312 East Laskey Road, Toledo, OH 43612.

Laboratory Procedures

Blood samples were allowed to clot at room temperature for several hours, and then stored in a refrigerator at 4 C for one or two days. Serum was collected by centrifuging blood samples at 2,500 rpm for 15 minutes and then decanting serum into 12 x 75 mm culture tubes[t] which were stored in a freezer at -20 C until required for assay.

Glucocorticoid Assay. Sera were initially extracted with 2,2,4-trimethyl pentane[u] to remove progestins. Total glucocorticoids were extracted with methylene chloride[v] and quantified by a dog plasma corticosteroid binding globulin (CBG) method, as validated by Smith et al. (1972, 1973). Blood was collected from a cow one hour before, and then immediately after, milking and the two sera were used as low glucocorticoid and high glucocorticoid standards. Both standards were run in every assay, being spaced at the beginning, in the middle, and at the end of the assay. The average intra-assay variations were 9.2% for the low glucocorticoid serum and 7.6% for the high glucocorticoid serum, with inter-assay variation being 11.2% and 18.1%, respectively.

---

[t]diSPo Culture Tubes, distributed by Scientific Products, McGaw Park, IL 60085.

[u]Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442.

[v]Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442.

Since metyrapone is an 11β-hydroxylase inhibitor, it suppresses the final step in cortisol and corticosterone production. Thus, when calves were treated with metyrapone, increases in 11-deoxycortisol and 11-deoxycorticosterone occurred in the serum. According to Gijzen (1977), 11-deoxycortisol has a 97% interference with cortisol in a competitive protein binding assay, and 11-deoxycorticosterone has a 62% interference. In order to demonstrate that 11-deoxycortisol was not being measured in the CBG assay, some pure 11-deoxycortisol[w] was obtained. To one liter of methanol[x] was added 100 mg of 11-deoxycortisol, giving a concentration of 100 μg/ml. One milliliter of this solution was then made up to a one liter solution with methanol to give a final concentration of 100 ng/ml. Duplicate aliquots of this solution were placed in 12 x 75 mm culture tubes, so that the tubes contained 0, 5, 10, 15, 20, 25, 50, 100, 150, 200, 250 and 500 ng of 11-deoxycortisol. After the methanol had been evaporated, one milliliter amounts of pooled sera with high concentrations of glucocorticoids were added to one of the duplicate sets of tubes, and one milliliter amounts of pooled sera with low concentrations of glucocorticoids were added to the other set. The tubes were vortexed and allowed to stand at room

---

[w] 4-Pregnen-17β,21-diol-3,20-dione, Steraloids, Inc., Wilton, NH.

[x] Methyl Alcohol, Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442.

temperature so that the 11-deoxycortisol could be bound to the corticosteroid binding globulin present in the serum. These samples were then routinely assayed for total glucocorticoids, and the results are shown in Table 2.

With the low glucocorticoid serum, only the last tube, with a concentration of 500 ng/ml of 11-deoxycortisol, was more than one standard deviation away from the mean. In the high group, both the first tube, with no 11-deoxycortisol added, and the last tube (500 ng/ml of 11-deoxycortisol) were more than one standard deviation removed from the mean. The low value for the zero tube in the high glucocorticoid group could not be explained, since in a previous assay this value was very close to that of the others. This earlier assay was repeated because the values of the low glucocorticoid group were off the standard curve. So it may be that the low concentration seen in the zero tube was due to experimental error.

The increased glucocorticoid concentrations present in the sera containing 500 ng/ml of 11-deoxycortisol could well be due to the added 11-deoxycortisol. But these increases of 10 to 12.9 ng/ml are minimal when compared with the extra 500 ng/ml in those sera. Thus, any 11-deoxycortisol present in the serum samples from the metyrapone-treated calves would not be measured in significant amounts. This fact is verified by the actual serum glucocorticoid concentrations in those calves (Table 7, Appendix Table A-4).

Table 2. Glucocorticoid concentrations of two groups of pooled sera with various amounts of 11-deoxycortisol added

Serum	Amount (ng) of 11-deoxycortisol added per ml of serum												Mean±S.D.
	0	5	10	15	20	25	50	100	150	200	250	500	
Low	36.1	35.8	42.0	42.9	39.8	37.5	37.7	39.9	37.1	40.5	45.7	53.6	40.7±5.0
High	53.9	73.9	77.4	75.2	67.7	66.4	65.4	65.1	75.6	73.6	73.1	80.7	70.7±7.3

Immunoglobulin G Assay. Serum IgG concentrations were quantified in samples from calves in experiment 7 by using radial immunodiffusion kits[y] (Johnston et al., 1977). The procedure was modified in that a 10 μl Hamilton syringe,[z] set at 2.5 μl, was used to measure the sera and standards to be placed in the wells. Sera collected in experiment 7 at 1.5, 3, 6, 12, 24 and 48 hours after birth were assayed for IgG concentrations (Table 8, Appendix Tables A5, A6, and A7).

Statistical Analysis. Data were analyzed by one-way analysis of variance. For IgG and glucocorticoid values, Dunnett's t-test was used to test the two treatment groups against the control. Tukey's test was also used to compare the individual treatment mean concentrations of IgG against each other.

---

[y]Quantitative Kit for Bovine IgG Determination 64-472-1. Miles Laboratories, Inc., Elkhart, IN 46514.

[z]Hamilton Company, P.O. Box 10030, Reno, NE 89510.

RESULTS

Experiments 1, 2 and 3

These preliminary experiments were conducted to establish a method to maintain elevated serum glucocorticoid concentrations in newborn calves. Corticosterone was infused at 0.34 mg per hour for four hours in calves A, B and C. The infusion was started at time 0, but the infused corticosterone failed to significantly increase serum glucocorticoids in these three calves (Table 3).

In experiment 2 the corticosterone was infused at a rate of 2.0 mg per hour in calf D for four hours. Even this six-fold increase in the amount of corticosterone failed to increase serum glucocorticoids in calf D during the experiment (Table 3).

In a third attempt to increase serum glucocorticoid concentrations, cortisol (7.0 mg/hour) was infused during a four-hour experiment. This cortisol infusion also failed to increase serum glucocorticoids (calf E, Table 3). Since neither corticosterone nor cortisol infusion raised serum glucocorticoids, it was decided to test the effect of adrenocorticotropic hormone on newborn calves as a method for maintaining elevated serum glucocorticoid concentrations.

Table 3. Serum glucocorticoid concentrations (ng/ml) during infusion of corticosterone or cortisol

Time (hours)	Calves				
	A*	B*	C*	D**	E‡
-0.3	16.8	11.8	15.4	8.7	10.4
0†	13.9	12.1	13.6	--	11.7
0.5	14.7	10.1	12.8	6.8	14.2
1.0	14.6	9.5	13.4	8.7	13.1
1.5	15.6	11.4	14.9	10.3	10.6
2.0	16.6	11.4	17.3	8.9	12.0
2.25	16.1	12.5	17.1	10.6	--
2.5	16.7	13.1	13.6	9.7	11.6
2.75	16.3	14.4	14.0	9.0	--
3.0	16.2	9.7	13.0	9.6	11.9
3.25	14.3	10.1	10.5	16.5	--
3.5	16.8	10.9	12.4	8.1	11.7
3.75	18.5	9.6	11.3	10.0	--
4.0δ	19.2	9.7	9.8	13.9	12.5
4.25	14.8	11.4	13.6	9.7	8.0
4.5	13.4	9.1	--	14.4	11.8

*Experiment 1. **Experiment 2. ‡Experiment 3.

†Start of infusion. δEnd of infusion.

Experiment 4

Serum glucocorticoid concentrations continued to decrease in the non-treated calf (F) as shown in Table 4. The other two calves (G and H) received pituitary extract ACTH (100 IU, intramuscularly) immediately after the first blood sample was collected and again 24 hours later. The ACTH treatment failed to cause increased serum glucocorticoids in calves G and H (Table 4). This unexpected result indicated a lack of potency for that particular lot of ACTH. The time of birth of these calves was estimated by the herdsman. However, they may have been up to 12 hours

older than the age given because the serum glucocorticoid concentrations were low in the initial serum samples from these three calves.

Table 4. Serum glucocorticoid concentrations (ng/ml) after intramuscular injection of 100 IU of pituitary extract ACTH

Age (hours)*	Calves		
	F	G	H
2	12.9	14.1**	7.4**
4	--	11.2	--
8	--	--	11.6
10	8.7	--	--
14	9.1	--	--
16	--	7.3	--
24	3.9	12.3**	11.3**
28	--	7.7	8.2
48	1.6	4.6	2.9

*Age was estimated by herdsman, since not seen at birth.

**Immediately after these blood samples were collected, 100 IU of pituitary extract ACTH was given by intramuscular injection.

Experiment 5

In this experiment a new lot of pituitary extract ACTH was used since the potency of the previous lot used in experiment 4 was in question. Calves treated with this new lot of ACTH at 200 IU per injection had increased serum glucocorticoid concentrations after treatment at birth and at 24 hours (Table 5 and Figure 1). The mean serum glucocorticoid concentrations for ACTH-treated calves are shown in Figure 1. Serum glucocorticoids decreased rapidly the

Table 5. Serum glucocorticoid concentrations (ng/ml) after intramuscular injection of 200 IU of pituitary extract ACTH

Time (hours)	Treated Calves						Control Calf
	I	J	K	L	M	N	O[δ]
0**	98.0†	104.1†	62.9†	81.0†	87.5†	94.4†	100.7
0.5	95.0	85.6	62.0	104.0	94.6	92.2	100.7
1	118.0	93.7	74.0	114.2	102.1	96.2	92.6
3	128.8	95.0	79.1	110.0	85.8	96.8	--
6	108.8	101.0	67.7	100.3	86.6	--	--
12	72.8	--	33.1	59.0	55.4	57.6	28.3
18	--	--	33.7	--	56.2	50.7	26.9
24**	28.6†	26.9†	27.2†	18.0†	34.7†	30.1‡	19.7
24.5	71.1	68.1	--	83.0	--	--	--
25	88.3	94.8	--	93.3	75.9	--	--
27	89.3	88.3	40.9	102.4	79.0	25.0	25.1
30	76.5	72.2	--	60.6	34.2	--	--
36	74.9	--	30.6	59.6	28.6	29.1	27.5
42	--	--	26.7	--	28.4	--	29.2
48	33.2	22.1	25.6	29.5	25.6	--	--

*Time adjusted so that both ACTH injections occur at 0 and 24 hours.

**Approximate time of ACTH injections.

†Calf received 200 IU of pituitary extract ACTH by intramuscular injection immediately following this blood sample.

‡Calf N did not receive an injection of ACTH at 24 hours.

δCalf O did not receive any ACTH injections.

first 12 hours in the non-treated calf (O) in marked contrast to the changes observed in the ACTH-treated calves. The values at 24 hours were close together for the non-treated and treated calves, but the second ACTH injection very rapidly produced a significant difference (P<.05) at 27 hours (three hours after the second injection). By 18

Figure 1. Mean serum glucocorticoid concentrations (ng/ml) after IM injection of 200 IU of pituitary extract ACTH.

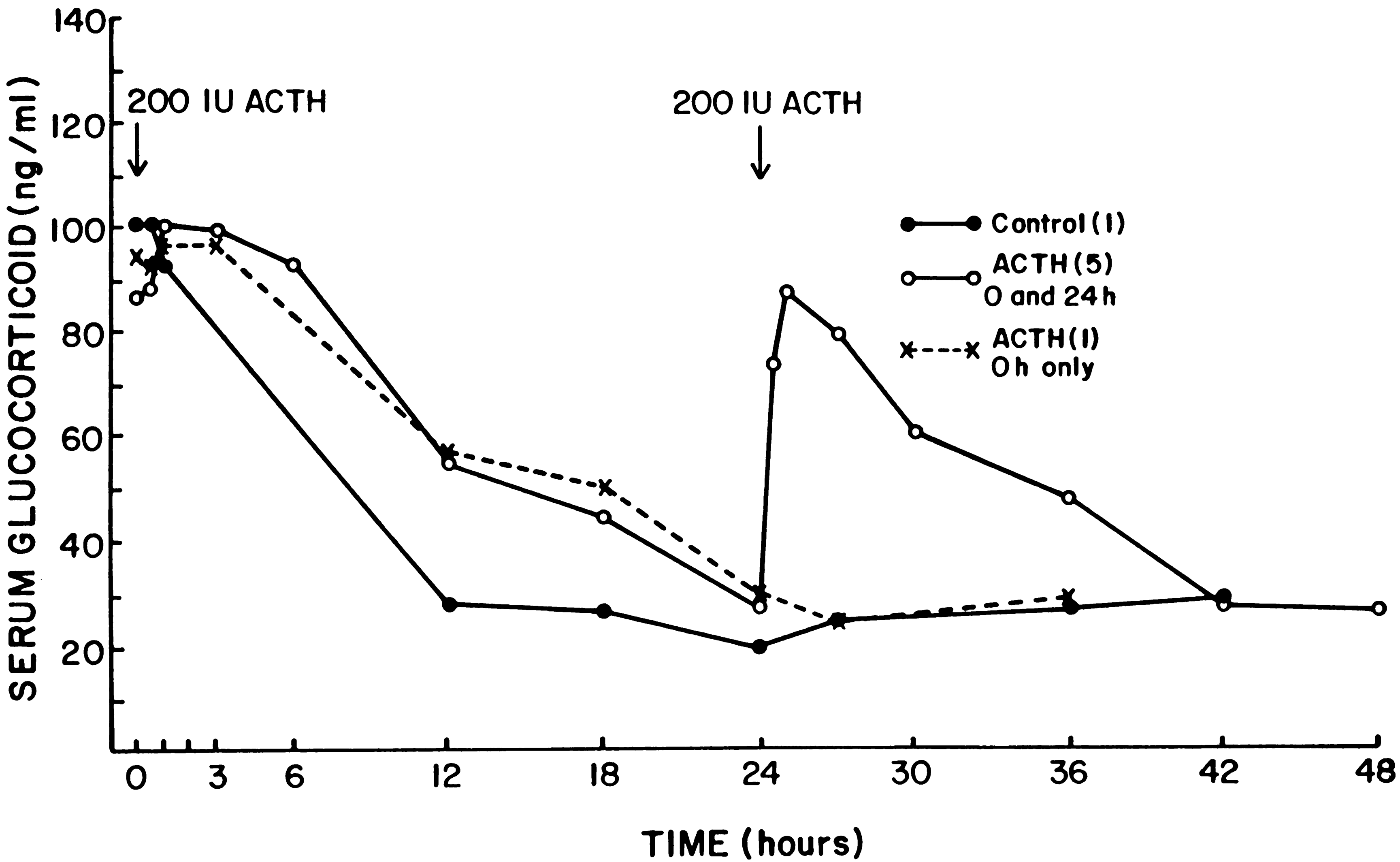


Figure 1

hours after the second injection of ACTH, the serum glucocorticoid concentrations for treated and non-treated calves were similar again. Calf N, which did not receive any ACTH at 24 hours, had nearly identical values to the control calf (O) from 27 to 48 hours.

Experiment 6

Although the pituitary extract ACTH used in experiment 5 produced significant elevations in serum glucocorticoid concentrations, we were unable to secure suitable amounts to conduct a larger experiment. Because of the supply problem, it was decided to use synthetic ACTH in an infusion, in an attempt to maintain consistently high serum glucocorticoid concentrations. Calves (P and R, Table 6) were infused with the synthetic ACTH, at 16.6 IU and 33.3 IU per hour for 36 hours, respectively, and the control calves (Q and S) received the diluent. Both the treated calves required assistance in their deliveries and therefore the time of birth was delayed. The births of calves Q and S were not observed, and their ages were estimated.

The serum glucocorticoid concentrations of these four calves are shown in Table 6. The infusion of synthetic ACTH at the rate of 16.6 IU/hour (calf P) maintained glucocorticoids between 33 and 53 ng/ml during the infusion in contrast to the decrease observed for calves infused with the diluent. Infusion of 33.3 IU/hour of synthetic ACTH also appeared to be effective in maintaining serum glucocorticoids for about 12 hours (calf R). This synthetic

Table 6. Serum glucocorticoid concentrations (ng/ml) during infusion of synthetic β^{1-24} ACTH or diluent

Age (hours)	Calves			
	P*	Q**	R†	S**
0	47.3	--	20.7	--
0.5	51.8	--	22.3	20.6
1	53.7	13.4	23.2	12.1
1.5	44.8	11.7	23.4	12.8
2	42.3	11.9	--	12.6
2.5	--	--	--	14.5
3	49.4	9.5	27.8	9.6
6	50.4	9.9	26.8	4.9
12	39.2	11.6	28.6	4.4
18	36.9	10.9	15.2	5.3
24	39.7	8.9	15.6	4.1
30	33.1	8.3	14.2	3.9
36‡	36.6	3.7	4.5	4.3
42	22.1	7.0	5.9	3.3
48	11.8	11.0	7.4	5.2
72	--	--	7.8	4.8

*Calf P was given 100 IU of synthetic ACTH by intramuscular injection at birth, and then received an infusion of 16.6 IU of this ACTH per hour.

**Calves Q and S received an intramuscular injection of 2.5 or 5.0 ml of diluent after the first blood sample had been taken, and then an intravenous infusion of diluent (0.1% gelatin in 0.85% saline) at the rate of 4.3 ml per hour.

†Calf R was given 200 IU of synthetic ACTH intramuscularly at birth, and then received an infusion of 33.3 IU each hour.

‡All infusions were stopped at 36 hours.

ACTH had been stored in the diluent (0.1% gelatin in 0.85% saline) at 4 C for several days before the infusion and may have lost some of its potency. However, it appeared that the synthetic ACTH could be used to maintain elevated serum glucocorticoid concentrations in newborn calves.

Experiment 7

Calves in this experiment received either synthetic ACTH to maintain elevated serum glucocorticoids, metyrapone to depress serum glucocorticoids, or diluent to serve as controls. Treatments were started at birth, repeated at three hours and six hours, and then continued every six hours for a total of seven treatments for each calf. Serum samples were collected from each calf to quantify serum glucocorticoid and immunoglobulin changes during the 48 hours following birth. The average concentrations of serum glucocorticoids for the three groups are shown in Table 7 and Figure 2. Individual calf serum glucocorticoid concentrations are in Appendix Tables A2, A3 and A4. There was a marked increase ($P<.05$) in serum glucocorticoids in the ACTH-treated calves within two hours following treatment when compared with the control calves. The ACTH-treated calves had significantly higher concentrations of glucocorticoids for at least 36 hours after birth. Glucocorticoid concentrations were significantly ($P<.05$) lower by one hour in calves treated with metyrapone when compared with the control calves (Table 7, Figure 2). The depressed serum glucocorticoid concentrations continued throughout the experiment in the metyrapone-treated calves, even though they were significantly lower than those of the control calves only through 12 hours. There were no significant differences in serum glucocorticoid concentrations between the three groups at 48 hours of age.

Table 7. Comparison of mean serum glucocorticoid concentrations (ng/ml) of the control and ACTH groups, and the control and metyrapone groups

Group	Age (hours) 0	0.25	0.5	1	1.5	2	2.5	3	6	12	18	24	30	36	42	48
ACTH	91.6* 8.0**	106.2 6.1	122.0 7.1	136.7 12.7	143.6 13.7	141.5 12.9	138.1 15.3	32.3 16.2	123.7 12.8	95.5 11.0	78.7 7.9	76.0 6.2	68.6 6.3	62.9 7.0	51.4 7.2	47.1 6.0
Sig.†	NS	.05	NS	NS	NS	.05	.01	.01	.01	.01	.01	.01	.01	.01	NS	NS
CON.‡	139.1* 19.7**	141.3 13.8	143.4 13.9	125.4 17.6	116.8 15.5	101.5 12.1	83.7 13.6	74.7 12.3	63.9 6.6	46.4 4.9	36.8 5.4	41.3 6.1	37.3 8.9	41.7 6.8	43.0 5.8	42.0 6.1
Sig.†	NS	NS	NS	.05	.01	.01	.05	.05	.01	.05	NS	NS	NS	NS	.05	NS
MET$^{\delta}$	138.4* 16.4**	125.5 16.0	114.3 16.2	73.1 9.3	61.2 6.8	47.0 6.3	41.5 5.1	36.3 5.0	21.7 4.5	24.1 4.9	25.2 6.4	25.6 5.0	24.6 5.1	26.8 5.6	25.2 4.5	29.3 5.7

*Mean serum glucocorticoid concentration (ng/ml).

**Standard error of the mean.

†Significant difference when the treatment means were tested individually against the control mean using Dunnett's t-test. NS = no significant difference at $P<.05$.

‡Control group.

$^{\delta}$Metyrapone group.

Figure 2. Mean serum glucocorticoid concentrations (ng/ml) of control, ACTH, and metyrapone groups. The values represent the mean for seven calves at each observation, except for values obtained during the first hour. Samples were obtained from two to seven calves in each group during the first hour.

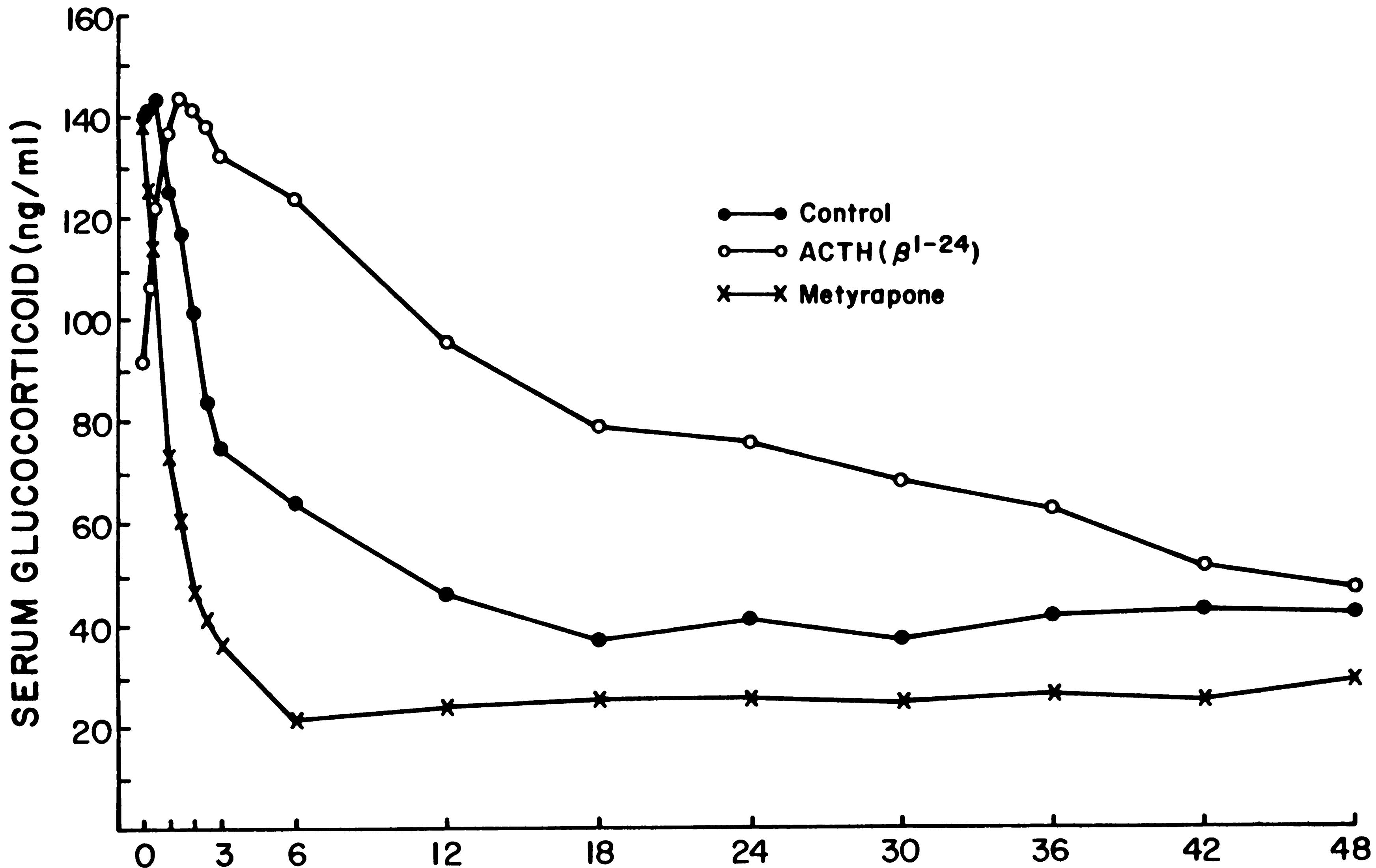


Figure 2

In the control calves serum glucocorticoid concentrations decreased more than 50% within six hours following birth. After this sharp decline in serum glucocorticoids, values remained unchanged from 12 through 48 hours of age. The results indicate that these treatments were effective in producing one group of calves (ACTH-treated) with significantly higher serum glucocorticoid concentrations than the control calves, and another group (metyrapone-treated) with significantly lower values.

A second pool of frozen colostrum was prepared when the first pool of colostrum proved insufficient to complete the experiment. Although the serum IgG concentrations tended to be lower in those calves fed from the second pool of colostrum (two calves each in the control and metyrapone groups, and three calves in the ACTH group), there was no significant difference between IgG values of calves fed with the different pools. Therefore, all calves were considered to have received the same colostrum, and the data were analyzed as such. Individual calf serum IgG concentrations are given in Appendix Tables A5, A6, and A7. There was a large variation in serum IgG concentrations within treatment groups even though calves were fed the same quality and quantity of pooled colostrum at identical times after birth. One calf (number 1) in the control group had a 48-hour value nearly 50% higher than any other calf. Two calves (numbers 9 and 22) on the metyrapone treatment had the lowest serum concentrations, being about 30% lower than the mean value for their group at 48 hours.

All three of these calves died within 24 hours of the end of the experiment.

Average serum IgG concentrations for all calves are shown in Table 8 and Figure 3. Serum IgG concentrations increased markedly during the first 24 hours for all calves. Even though the 48-hour concentrations of the control and ACTH calves were higher than those of the metyrapone group, the large error mean square values due to the large variations within treatment groups prevented any significant difference being observed using either Tukey's test to compare all treatment groups with each other, or Dunnett's t-test to compare each treatment mean with the control mean.

Table 8. Mean ± S.E. of serum IgG concentrations (mg/ml)

Group	Time after Birth (hours)					
	1.5	3	6	12	24	48
Control	0.25 ±0.16	0.65 ±0.18	4.05 ±0.58	11.57 ±1.47	15.50 ±1.68	16.25 ±2.67
ACTH	0	0.84 ±0.15	3.97 ±0.65	10.16 ±1.08	15.88 ±1.05	16.94 ±0.80
Metyrapone	0.10 ±0.10	0.68 ±0.18	3.74 ±0.66	9.23 ±1.01	12.64 ±1.16	11.96 ±1.28

However, if the values of those three calves (numbers 1, 9 and 22) which died within 24 hours of the conclusion of the experiment had been excluded (Table 9 and Figure 4), the mean serum IgG concentration of the ACTH-treated calves

Figure 3. Mean serum IgG concentrations (mg/ml) of control, ACTH, and metyrapone groups.

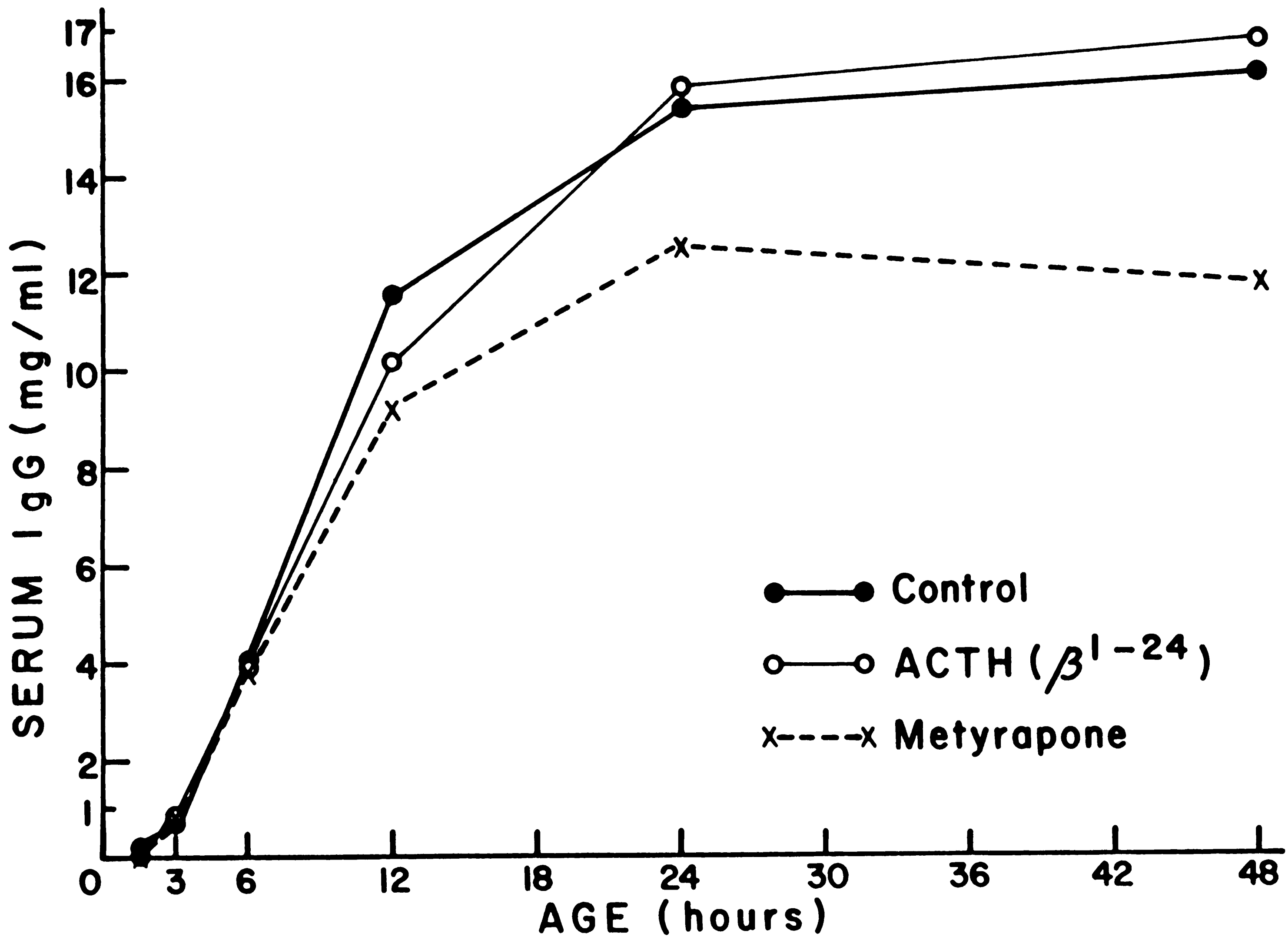


Figure 3

Table 9. Mean ± S.E. of serum IgG concentrations (mg/ml), excluding three calves which died within 24 hours of end of experiment

Group	Time after Birth (hours)					
	1.5	3	6	12	24	48
Control	0.29 ±0.19	0.76 ±0.17	4.17 ±0.67	10.90 ±1.55	14.30 ±1.39	13.82* ±1.29
ACTH	0	0.84 ±0.15	3.97 ±0.65	10.16 ±1.08	15.88 ±1.05	16.94* ±0.80
Metyrapone	0.15 ±0.15	0.95 ±0.07	4.62 ±0.44	10.38 ±0.98	13.62 ±1.34	13.46 ±1.21

*Significant difference ($P<.05$) between these two means, using Dunnett's t-test.

(16.94 mg/ml) would have been significantly ($P<.05$) greater than that of the control calves (13.82 mg/ml) at 48 hours, as determined by Dunnett's t-test. There would be no significant difference between the metyrapone group and the ACTH group, or between the metyrapone group and the control group. Because of the large variation in serum IgG concentrations within groups, even seven calves in each group was not sufficient to obtain significant differences between treatment means.

Figure 4. Mean serum IgG concentrations (mg/ml) of control, ACTH, and metyrapone groups, excluding three calves which died within 24 hours of the end of the experiment.

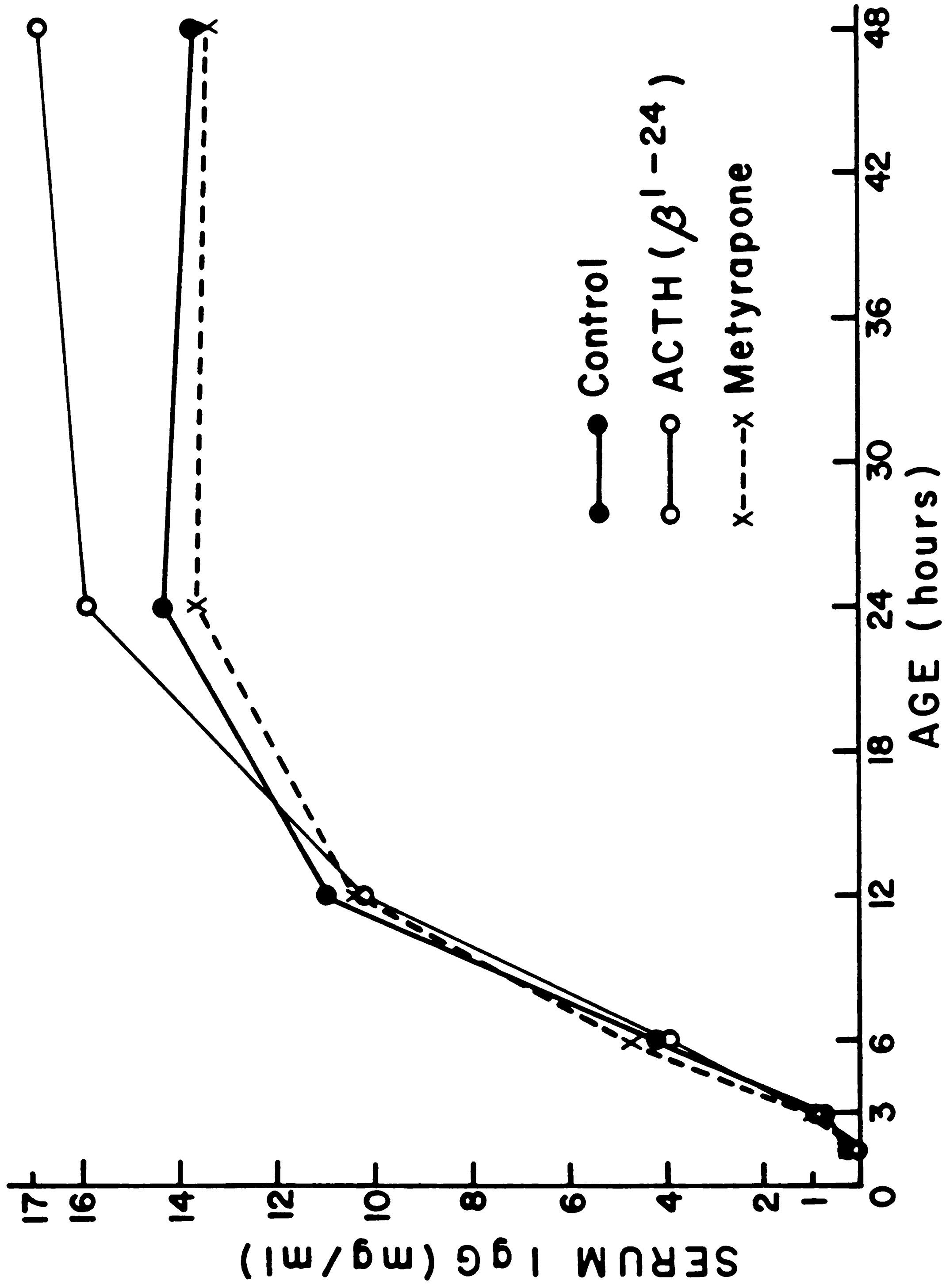


Figure 4

DISCUSSION

The initial calculations for the rate of infusion of corticosterone into newborn calves were based on data from prolactin infusions (Tucker et al., 1973) and the assumption that blood volume was approximately 7% of body weight of the newborn calf. An infusion rate of 0.25 mg per hour was expected to increase the serum glucocorticoid concentration by about 40 ng/ml. When the first two experiments showed no increase in serum glucocorticoid concentrations, it was realized that, whereas prolactin remains primarily within the vascular system, steroids are distributed throughout the extravascular system as well. For the cortisol infusion (experiment 3), the rate of infusion was increased by a factor of 100/7 to allow for distribution of steroids throughout the body fluids, and multiplied by two to hopefully produce an increase of up to 80 ng/ml in serum glucocorticoid concentrations.

The lack of any increase in the serum glucocorticoid concentrations was surprising because Comline et al. (1974) reported concentrations of 1,000 to 10,000 ng/ml in a bovine fetus they were treating with 100 mg of cortisol four times a day. Over a 24-hour period they were giving 16.7 mg of cortisol per hour to that fetus, yet an infusion of 7 mg per hour to the newborn calf in experiment 3 did not alter

the serum glucocorticoid concentrations (Table 3). Perhaps the cortisol had been altered in storage or by the ethanol diluent so that it could not bind to the CBG used to quantify glucocorticoids, but this does not seem likely. Comline et al. (1974) did not report which diluent they had used for the cortisol.

Because of the disappointing results with infusions of glucocorticoids, it was decided to use a pituitary extract ACTH to increase the serum glucocorticoid concentrations in newborn calves (experiment 4). Even though these calves may have been older than estimated, the injections of 100 IU of ACTH should have elicited a significant increase in serum glucocorticoids. The ACTH did not appear to have any activity because the serum glucocorticoids did not increase, but actually declined in a similar fashion to the control calf. This decrease is normal in newborn calves (Hudson et al., 1976). These unexpected results could be attributed to a loss of potency in the ACTH or inability of the adrenal gland to respond to the ACTH, and loss of potency appeared to be the problem.

The ages of the calves were more accurately known in experiment 5. Calves in this experiment were treated within one hour of birth and therefore serum glucocorticoid concentrations were higher at the time calves were first treated. The expected normal decline in serum glucocorticoid concentration, which occurred within one hour of birth, was altered to an increase in all the calves treated with this pituitary extract ACTH. In addition, a second treatment 24

hours later produced a similar increase in serum glucocorticoids, indicating that repeated ACTH treatment could be used to maintain elevated serum glucocorticoid concentrations in newborn calves. However, it was impossible to obtain larger amounts of this pituitary extract ACTH and thus a synthetic ACTH was investigated.

In calves treated with synthetic β^{1-24} ACTH in experiment 6, serum glucocorticoids remained elevated for about 30 hours. It appeared that synthetic ACTH treatment of newborn calves could delay the normal decrease in serum glucocorticoids but would not prevent that decrease. The dose rate of 200 IU every six hours was expected to maintain high glucocorticoid concentrations since previous work (experiment 5) had shown that 200 IU of ACTH would keep the glucocorticoid concentration at or above the pre-injection concentration for at least six hours. The dose rate of 200 IU every six hours was 111.1 $ng.kg^{-1}min^{-1}$ for a 50 kg calf. This rate fell between the high (50 $ng.kg^{-1}min^{-1}$) and the very high (500 $ng.kg^{-1}min^{-1}$) infusion rates used by Edwards et al. (1974, 1975) in neonatal calves, and was expected to produce a near-maximum response.

Metyrapone is an 11β-hydroxylase inhibitor, and thus suppresses the final step in cortisol and corticosterone production (Carballeira et al., 1976). It also inhibits pregnenolone synthesis from cholesterol (Carballeira et al., 1976) and shortens the half-life of cortisol (Bruno et al., 1971; Blichert-Toft et al., 1972). For these reasons it was used in experiment 7 to cause a rapid decrease in the

serum glucocorticoid concentrations of the newborn calves. It can be seen from Table 7 that the serum glucocorticoid concentrations decreased to less than 50% of those at birth by 1.5 hours in the metyrapone-treated calves, compared with six hours in the control calves. The dose rate of 250 mg was based on that used by Patt and Eberhart (1976) in newborn piglets, which was 5 mg per kg of body weight.

Synthetic ACTH and metyrapone treatments were selected to accomplish the first objective of experimentally changing serum glucocorticoid concentrations in newborn calves. The ACTH-treated calves had significantly higher serum glucocorticoid concentrations than the control calves from two hours through 36 hours after birth, while the metyrapone group had significantly lower serum glucocorticoids than controls from one hour through 12 hours. The decrease over time seen in the control group values was similar to that reported by Hudson et al. (1976) and appeared to be intermediate to their healthy and their diarrheic calves.

One of the reasons for conducting this experiment was that many calves which had received colostrum were later found to be deficient in serum immunoglobulins (Gay, 1965; Smith et al., 1967; Klaus et al., 1969; Fey, 1971; McBeath et al., 1971; Irwin, 1974; Logan et al., 1974a). The cause of this decreased ability to absorb immunoglobulins is unknown, but glucocorticoids appear to be involved in the premature cessation of absorption of macromolecules in other species and may have some role in the calf (Simpson-Morgan and Smeaton, 1972). It was expected that the

ACTH-treated calves, with high concentrations of glucocorticoids, would be unable to absorb adequate amounts of colostral immunoglobulins. Halliday (1959) had reported this in rats, and Stott et al. (1976) had found that heat-stressed newborn calves had higher glucocorticoid and lower IgG concentrations than did the control calves, all having been fed fresh pooled colostrum. Calves that had been removed from their dams at birth, perhaps a stressful experience, absorbed less immunoglobulin than those which had remained with their dams and received some "mothering" (Selman et al., 1971a,b). Hudson et al. (1976) had shown that calves which had diarrhea possessed higher serum glucocorticoid concentrations in the first few days of life than those which had stayed healthy. Although serum IgG concentrations had not been measured, this proposed hypothesis would suggest that the higher serum glucocorticoid concentrations over the first few days had resulted in lower serum IgG concentrations, which in turn had made these calves somewhat more susceptible to disease. Metyrapone was used to provide a group of calves with lowered glucocorticoid concentrations, and theoretically, to allow increased absorption of colostral immunoglobulins.

Since IgG (IgG_1 and IgG_2) makes up 85% of the immunoglobulin in colostrum (Porter, 1976), the measurement of serum IgG concentrations in the calves was regarded as a good indicator of absorption of colostral immunoglobulin. Individual calves within groups, fed the same amount of pooled colostrum at the same times after birth, still had

wide variation in their serum IgG concentrations. Selman et al. (1971b) fed pooled colostrum at fixed rates and times post-partum to newborn calves and reported a "marked uniformity" in the 48-hour serum immune lactoglobulin concentrations, as measured by the zinc sulfate turbidity test. Their standard deviations were similar to those obtained in this experiment for the ACTH and metyrapone groups (2.12 and 3.38, respectively), although the standard deviation for the control group (7.07) was greater. So it appears that considerable variation occurs even when calves are fed the same quality and quantity of pooled colostrum at identical times after birth. This variation may be due to some intrinsic factor(s), or it may be due to differences in body weight and therefore differences in plasma and extravascular volumes. McDougall and Mulligan (1969), using labeled IgG, found an average extravascular-intravascular ratio of 1.2 to 1.0 for the distribution of IgG in the neonatal calf.

Serum IgG was quantified in the final experiment to evaluate colostral immunoglobulin absorption. At 48 hours after birth the mean serum IgG concentrations of the control group and the ACTH group were similar (16.25 and 16.94 mg/ml, respectively), and no significant difference could be shown between them and the metyrapone mean value of 11.96 mg/ml because of the large variation present in each group, and especially in the control group. This variation was mainly due to the high value of calf number 1 (30.88 mg/ml), and if this calf was excluded from the experiment

the ACTH mean serum IgG concentration would be significantly ($P<.05$) greater than that of the control group. The ACTH group mean would not be significantly greater than the metyrapone group mean. The interpretation of the results would not have been altered by removing the values of the two metyrapone calves (numbers 9 and 22) which had low serum IgG concentrations.

Eighteen of the 21 calves experienced diarrhea during the experiment, and there were many other variables which could not be controlled. Thus, it was decided not to exclude these three calves (numbers 1, 9 and 22) which died within 24 hours of the conclusion of the experiment. They showed no clinical differences to the other calves, and their glucocorticoid concentrations were not different from the other calves in their groups.

The results of this experiment suggest that increased glucocorticoid concentrations maintained in newborn calves do not decrease the serum IgG concentrations at 48 hours after birth when compared with control calves. There is also the suggestion that decreased glucocorticoid concentrations may tend to lower the serum IgG concentrations at 48 hours in newborn calves. Therefore, these results did not agree with the hypothesis that had been proposed, but rather tended to support the results of Patt and Eberhart (1976) in newborn pigs. Patt and Eberhart (1976) reported that newborn pigs injected with ACTH absorbed similar amounts of bovine IgG as did the control pigs, and the metyrapone-treated pigs absorbed significantly less. The

results obtained in experiment 7 seem to be similar, with the metyrapone-treated calves apparently having lower serum IgG concentrations at 48 hours than the control calves, and the ACTH-treated calves having similar concentrations, or even greater if calf number 1 had been excluded, than the control calves. In this present experiment, however, no significant difference was obtained between the control and metyrapone groups.

Halliday (1959) found that the administration of cortisone acetate to 15-day-old rats caused an increase in immunoglobulin absorption 24 hours later, but by 48 hours absorption was very much reduced or had stopped. This could be one reason for the very good absorption seen in the ACTH-treated calves, since absorption only lasts for 24 hours in the calf anyway. But Morris and Morris (1974) repeated Halliday's work and found that the cortisone acetate caused a reduced gastric emptying and a decreased intestinal absorption at 24 hours as well as at 50 hours.

Halliday (1959) also used deoxycorticosterone acetate to cause premature gut closure. Since metyrapone blocks cortisol and corticosterone synthesis in the calf, the effect of the rapid decrease in their serum concentration will stimulate increased release of ACTH from the anterior pituitary (Strott et al., 1969). This ACTH will induce production and secretion of large amounts of 11-deoxycortisol and 11-deoxycorticosterone because of the 11β-hydroxylase block. This increase in 11-deoxycortisol and 11-deoxycorticosterone may have had some effect on the

ability of the metyrapone-treated calves to absorb immunoglobulins from the colostrum. However, Gillette and Filkins (1966) could not shorten the absorptive period in newborn puppies with metyrapone. Since 11-deoxycortisol and 11-deoxycorticosterone are not normally present in calf serum in significant amounts, they probably are not involved.

Plog (unpublished data) measured serum glucocorticoid and IgG concentrations in 28 newborn calves at 0, 12, 24, and 48 hours after birth, and determined that when the glucocorticoid concentration dropped rapidly the IgG concentration reached a peak by the next serum sample. This decrease in serum glucocorticoid concentration occurred dramatically in the metyrapone-treated calves to reach a basal concentration within the first six hours, and five of these seven calves reached their maximum IgG concentration at 24 hours. This is in contrast to the ACTH-treated calves where only one of the seven calves reached its maximum IgG concentration by that time. Perhaps the sudden decline in glucocorticoid concentrations in the serum of the newborn calf triggers the mechanism that leads to the cessation of immunoglobulin absorption. The results of the present experiment suggest that metyrapone, in rapidly lowering the serum glucocorticoid concentration, may have reduced the ability of, or the time available for, the newborn calf to absorb immunoglobulins from colostrum.

The general view in the literature is that high concentrations of serum immunoglobulins protect calves from death due to septicemia or diarrhea, although some calves

with high IgG concentrations may still die from diarrhea (Gay et al., 1965; Penhale, 1965; McEwan et al., 1970b; Penhale et al., 1970; Selman et al., 1971c; Penhale et al., 1973; Irwin, 1974; Woode et al., 1975; McNulty et al., 1976; Johnston et al., 1977). Of the five calves which died before, or within 24 hours after, the end of the present experiment, three had very high serum IgG concentrations (22.74, 18.06, and 24.03 mg/ml at 24 hours, and 30.88 mg/ml at 48 hours) and two had low serum IgG concentrations (8.21 and 8.22 mg/ml at 48 hours). So obviously other factors, such as contamination of their environment, were involved in the death of these calves. There was no difference between groups in the calves which died, with one being from the control group, and two each being from the ACTH and metyrapone groups. Certainly the small numbers of calves in each group and the number of factors which can cause death in newborn calves prevents drawing significant conclusions as to the effects of the treatments in preventing mortality. One calf from each group did not have diarrhea at any time during the 48 hours of the experiment.

The results of the present experiment taken with results of some other researchers produce an interesting idea for speculation. Several reports have indicated that a prolonged birth process for calves was closely correlated with an increased neonatal mortality rate (Laster and Gregory, 1973). Plog (unpublished data) found that calves which required assistance due to a long labor had

significantly lower serum glucocorticoid concentrations at birth than calves born naturally (50.0 ng/ml compared with 77.1 ng/ml, respectively). Serum glucocorticoid concentrations increase to a peak around the time of onset of labor and then decline (Comline et al., 1974). Thus, any delay in the birth process results in lower glucocorticoid concentrations at the time of delivery, and perhaps a subsequent decrease in the ability of the calf to absorb colostral immunoglobulins, as suggested by the metyrapone-treated calves. Delaying the fall in serum glucocorticoid concentrations by treating the newborn calf with ACTH did not cause a decrease in serum IgG concentrations, and may even have caused an increase. Additional research will be required to verify the effect of serum glucocorticoid concentrations on the absorption of colostral immunoglobulins.

CONCLUSIONS

1. Synthetic B^{1-24} ACTH, administered intramuscularly to newborn calves at the rate of 200 IU every six hours for 30 hours, was able to maintain serum glucocorticoid concentrations at levels significantly higher than control calves from two hours of age through 36 hours. The ACTH treatment delayed the normal decrease in serum glucocorticoid concentrations, but could not prevent it.

2. Metyrapone, given as intramuscular injections of 250 mg to newborn calves every six hours for 30 hours, caused a rapid decrease in serum glucocorticoid concentrations which reached basal levels within six hours of birth. Serum glucocorticoid concentrations in metyrapone-treated calves were significantly lower than those of control calves from one hour of age through 12 hours.

3. Frozen, pooled colostrum, thawed and fed to newborn calves at the rate of one liter every six hours for 30 hours, increased serum immunoglobulin G (IgG) concentrations from 0.25 mg/ml at 1.5 hours after birth to 16.25 mg/ml at 48 hours in control calves.

4. The feeding of one liter of colostrum to newborn calves before two hours of age did not prevent diarrhea in these calves.

5. Treatment of newborn calves with synthetic β^{1-24} ACTH did not decrease the serum IgG concentrations at 48 hours of age compared with the control calves.

6. Newborn calves treated with metyrapone did not have significantly lower serum IgG concentrations at 48 hours after birth compared with the control calves.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adinolfi, M. C. and Billington, W. D.: Ontogeny of Acquired Immunity and Feto-Maternal Immunological Interactions. In, *Fetal Physiology and Medicine. The Basis of Perinatology*. Ed. R. W. Beard and P. W. Nathanielsz. W. B. Saunders Co. Ltd., Philadelphia, PA, (1976): 17-42.

Aschaffenburg, R.: The Nutritive Value of Colostrum for the Calf. 3. Changes in the Serum Protein of the Newborn Calf Following the Ingestion of Small Quantities of the Non-Fatty Fraction. Br. J. Nutr., 3, (1949): 200-205.

Aschaffenburg, R., Bartlett, S., Kon, S. K., Terry, P., Thompson, S. Y., Walker, D. M., Briggs, C., Cotchin, E., and Lovell, R.: The Nutritive Value of Colostrum for the Calf. I. The Effect of Different Fractions of Colostrum. Br. J. Nutr., 3, (1949a): 187-196.

Aschaffenburg, R., Bartlett, S., Kon, S. K., Walker, D. M., Briggs, C., Cotchin, E., and Lovell, R.: The Nutritive Value of Colostrum for the Calf. 2. The Effect of Small Quantities of the Non-Fatty Fraction. Br. J. Nutr., 3, (1949b): 196-200.

Bailey, L. F., McLennan, M. W., McLean, D. M., Hartford, P. R., and Munro, G. L.: The Use of Dexamethasone Trimethylacetate to Advance Parturition in Dairy Cows. Aust. Vet. J., 49, (1973): 567-573.

Baker, J. R. J., Bennett, H. P. J., Hudson, A. M., McMartin, C., and Purdon, G. E.: On the Metabolism of Two Adrenocorticotrophin Analogues. Clin. Endocr., 5, (1976), Suppl.: 61-72s.

Balconi, I. R., and Lecce, J. G.: Intestinal Absorption of Homologous Lactic Dehydrogenase Isoenzymes by the Neonatal Pig. J. Nutr., 88, (1966): 233-238.

Balfour, W. E. and Comline, R. S.: Acceleration of the Absorption of Unchanged Globulins in the Newborn Calf by Factors in the Colostrum. J. Physiol., 147, (1959a): 22-23P.

Balfour, W. E. and Comline, R. S.: The Specificity of the Intestinal Absorption of Large Molecules by the Newborn Calf. J. Physiol., 148, (1959b): 77-78P.

Balfour, W. E. and Comline, R. S.: Acceleration of the Absorption of Unchanged Immunoglobulin in the Newborn Calf by Factors in Colostrum. J. Physiol., 160, (1962): 234-257.

Bangham, D. R., Ingram, P. L., Roy, J. H. B., Shillam, K. W. G., and Terry, R. J.: The Absorption of ^{131}I-Labelled Serum and Colostral Proteins from the Gut of the Young Calf. Proc. Roy. Soc., B, 149, (1958): 184-191.

Bassett, J. M. and Thorburn, G. D.: Foetal Plasma Corticosteroids and the Initiation of Parturition in Sheep. J. Endocr., 44, (1969): 285-286.

Baumwart, A. L., Bush, L. J., Mungle, M., and Corley, L. D.: Effect of Potassium Isobutyrate on Absorption of Immunoglobulins from Colostrum by Calves. J. Dairy Sci., 60, (1977): 759-762.

Beardsley, G. L., Muller, L. D., Ellis, R. P., Reed, D. E., and Owens, M. J.: Initiation of Parturition in Dairy Cows with Dexamethasone. II. Calf Performance. J. Dairy Sci., 56, (1973b): 640-641 (Abstr).

Beardsley, G. L., Muller, L. D., Garverick, H. A., Ludens, F. C., and Tucker, W. L.: Initiation of Parturition in Dairy Cows. III. Response to Dexamethasone in Combination with Estradiol Benzoate. J. Dairy Sci., 59, (1976): 241-247.

Beardsley, G. L., Muller, L. D., and Owens, M. J.: Initiation of Parturition in Dairy Cows. I. Cow Response and Performance. J. Dairy Sci., 56, (1973a): 640 (Abstr).

Blichert-Toft, M., Folke, K., and Nielsen, M. L.: Effects of Metyrapone and Corticotropin Infusion on Cortisol Disappearance Rate in Man. J. Clin. Endocr. Metab., 35, (1972): 59-62.

Brambell, F. W. R.: The Passive Immunity of the Young Mammal. Biol. Rev., 33, (1958): 488-531.

Brambell, F. W. R.: The Transmission of Immunity from Mother to Young and the Catabolism of Immunoglobulins. Lancet, II, (1966): 1087-1093.

Brambell, F. W. R.: The Transmission of Passive Immunity from Mother to Young. Frontiers of Biology, Vol. 18. American Elsevier Publishing Company, New York, NY, 1970.

Brambell, F. W. R., Hemmings, W. A., Henderson, M., Oakley, C. L., and Rowlands, W. T.: The Accumulation of Antibodies in the Stomach Contents of Foetal Rabbits. Proc. Roy. Soc., B, 138, (1951): 195-204.

Brambell, F. W. R., Hemmings, W. A., and Morris, I. G.: A Theoretical Model of γ-Globulin Metabolism. Nature, 203, (1964): 1352-1355.

Brandon, M. R.: Selective Transfer and Catabolism of IgG in the Ruminant. In, *Maternofoetal Transmission of Immunoglobulins*. Ed. W. A. Hemmings, Cambridge University Press, Cambridge, (1975): 437-448.

Brandon, M. R. and Lascelles, A. K.: Relative Efficiency of Absorption of IgG_1, IgG_2, IgA and IgM in the Newborn Calf. Aust. J. Exp. Biol. Med. Sci., 49, (1971): 629-633.

Brandon, M. R. and Lascelles, A. K.: The Effect of Pre-Partum Milking on the Transfer of Immunoglobulin into Mammary Secretion of Cows. Aust. J. Exp. Biol. Med. Sci., 53, (1975): 197-204.

Brandon, M. R., Watson, D. L., and Lascelles, A. K.: The Mechanism of Transfer of Immunoglobulin into Mammary Secretion of Cows. Aust. J. Exp. Biol. Med. Sci., 49, (1971): 613-623.

Brock, J. H., Arzabe, F. R., Pineiro, A., and Olivito, A.: The Effect of Trypsin and Chymotrypsin on the Bactericidal Activity and Specific Antibody Activity of Bovine Colostrum. Immunology, 32, (1977a): 207-213.

Brock, J. H., Arzabe, F. R., Ortega, F., and Pineiro, A.: The Effect of Limited Proteolysis by Trypsin and Chymotrypsin on Bovine Colostral IgG_1. Immunology, 32, (1977b): 215-219.

Brodish, A.: Tissue Corticotropin Releasing Factors. Fed. Proc., 36, (1977): 2088-2093.

Broughton, C. W. and Lecce, J. G.: Electron-Microscopic Studies of the Jejunal Epithelium from Neonatal Pigs Fed Different Diets. J. Nutr., 100, (1970): 445-449.

Bruno, O. D., Lecleriq, R., Virasoro, E., and Copinschi, G.: Extra-Adrenal Actions of Metyrapone in Man. Effects on Plasma Cortisol Disappearance, Growth Hormone Secretion and Glucose Metabolism. J. Clin. Endocr. Metab., 32, (1971): 260-265.

Bruno, O. D., Metzger, P., and Malaisse, I. J.: Inhibitory Effect of Metyrapone on Glucose Utilization by Brain and Muscle and on Insulin Release by the Pancreas. Acta Endocr., 70, (1972): 710-718.

Bush, L. J., Aguilera, M. A., Adams, G. D., and Jones, E. W.: Absorption of Colostral Immunoglobulins by Newborn Dairy Calves. J. Dairy Sci., 54, (1971): 1547-1549.

Butler, J. E.: Review of the Bovine Immunoglobulins. J. Dairy Sci., 54, (1971): 1315-1316.

Butler, J. E.: Synthesis and Distribution of Immunoglobulins. J. Am. Vet. Med. Assoc., 163, (Oct. 1, 1973): 795-798.

Butler, J. E., Kiddy, C. A., Pierce, C. S., and Rock, C. A.: Quantitative Changes Associated with Calving in the Levels of Bovine Immunoglobulins in Selected Body Fluids. I. Changes in the Levels of IgA, IgG, and Total Protein. Canad. J. Comp. Med., 36, (1971a): 234-242.

Butler, J. E., Maxwell, C. F., Pierce, C. S., Hylton, M. B., Asofsky, R., and Kiddy, C. A.: Studies on the Relative Synthesis and Distribution of IgA and IgG_1 in Various Tissues and Body Fluids of the Cow. J. Immunol., 109, (1972): 38-46.

Butler, W. T., Rossen, R. D., and Waldmann, T. A.: The Mechanism of Appearance of Immunoglobulin A in Nasal Secretions in Man. J. Clin. Invest., 46, (1967): 1883-1893.

Cambell, B., Porter, R. M., and Peterson, W. E.: Plasmacytosis of the Bovine Udder During Colostrum Secretion and Experimental Cessation of Milking. Nature, 166, (1950): 913.

Carballeira, A., Fishman, L. M., and Jacobi, J. D.: Dual Sites of Inhibition by Metyrapone of Human Adrenal Steroidogenesis: Correlation of *in vivo* and *in vitro* Studies. J. Clin. Endocr. Metab., 42, (1976): 687-695.

Chart, J. J., Shepard, H., Allen, M. J., Bencze, W. L., and Gaunt, R.: New Amphenone Analogs as Adrenocortical Inhibitors. Experientia, 14, (1958): 151-153.

Chouraqui, J. and Weniger, J. P.: Identification des Corticosteroides Secretes par les Sunenales Embryonnaires de Veau, Lapin et Souris Culturees *in vitro*. Acta Endocr., 65, (1970): 650-662.

Ciupercescu, D. D.: Dynamics of Serum Immunoglobulin Concentrations in Sheep During Pregnancy and Lactation. Res. Vet. Sci., 22, (1977): 23-27.

Clark, S. L.: The Ingestion of Proteins and Colloidal Materials by Columnar Absorptive Cells of the Small Intestine in Suckling Rats and Mice. J. Biophys. Biochem. Cytol., 5, (1959): 41-48.

Clarke, R. M. and Hardy, R. N.: An Analysis of the Mechanism of Cessation of Uptake of Macromolecular Substances by the Intestine of the Young Rat ("Closure"). J. Physiol., 204, (1969): 127-134.

Clarke, R. M. and Hardy, R. N.: Histological Changes in the Small Intestine of the Young Pig and Their Relation to Macromolecular Uptake. J. Anat., 108, (1971a): 63-77.

Clarke, R. M. and Hardy, R. N.: Structural Changes and the Uptake of Polyvinyl Pyrrolidone in the Small Intestine of the Young Goat. J. Anat., 108, (1971b): 79-87.

Collins, J.D., Carroll, E. J., Jasper, D. E., and Jain, N. C.: Observations on the *in vitro* Bactericidal Activity of Sera from Pre-Natal and Post-Natal Calves. Vet. Rec., 86, (1970): 93-96.

Comline, R. S., Hall, L. W., Lavelle, R. B., Nathanielsz, P. W., and Silver, M.: Parturition in the Cow: Endocrine Changes in Animals with Chronically Implanted Catheters in the Foetal and Maternal Circulations. J. Endocr., 63, (1974): 451-472.

Comline, R. S., Roberts, H. E., and Titchen, D. A.: Route of Absorption of Colostrum Globulin in the Newborn Animal. Nature, 167, (1951a): 561-562.

Comline, R. S., Roberts, H. E., and Titchen, D. A.: Histological Changes in the Epithelium of the Small Intestine During Protein Absorption in the Newborn Animal. Nature, 168, (1951b): 84-85.

Conner, G. H. and Carter, G. R.: Response of the Bovine Fetus to Reovirus. Vet. Med. Small Anim. Clin., 70, (1975): 1463-1464.

Conner, G. H., Richardson, M., and Carter, G. R.: Prenatal Immunization and Protection of the Newborn: Ovine and Bovine Fetuses Vaccinated with *Escherichia coli* Antigen by the Oral Route and Exposed to Challenge Inoculum at Birth. Am. J. Vet. Res., 34, (June, 1973): 737-741.

Conner, G. H., Richardson, M., Carter, G. R., and Wamukoya, J. P. O.: Immune Responses of the Bovine Fetus. J. Dairy Sci., 60, (1977): 289-293.

Corley, L. D., Staley, T. E., Bush, L. J., and Jones, E. W.: Influence of Colostrum on Transepithelial Movement of *Escherichia coli* 055. J. Dairy Sci., 60, (1977): 1416-1421.

Cripps, A. W., Fulkerson, W. J., Griffiths, D. A., McDowell, G. H., and Lascelles, A. K.: The Relationship Between the Transfer of Immunoglobulins, Sodium and Potassium into Mammary Secretion of the Parturient Ewe. Aust. J. Exp. Biol. Med. Sci., 54, (1976): 337-348.

Cripps, A. W., Husband, A. J., and Lascelles, A. K.: The Origin of Immunoglobulins in Intestinal Secretions of Sheep. Aust. J. Exp. Biol. Med. Sci., 52, (1974): 711-716.

Cunningham, B.: The Transfer of *Brucella abortus* Antibodies from Dam to Calf. Vet. Rec., 100, (1977): 522-524.

Curtain, C. C., Clark, B. L., and Duffy, J. M.: The Origin of Immunoglobulins in the Mucous Secretions of Cattle. Clin. Exp. Immunol., 8, (1971): 335-344.

Dam, A.: Studies on the Gammaglobulin Levels in Sera of Calves from Herds with Colisepticemia as a Problem, and Some Investigations on the Content of Specific Antibodies in Colostrum. Nord. Vet. Med., 20, (1968): 449-457.

Daniels, V. G., and Hardy, R. N.: Adrenal Gland in the Control of Intestinal Absorption of Macromolecules by the Young Rat. Experientia, 28, (1972): 272.

Daniels, V. G., Hardy, R. N., Malinowska, K. W., and Nathanielsz, P. W.: Adrenocortical Hormones and Absorption of Macromolecules by the Small Intestine of the Young Rat. J. Endocr., 52, (1972): 405-406.

DeLemos, R. A., Shermata, D. W., Knelson, J. H., Kotas, R., and Avery, M. E.: Acceleration of Appearance of Pulmonary Surfactant in the Fetal Lamb by Administration of Corticosteroids. Am. Rev. Resp. Dis., 102, (1970): 459-461.

Deutsch, H. F., and Smith, V. R.: Intestinal Permeability to Proteins in the Newborn Herbivore. Am. J. Physiol., 191, (1957): 271-276.

Dvorak, M.: Adrenocortical Function in Foetal, Neonatal and Young Pigs. J. Endocr., 54, (1972): 473-481.

Edwards, A. V., Hardy, R. N., and Malinowska, K. W.: The Effects of Infusions of Synthetic Adrenocorticotrophin in the Conscious Calf. J. Physiol., 239, (1974): 477-498.

Edwards, A. V., Hardy, R. N., and Malinowska, K. W.: The Sensitivity of Adrenal Responses to Synthetic Adrenocorticotrophin in the Conscious Unrestrained Calf. J. Physiol., 245, (1975): 639-653.

El-Nageh, M. M.: Voies d'Absorption des Gammaglobulines du Colostrum au Niveau de l'Intestine Grêle du Veau Noveau-né. Ann. Méd. Vét., 111, (1967a): 384-390.

El-Nageh, M. M.: Relation Entre l'Arrêt de la Resorption Intestinale des Anticorps et le Renouvellement de l'Epithélium Intestinal. Ann. Méd. Vét., 111, (1967b): 400-405.

Ehrlich, P.: Uber Immunitat durch Vererbung und Saugung. Zeitschrift Hyg. Intektionskrankheiten, 12, (1892): 183-203.

Fahey, K. J.: Immunological Reactivity in the Foetus and the Structure of Foetal Lymphoid Tissues. In, *Progress in Immunology II*, Vol. 3. Ed. L. Brent and J. Holborow, North-Holland Publishing Company, Amsterdam, Holland, (1974): 49-60.

Famulener, L. W.: On the Transmission of Immunity from Mother to Offspring. A Study Upon Serum Hemolysins in Goats. J. Infect. Dis., 10, (1912): 332-368.

Ferris, T. A. and Thomas, J. W.: Relationship of Immunoglobulin to Dairy Calf Mortality and Influence of Herd Environment. J. Dairy Sci., 57, (1974): 641 (Abstr).

Fey, H.: Immunology of the Newborn Calf: Its Relationship to Colisepticemia. Ann. N.Y. Acad. Sci., 176, (1971): 49-63.

Fey, H., and Margadant, A.: Zur Pathogenese der Kälber-Colisepsis. IV. Agammaglobulinamie als disponierender Faktor. Zentral Vet. Med., 9, (1962): 653-663.

Ganong, W. F. and Gold, E. M.: Changes in Blood ACTH Levels Following Administration of SU-4885 to Adrenalectomized Dogs. Physiologist, 3, (1960): 63 (Abstr).

Gay, C. C.: *Escherichia coli* and Neonatal Disease of Calves. Bact. Rev., 29, (1965): 75-101.

Gay, C. C.: Problems of Immunization in the Control of *Escherichia coli* Infection. Ann. N.Y. Acad. Sci., 176, (1971): 336-349.

Gay, C. C.: *In utero* Immunization of Calves Against Colisepticemia. Am. J. Vet. Res., 36, (May, 1975): 625-630.

Gay, C. C., Anderson, N., Fisher, E. W., and McEwan, A. D.: Gammaglobulin Levels and Neonatal Mortality in Market Calves. Vet. Rec., 77, (1965): 148-149.

Gay, C. C., McKay, K. A., and Barnum, D. A.: Studies on Colibacillosis of Calves. III. The Experimental Reproduction of Colibacillosis. Canad. Vet. J., 5, (1964): 314-325.

Gijzen, A. H. J.: Specificity and Sensitivity of Binding Proteins in the Radioimmunoassay of Cortisol. A Comparative Study with Several Protein Binding Agents in Reactions with Many Steroids. J. Molec. Med., 2, (1977): 113-118.

Gillette, D. D. and Filkins, M.: Factors Affecting Antibody Transfer in the Newborn Puppy. Am. J. Physiol., 210, (1966): 419-422.

Grosser, O.: Vergleichende Anatomie und Entwicklungs Geschichte der Eihaute und Placenta. Braumüller, Vienna, 1909. Cited by Schlamowitz, M.: Membrane Receptors in the Specific Transfer of Immunoglobulins from Mother to Young. Immunol. Communications, 5, (1976): 481-500.

Grosser, O.: Frühentwicklung, Eihautbildung und Placentation der Menschen und der Säugetiere. Bergman, München, 1927. Cited by Schlamowitz, M.: Membrane Receptors in the Specific Transfer of Immunoglobulins from Mother to Young. Immunol. Communications, 5, (1976): 481-500.

Halliday, R.: The Effect of Steroid Hormones on the Absorption of Antibody by the Young Rat. J. Endocr., 18, (1959): 56-66.

Halliday, R. and Williams, M. R.: Passive Immunity in the Lamb. Effects of a Second Feed of Colostrum on Antibody Absorption from the First Feed. Res. Vet. Sci., 21, (1976): 173-175.

Hammer, D. K., Kickhöfen, B., and Hemming, G.: Molecular Classes and Properties of Antibodies in Cattle Serum and Colostrum Synthesized During the Primary and Secondary Response to Protein Antigens. Europ. J. Biochem., 6, (1968): 443-454.

Hardy, R. N.: The Acceleration by Certain Anions of the Absorption of Macromolecular Substances from the Small Intestine of the Newborn Calf. J. Physiol., 194, (1968): 45-46P.

Hardy, R. N.: 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, (1969): 607-623.

Hardy, R. N.: Absorption of Macromolecules from the Intestine of the Newborn Animal. In, *Physiology of Digestion and Metabolism in the Ruminant*. Ed. A. T. Phillipson, Oriel Press Limited, Newcastle-upon-Tyne, England, (1970): 150-165. Proc. Third International Symposium, Cambridge, England, August, 1969.

Hemmings, W. A., and Williams, E. W.: The Attachment of IgG to Cell Components of Transporting Membranes. In, *Maternofoetal Transmission of Immunoglobulins*. Ed. W. A. Hemmings, Cambridge University Press, Cambridge, (1975): 91-111.

Hill, K. J.: Gastric Development and Antibody Transference in the Lamb, with Some Observations on the Rat and Guinea Pig. Quart. J. Exp. Physiol., 41, (1956): 421-432.

Hoerlein, A. B. and Jones, D. L.: Bovine Immunoglobulins Following Induced Parturition. J. Am. Vet. Med. Assoc., 170, (Feb. 1, 1977): 325-326.

Holt, P. G. and Oliver, I. T.: Plasma Corticosterone Concentrations in the Perinatal Rat. Biochem. J., 108, (1968): 339-341.

Howe, P. E.: An Effect of the Ingestion of Colostrum upon the Composition of the Blood in New-Born Calves. J. Biol. Chem., 49, (1921): 115-118.

Howe, P. E.: The Relation Between Age and the Concentration of Protein Fractions in the Blood of the Calf and Cow. J. Biol. Chem., 53, (1922): 479-494.

Howe, P. E.: The Relation Between the Ingestion of Colostrum or Blood Serum and the Appearance of Globulins and Albumin in the Blood and Urine of the Newborn Calf. J. Exp. Med., 39, (1924): 313-320.

Hudson, S., Mullord, M., Whittlestone, W. G., and Payne, E.: Plasma Corticoid Levels in Healthy and Diarrhoeic Calves from Birth to 20 Days of Age. Br. Vet. J., 132, (1976): 551-556.

Hunter, J. T., Fairclough, R. J., Peterson, A. J., and Welch, R. A. S.: Foetal and Maternal Hormonal Changes Preceding Normal Bovine Parturition. Acta Endocr., 84, (1977): 653-662.

Husband, A. J., Brandon, M. R., and Lascelles, A. K.: Absorption and Endogenous Production of Immunoglobulins in Calves. Aust. J. Exp. Biol. Med. Sci., 50, (1972): 491-498.

Husband, A. J., Brandon, M. R., and Lascelles, A. K.: The Effect of Corticosteroid on Absorption and Endogenous Production of Immunoglobulins in Calves. Aust. J. Exp. Biol. Med. Sci., 51, (1973): 707-710.

Irwin, V. C. R.: Incidence of Disease in Colostrum-Deprived Calves. Vet. Rec., 94, (1974): 105-106.

Jacobson, N. L.: The Mammary Gland and Lactation. In, *The Physiology of Domestic Animals*, Eighth Edition. Ed. M. J. Swenson, Cornell University Press, Ithaca, NY, (1970): 1356-1382.

Jameson, E., Alvarez-Tostado, C., and Sortor, H. H.: Electrophoretic Studies on New-Born Calf Serum. Proc. Soc. Exp. Biol. NY, 51, (1942): 163-165.

Johnson, P. and Pierce, A. E.: Ultracentrifugal and Electrophoretic Studies on Neonatal Calf Sera and Maternal Colostrum. J. Hyg. Camb., 57, (1959): 309-320.

Johnston, N. E., Estrella, R. A., and Oxender, W. D.: Resistance of Neonatal Calves Given Colostrum Diet to Oral Challenge with a Septicemia-Producing *Escherichia coli*. Am. J. Vet. Res., 38, (Sept., 1977): 1323-1326.

Jones, C. T., Luther, E., Ritchie, J. W. K., and Worthington, D.: The Clearance of ACTH from the Plasma of Adult and Fetal Sheep. Endocrinology, 96, (1975): 231-234.

Khan, M. A., Dickson, W. M., and Meyers, K. M.: The Effect of Low Environmental Temperature on Plasma Corticosteroid and Glucose Concentrations in the Newborn Calf. J. Endocr., 48, (1970): 355-363.

Kiddy, C. A., McCann, R., Maxwell, C., Pock, C., Pierce, C., and Butler, J. E.: Changes in Levels of Immunoglobulins in Serum and Other Body Fluids Immediately Before and After Parturition. J. Dairy Sci., 54, (1971): 1325-1327.

Klaus, G. G. B., Bennet, A., and Jones, E. W.: A Quantitative Study of the Transfer of Colostral Immunoglobulins to the Newborn Calf. Immunology, 16, (1969): 293-299.

Kniazeff, A. J., Rimer, V., and Gaeta, L.: Gamma Globulin in Foetal Bovine Sera; Significance in Virology. Nature, 214, (1967): 805-806.

Kraehenbuhl, J. P. and Campiche, M. A.: Early Stages of Intestinal Absorption of Specific Antibodies in the Newborn. An Ultrastructural, Cytochemical, and Immunological Study in the Pig, Rat, and Rabbit. J. Cell Biol., 42, (1969): 345-365.

Kraehenbuhl, J. P., Gloor, E., and Blanc, B.: Résorption Intestinale de la Ferritine chez Deux Espèces Animales aux Possibilités d'Absorption Protéique Néonatale Différentes. Zeitschrift für Zeliforschung, 76, (1967): 170-186.

Kruse, V.: Absorption of Immunoglobulin from Colostrum in Newborn Calves. Anim. Prod., 12, (1970): 627-638.

Kunkel, H. G.: Estimation of Alterations of Serum Gamma Globulin by Turbidimetric Technique. Proc. Soc. Exp. Biol. Med., 66, (1947): 217-224.

LaMotte, G. B.: Total Serum Protein, Serum Protein Fractions and Serum Immunoglobulins in Colostrum-Fed and Colostrum-Deprived Calves. Am. J. Vet. Res., 38, (Feb., 1977): 263-268.

LaMott, G. B., and Eberhart, R. J.: Blood Leukocytes, Neutrophil Phagocytosis, and Plasma Corticosteroids in Colostrum-Fed and Colostrum-Deprived Calves. Am. J. Vet. Res., 37, (Oct., 1976): 1189-1193.

Lascelles, A. K.: Immunoglobulin Secretion into Ruminant Colostrum. In, *Lactogenesis: The Initiation of Milk Secretion at Parturition.* Proceedings of a Symposium, Satellite to XXIV International Congress of Physiological Sciences. Edited by M. Reynolds and S. J. Folley, University of Pennsylvania Press, Philadelphia, PA, (1969): 131-136.

Lascelles, A. K.: Mechanism of Milk Synthesis and Secretion. Proc. 18th International Dairy Congress, 11, (1971): 514-524.

Laster, D. B. and Gregory, K. E.: Factors Influencing Peri- and Early Postnatal Calf Mortality. J. Anim. Sci., 37, (1973): 1092-1097.

Lecce, J. G.: Glucose Milli-Equivalents Eaten by the Neonatal Pig and Cessation of Intestinal Absorption of Large Molecules (Closure). J. Nutr., 90, (1966a): 240-244.

Lecce, J.G.: Absorption of Macromolecules by Neonatal Intestine. Biol. Neonat., 9, (1966b): 50-61.

Lecce, J. G.: Selective Absorption of Macromolecules into Intestinal Epithelium and Blood by Neonatal Mice. J. Nutr., 102, (1972): 69-76.

Lecce, J. G.: Effect of Dietary Regimen on Cessation of Uptake of Macromolecules by Piglet Intestinal Epithelium (Closure) and Transport to the Blood. J. Nutr., 103, (1973): 751-756.

Lecce, J. G., Matrone, G., and Morgan, D. O.: Porcine Neonatal Nutrition: Absorption of Unaltered Non-Porcine Proteins and Polyvinylpyrrolidone from the Gut of Piglets and the Subsequent Effect on the Maturation of the Serum Protein Profile. J. Nutr., 73, (1961): 158-166.

Lecce, J. G. and Morgan, D. O.: Effect of Dietary Regimen on Cessation of Intestinal Absorption of Large Molecules (Closure) in the Neonatal Pig and Lamb. J. Nutr., 78, (1962): 263-267.

Liddle, G. W., Island, D., Lance, E. M., and Harris, A. P.: Alterations of Adrenal Steroid Patterns in Man Resulting from Treatment with a Chemical Inhibitor of 11 -Hydroxylation. J. Clin. Endocr., 18, (1958): 906-912.

Liggins, G. C.: Premature Parturition after Infusion of Corticotrophin or Cortisol into Foetal Lambs. J. Endocr., 42, (1968): 323-329.

Liggins, G. C.: The Foetal Role in the Initiation of Parturition in the Ewe. In, *Ciba Foundation Symposium on Foetal Autonomy*. Ed. G. E. W. Wolstenholme and M. O'Connor, J. and A. Churchill Ltd., London, (1969): 218-231.

Liggins, G. C., Fairclough, R. J., Grieves, S. A., Kendall, J. Z., and Knox, B. S.: The Mechanism of Initiation of Parturition in the Ewe. Rec. Prog. Horm. Res., 29, (1973): 111-159.

Lin, Y. C., Oxender, W. D., and Hafs, H. D.: Cortisol and Corticosterone Concentrations in the Bovine Fetus and Dam. Biol. Reprod. (in press).

Little, R. B. and Orcutt, M. L.: The Transmission of Agglutinins of *Bacillus abortus* from Cow to Calf in the Colostrum. J. Exp. Med., 35, (1922): 161-171.

Logan, E. F.: Colostral Immunity to Colibacillosis in the Neonatal Calf. Br. Vet. J., 130, (1974): 405-412.

Logan, E. F.: The Influence of Husbandry on Colostrum Yield and Immunoglobulin Concentration in Beef Cows. Br. Vet. J., 133, (1977): 120-125.

Logan, E. F. and Gibson, T.: Serum Immunoglobulin Levels in Suckled Beef Calves. Vet. Rec., 97, (1975): 229-230.

Logan, E. F., McBeath, D. G., and Lowman, B.: Quantitative Studies on Serum Immunoglobulin Levels in Suckled Calves from Birth to Five Weeks. Vet. Rec., 94, (1974a): 367-370.

Logan, E. F. and Penhale, W. J.: Studies on the Immunity of the Calf to Colibacillosis. I. The Influence of Colostral Whey and Immunoglobulin Fractions on Experimental Colisepticemia. Vet. Rec., 88, (1971a): 222-228.

Logan, E. F. and Penhale, W. J.: Studies on the Immunity of the Calf to Colibacillosis. III. The Local Protective Activity of Colostrum within the Gastro-Intestinal Tract. Vet. Rec., 89, (1971b): 628-632.

Logan, E. F. and Penhale, W. J.: Studies on the Immunity of the Calf to Colibacillosis. IV. The Prevention of Experimental Colisepticaemia by the Intravenous Administration of a Bovine Serum IgM-Rich Fraction. Vet. Rec., 89, (1971c): 663-667.

Logan, E. F. and Penhale, W. J.: Studies on the Immunity of the Calf to Colibacillosis. V. The Experimental Reproduction of Enteric Colibacillosis. Vet. Rec., 91, (1972): 419-423.

Logan, E. F., Penhale, W. J., and Jones, R. A.: Changes in the Serum Immunoglobulin Levels of Colostrum-Fed Calves During the First 12 Weeks *Postpartum*. Res. Vet. Sci., 14, (1972): 394-397.

Logan, E. F., Stenhouse, A., Ormrod, D. J., and Penhale, W. J.: The Role of Colostral Immunoglobulins in Intestinal Immunity to Enteric Colibacillosis in the Calf. Res. Vet. Sci., 17, (1974b): 290-301.

Logan, E. F., Stenhouse, A., Ormrod, D. J., Penhale, W. J., and Armishaw, M.: Studies on the Immunity of the Calf to Colibacillosis. VI. The Prophylactic Use of a Pooled Serum IgM-Rich Fraction Under Field Conditions. Vet. Rec., 94, (1974c): 386-389.

Mach, J.-P.: *In vitro* Combination of Human and Bovine Secretory Component with IgA of Various Species. Nature, 228, (1970): 1278-1282.

Mach, J.-P. and Pahud, J.-J.: Secretory IgA, A Major Immunoglobulin in Most Bovine External Secretions. J. Immunol., 106, (1971): 552-563.

Madill, D. and Bassett, J. M.: Corticosteroid Release by Adrenal Tissue from Foetal and Newborn Lambs in Response to Corticotrophin Stimulation in a Perifusion System *in vitro*. J. Endocr., 58, (1973): 75-87.

Malinowska, K. W., Hardy, R. N., and Nathanielsz, P. W.: Neonatal Adrenocortical Function and Its Possible Relation to the Uptake of Macromolecules by the Small Intestine of the Guinea-Pig and Rabbit. J. Endocr., 55, (1972): 397-404.

Martinsson, K. and Jönsson, L.: The Uptake of Macromolecules in the Ileum of Piglets after Intestinal "Closure." Zbl. Vet. Med., 23, (1976): 277-282.

Mason, J. D., Dalling, T., and Gordon, W. S.: Transmission of Maternal Immunity. J. Path. Bact., 33, (1930): 783-796.

McBeath, D. G.: Prophylactic Use of Hyperimmune Serum in Experimental Colisepticemia in Calves. Vet. Rec., 100, (1977): 259-262.

McBeath, D. G., Penhale, W. J., and Logan, E. F.: An Examination of the Influence of Husbandry on the Plasma Immunoglobulin Level of the Newborn Calf, Using a Rapid Refractometer Test for Assessing Immunoglobulin Content. Vet. Rec., 88, (1971): 266-270.

McDougall, D. F. and Mulligan, W.: The Distribution and Metabolism of Fast IgG Immunoglobulin in the Neonatal Calf. J. Physiol., 201, (1969): 77-78P.

McEwan, A. D., Fisher, E. W., and Selman, I. E.: An Estimation of the Efficiency of the Absorption of Immune Globulins from Colostrum by Newborn Calves. Res. Vet. Sci., 11, (1970a): 239-243.

McEwan, A. D., Fisher, E. W., and Selman, I. E.: Observations on the Immune Globulin Levels of Neonatal Calves and Their Relationship to Disease. J. Comp. Path., 80, (1970b): 259-265.

McEwan, A. D., Fisher, E. W., Selman, I. E., and Penhale, W. J.: A Turbidity Test for the Estimation of Immune Globulin Levels in Neonatal Calf Serum. Clin. Chim. Acta, 27, (1970c): 155-163.

McGuire, T. C., Crawford, T. B., Hallowell, A. L., and Macomber, L. E.: Failure of Colostral Immunoglobulin Transfer as an Explanation for Most Infections and Deaths of Neonatal Foals. J. Am. Vet. Med. Assoc., 170, (June 1, 1977): 1302-1304.

McMartin, C. and Peters, J.: Levels of Corticotrophin Analogues in the Blood After Infusion into Rats. J. Endocr., 67, (1975): 41-48.

McNulty, M. S., McFerran, J. B., Bryson, D. G., Logan, E. B., and Curran, W. L.: Studies on Rotavirus Infection and Diarrhoea in Young Calves. Vet. Rec., 99, (1976): 229-230.

Merriman, M. J. G. S.: Serum Immunoglobulins in Newborn Calves Before and After Colostrum Feeding. Can. J. Comp. Med., 35, (1971): 269-273.

Micusan, V. V. and Borduas, A. G.: Preferential Transport into Colostrum of Fc Fragment Derived from Serum IgG_1 Immunoglobulin in the Goat. Res. Vet. Sci., 21, (1976): 150-154.

Miller, J. F. A. P.: Immunity in the Fetus and the Newborn. Br. Med. Bull., 22, (1966): 21-26.

Miller, W. J. and Hubbert, W. T.: Naturally Occurring Antibody in Bovine Fetal Serum: Reactivity Against Homologous and Heterologous Species Erythrocytes. Anim. Blood Grps. Biochem. Genet., 3, (1972): 3-17.

Moncloa, F., Velazco, I., and Beteta, L.: Biological Half-Life of a Synthetic Corticotrophin in Men. Acta Endocr., 52, (1966): 337-340.

Moog, F.: The Functional Differentiation of the Small Intestine. III. The Influence of the Pituitary-Adrenal System on the Differentiation of Phosphatase in the Duodenum of the Suckling Mouse. J. Exp. Zool., 124, (1953): 329-346.

Moog, F.: Development Adaptations of Alkaline Phosphatases in the Small Intestine. Fed. Proc., 21, (1962): 51-56.

Moog, F. and Thomas, E. R.: The Influence of Various Adrenal and Gonadal Steroids on the Accumulation of Alkaline Phosphatase in the Duodenum of the Suckling Mouse. Endocrinology, 56, (1955): 187-196.

Moon, H. W. and Joel, D. D.: Epithelial Cell Migration in the Small Intestine of Sheep and Calves. Am. J. Vet. Res., 36, (Feb., 1975): 187-189.

Morris, B. and Morris, R.: The Effect of Cortisone Acetate on Stomach Evacuation and the Absorption of ^{125}I-Labelled Globulins in Young Rats. J. Physiol., 240, (1974): 77-89.

Morris, B. and Steel, E. D.: The Absorption of Antibody by Young Hedgehogs after Treatment with Cortisone Acetate. J. Endocr., 30, (1964): 195-203.

Morris, I. G.: Intestinal Transmission of IgG Subclasses in Suckling Rats. In, *Maternofoetal Transmission of Immunoglobulins*. Ed. W. A. Hemmings, Cambridge University Press, Cambridge, (1975): 341-357.

Moscona, A. A.: Hydrocortisone-Mediated Regulation of Gene Expression in Embryonic Neural Retina: Induction of Glutamine Synthetase. J. Steroid Biochem., 6, (1975): 633-638.

Muller, L. D., Beardsley, G. L., Ellis, R. P., Reed, D. E., and Owens, M. J.: Calf Response to the Initiation of Parturition in Dairy Cows with Dexamethasone or Dexamethasone with Estradiol Benzoate. J. Anim. Sci., 41, (1975): 1711-1716.

Munn, E. A. and Smith, M. W.: Uptake of Albumin by Neonatal Pig Ileum Incubated *in vitro*. J. Physiol., 242, (1974): 30-32P.

Murphy, B. E. P.: Does the Human Fetal Adrenal Play a Role in Parturition? Am. J. Obstet. Gynecol., 115, (1973): 521-525.

Naylor, J. M. and Kronfeld, D. S.: Refractometry as a Measure of the Immunoglobulin Status of the Newborn Dairy Calf: Comparison with the Zinc Sulfate Turbidity Test and Single Radial Immunodiffusion. Am. J. Vet. Res., 38, (Sept., 1977): 1331-1334.

Naylor, J. M., Kronfeld, D. S., Bech-Nielsen, S., and Bartholomew, R. C.: J. Am. Vet. Med. Assoc., 171, (Oct. 1, 1977): 635-638.

Needham, J.: *Clinical Embryology, Vol. 3, Biochemistry of the Placental Barrier*. Cambridge University Press, Cambridge, 1931. Cited by Schlamowitz, M.: Membrane Receptors in the Specific Transfer of Immunoglobulins from Mother to Young. Immunol. Communications, 5, (1976): 481-500.

Newby, T. J. and Bourne, F. J.: The Nature of the Local Immune System of the Bovine Small Intestine. Immunology, 31, (1976a): 475-480.

Newby, T. J. and Bourne, F. J.: Relative Resistance of Bovine and Porcine Immunoglobulins to Proteolysis. Immunol. Communications, 5, (1976b): 631-635.

Olson, D. P. and Waxler, G. L.: Immune Responses of the Bovine Fetus and Neonate to *Escherichia coli*: Plaque-Forming and Intestinal Immune Responses. Am. J. Vet. Res., 38, (Aug., 1977): 1177-1181.

Olsson, B.: Studies on the Formation and Absorption of Antibodies and Immune Globulins in Piglets. II. The Intestinal Absorption of Antibodies and Immune Globulins by Newborn Piglets after the Administration of Bovine Colostrum. Nord. Vet.-Med., 11, (1959a): 375-390.

Olsson, B.: Studies on the Formation and Absorption of Antibodies and Immune Globulins in Piglets. III. The Intestinal Absorption of Heterologous Antibodies and Serum Proteins in Newborn Piglets. Nord. Vet.-Med., 11, (1959b): 441-460.

Orcutt, M. L. and Howe, P. E.: The Relation Between the Accumulation of Globulins and the Appearance of Agglutinins in the Blood of New-Born Calves. J. Exp. Med., 36, (1922): 291-308.

Orlowski, J. P., Sieger, L., and Anthony, B. F.: Bactericidal Capacity of Monocytes of Newborn Infants. J. Pediat., 89, (1976): 797-801.

Osburn, B. I.: Immune Responsiveness of the Fetus and Neonate. J. Am. Vet. Med. Assoc., 163, (Oct. 1, 1973): 801-803.

Osburn, B. I. and Stabenfeldt, G. H.: Immune Responsiveness in Perinatal Calves. J. Am. Vet. Med. Assoc., 163, (Nov. 15, 1973): 1188 (Abstr).

Osburn, B. I., Stabenfeldt, G. H., Ardans, A. A., Trees, C., and Sawyer, M.: Perinatal Immunity in Calves. J. Am. Vet. Med. Assoc., 164, (Feb. 1, 1974): 295-298.

Oxender, W. D., Newman, L. E., and Morrow, D. A.: Factors Influencing Dairy Calf Mortality in Michigan. J. Am. Vet. Med. Assoc., 162, (March 15, 1973): 458-460.

Paape, M. J., Desjardins, C., Guidry, A. J., Miller, R. H., and Smith, V. R.: Response of Plasma Corticosteroids and Circulating Leukocytes in Cattle Following Intravenous Injection of Different Doses of Adrenocorticotropin. Am. J. Vet. Res., 38, (Sept., 1977): 1345-1348.

Papaikonomou, E.: Rat Adrenocortical Dynamics. J. Physiol., 265, (1977): 119-131.

Patt, J. A. and Eberhart, R. J.: Effects of Metyrapone and ACTH on Intestinal Absorption of Immunoreactive Bovine IgG in Cesarian-Derived Pigs. Am. J. Vet. Res., 37, (Dec., 1976): 1409-1413.

Payne, L. C. and Marsh, C. L.: Gammaglobulin Absorption in the Baby Pig: The Non-Selective Absorption of Heterologous Globulins and Factors Influencing Absorption Time. J. Nutr., 76, (1962a): 151-158.

Payne, L. C. and Marsh, C. L.: Absorption of Gammaglobulin by the Small Intestine. Fed. Proc., 21, (1962b): 909-912.

Penhale, W. J.: Gamma Globulin Levels and Neonatal Mortality in Market Calves. Vet. Rec., 77, (1965): 322-323.

Penhale, W. J., Christie, G., McEwan, A. D., Fisher, E. W., and Selman, I. E.: Quantitative Studies on Bovine Immunoglobulins. II. Plasma Immunoglobulin Levels in Market Calves and Their Relationship to Neonatal Infection. Br. Vet. J., 126, (1970): 30-37.

Penhale, W. J., Logan, E. F., Selman, I. E., Fisher, E. W., and McEwan, A. D.: Observations on the Absorption of Colostral Immunoglobulins by the Neonatal Calf and Their Significance in Colibacillosis. Ann. Rech. Vétér., 4, (1973): 223-233.

Penhale, W. J., Logan, E. F., and Stenhouse, A.: Studies on the Immunity of the Calf to Colibacillosis. II. Preparation of an IgM-Rich Fraction from Bovine Serum and its Prophylactic Use in Experimental Colisepticemia. Vet. Rec., 89, (1971): 623-627.

Pfeiffer, N. E. and McGuire, T. C.: A Sodium Sulfite-Precipitation Test for Assessment of Colostral Immunoglobulin Transfer to Calves. J. Am. Vet. Med. Assoc., 170, (April 15, 1977): 809-811.

Pfeiffer, N. E., McGuire, T. C., Bendel, R. B., and Weikel, J. M.: Quantitation of Bovine Immunoglobulins: Comparison of Single Radial Immunodiffusion, Zinc Sulfate Turbidity, Serum Electrophoresis, and Refractometer Methods. Am. J. Vet. Res., (May, 1977): 693-698.

Pierce, A. E.: Electrophoretic and Immunological Studies on Sera from Calves from Birth to Weaning. I. Electrophoretic Studies. J. Hyg. Camb., 53, (1955): 247-260.

Pierce, A. E.: Studies on the Proteinuria of the New-Born Calf. J. Physiol., 148, (1959): 469-488.

Pierce, A. E.: Further Studies on Proteinuria in the New-born Calf. J. Physiol., 156, (1961): 136-149.

Pierce, A. E. and Feinstein, A.: Biophysical and Immunological Studies on Bovine Immune Globulins with Evidence for Selective Transport within the Mammary Gland from Maternal Plasma to Colostrum. Immunology, 8, (1965): 106-123.

Pierce, A. E., Risdall, P. C., and Shaw, B.: Absorption of Orally Administered Insulin by the Newly Born Calf. J. Physiol., 171, (1964): 203-215.

Playfair, J. H. L.: Strain Differences in the Immune Response of Mice. I. The Neonatal Response to Sheep Red Cells. Immunology, 15, (1968a): 35-50.

Playfair, J. H. L.: Strain Differences in the Immune Response of Mice. II. Responses by Neonatal Cells in Irradiated Adult Hosts. Immunology, 15, (1968b): 815-826.

Plog, J.: Unpublished data, 1974.

Porter, P.: Immunoglobulin IgA in Bovine Mammary Secretions and Serum of the Neonatal Calf. Biochim. Biophys. Acta, 236, (1971): 664-674.

Porter, P.: Immunoglobulins in Bovine Mammary Secretions. Quantitative Changes in Early Lactation and Absorption by the Neonatal Calf. Immunology, 23, (1972): 225-238.

Porter, P.: The Functional Role of Secretory Antibody Systems in Early Development of the Pig and Calf. Proc. Nutr. Soc., 32, (1973a): 217-222.

Porter, P.: Functional Heterogeneity of the Bovine Immune System. J. Am. Vet. Med. Assoc., 163, (Oct. 1, 1973b): 789-794.

Porter, P.: Immunoglobulin Mechanisms in Health and Nutrition from Birth to Weaning. Proc. Nutr. Soc., 35, (1976): 273-282.

Porter, P., Noakes, D. E., and Allen, W. D.: Intestinal Secretion of Immunoglobulins in the Preruminant Calf. Immunology, 23, (1972): 299-312.

Reisenger, R. C.: Pathogenesis and Prevention of Infectious Diarrhea (Scours) of Newborn Calves. J. Am. Vet. Med. Assoc., 147, (Dec. 15, 1965): 1377-1386.

Renshaw, H. W., Eckblad, W. P., Everson, D. O., Tassinari, P. D., and Amos, D.: Ontogeny of Immunocompetence in Cattle: Evaluation of Phytomitogen-Induced *in vitro* Bovine Fetal Lymphocyte Blastogenesis, Using a Whole Blood Culture Technique. Am. J. Vet. Res., 38, (Aug., 1977): 1141-1150.

Renshaw, H. W., Eckblad, W. P., Thacker, D. L., and Frank, F. W.: Antibacterial Host Defense: *In vitro* Interaction of Bacteria, Serum Factors, and Leukocytes from Precolostral Dairy Calves and Their Dams. Am. J. Vet. Res., 37, (Nov., 1976): 1267-1274.

Rodewald, R.: Selective Antibody Transport in the Proximal Small Intestine of the Neonatal Rat. J. Cell Biol., 45, (1970): 635-640.

Rodewald, R.: Intestinal Transport of Antibodies in the Newborn Rat. J. Cell Biol., 58, (1973): 189-211.

Rodewald, R.: Intestinal Transport of Peroxidase-Conjugated IgG Fragments in the Neonatal Rat. In, *Maternofoetal Transmission of Immunoglobulins*. Ed. W. A. Hemmings, Cambridge University Press, Cambridge, (1975): 123-135.

Rossi, C. R., Kiesel, G. K., and Hubbert, W. T.: Immunoglobulin Concentrations and Bovine Enterovirus Inhibitors in Fetal Bovine Fluids. Cornell Vet., 66, (1976): 381-386.

Roy, J. H. B.: *The Calf, Vol. 1, Management and Feeding*. Newnes-Butterworths, London, 1970.

Rundell, J. O. and Lecce, J. G.: Independence of Intestinal Epithelial Cell Turnover from Cessation of Macromolecules (Closure) in the Neonatal Mouse, Rabbit, Hamster, and Guinea Pig. Biol. Neonate, 20, (1972): 51-57.

Saffran, M. and Schally, A. V.: The Release of Corticotropin by Anterior Pituitary Tissue *in vitro*. Can. J. Biochem., 33, (1955): 408-415.

Sakamoto, S., Kigawa, T., Mizuno, M., Minaguchi, H., Satoh, K., Jimbo, T., Nakai, T., and Kuwabara, Y.: Adaptation Mechanism in the Fetus, with Special Reference to Fetal Endocrinology. Acta Obstet. Gynecol. Scand., Suppl. 63, (1977): 1-19.

Sawyer, M., Osburn, B. I., Knight, H. D., and Kendrick, J. W.: A Quantitative Serologic Assay for Diagnosing Congenital Infections of Cattle. Am. J. Vet. Res., 34, (Oct., 1973): 1281-1284.

Shally, A. V., Arimura, A., Redding, T. W., Chihara, K., Gordin, A., Huang, W.-Y., and Saffran, M.: Purification of Corticotropin Releasing Factor from Porcine Hypothalami. 59th Ann. Meeting, The Endocrine Society, (1977): 95 (Abstr).

Schimmel, D.: Agglutine und β-Globuline in Kalberserum vor Aufnahme von Kolostralmilch. Arch. Exp. Vet. Med., 21, (1967): 29-33.

Schlamowitz, M.: Membrane Receptors in the Specific Transfer of Immunoglobulins from Mother to Young. Immunol. Communications, 5, (1976): 481-500.

Schultz, R. D., Dunne, H. W., and Heist, C. E.: Ontogeny of the Bovine Immune Response. Infect. Immunity, 7, (1973): 981-991.

Schwyzer, R. and Sieber, P.: Total Synthesis of Adrenocorticotrophic Hormone. Nature, 199, (1963): 172-174.

Selman, I. E.: The Absorption of Colostral Globulins by Newborn Calves. Ann. Rech. Vétér., 4, (1973): 213-221.

Selman, I. E., de la Fuente, G. H., Fisher, E. W., and McEwan, A. D.: The Serum Immune Globulin Concentrations of Newborn Dairy Heifer Calves: A Farm Survey. Vet. Rec., 88, (1971c): 460-464.

Selman, I. E., McEwan, A. D., and Fisher, E. W.: Studies on Natural Suckling in Cattle During the First Eight Hours Post Partum. I. Behavioural Studies (Dams). Anim. Behav., 18, (1970a): 276-283.

Selman, I. E., McEwan, A. D., and Fisher, E. W.: Studies on Natural Suckling in Cattle During the First Eight Hours Post Partum. II. Behavioural Studies (Calves). Anim. Behav., 18, (1970b): 284-289.

Selman, I. E., McEwan, A. D., and Fisher, E. W.: Serum Immune Globulin Concentrations of Calves Left with Their Dams for the First Two Days of Life. J. Comp. Path., 80, (1970c): 419-427.

Selman, I. E., McEwan, A. D., and Fisher, E. W.: Studies on Dairy Calves Allowed to Suckle Their Dams at Fixed Times Post Partum. Res. Vet. Sci., 12, (1971a): 1-6.

Selman, I. E., McEwan, A. D., and Fisher, E. W.: Absorption of Immune Lactoglobulin by Newborn Dairy Calves. Res. Vet. Sci., 12, (1971b): 205-210.

Silverstein, A. M., Uhr, J. W., Kraner, K. L., and Lukes, R. J.: Fetal Response to Antigenic Stimulus: II. Antibody Production by the Fetal Lamb. J. Exp. Med., 117, (1963): 799-812.

Simpson-Morgan, M. W., and Smeaton, T. C.: The Transfer of Antibodies by Neonates and Adults. Adv. Vet. Sci. Comp. Med., 16, (1972): 355-386.

Smeaton, T. C.: Studies of Gamma-Globulin Metabolism in the Foetal and Neonatal Lamb. Ph.D. Thesis, Australian National University, Canberra, Australia (1969). Cited by Simpson-Morgan, M. W. and Smeaton, T. C.: The Transfer of Antibodies by Neonates and Adults. Adv. Vet. Sci. Comp. Med., 16, (1972): 355-386.

Smith, A. N. and Ingram, D. G.: Immunological Responses of Young Animals. II. Antibody Production in Calves. Canad. Vet. J., 6, (1965): 226-232.

Smith, E. L. and Holm, A.: Transference of Immunity to the Newborn Calf from Colostrum. J. Biol. Chem., 175, (1948): 349-357.

Smith, H. W.: Observations in the Aetiology of Neonatal Diarrhoea (Scours) in Calves. J. Path. Bact., 84, (1962): 147-168.

Smith, H. W. and Linggood, M. A.: Further Observations on *Escherichia coli* Enterotoxins with Particular Regard to Those Produced by Atypical Piglet Strains and by Calf and Lamb Strains: The Transmissible Nature of These Enterotoxins and of a K Antigen Possessed by Calf and Lamb Strains. J. Med. Microbiol., 5, (1972): 243-250.

Smith, H. W., O'Neil, J. A., and Simmons, E. J.: The Immune Globulin Content of the Serum of Calves in England. Vet. Rec., 80, (1967): 664-666.

Smith, I. D. and Alexander, G.: Perinatal Mortality in Shorthorn Cattle in Northern Queensland. Proc. Aust. Soc. Anim. Prod., 6, (1966): 63-65.

Smith, M. W., Munn, E. A., Burton, K. A., and Henriques de Jesus, C.: Functional Changes in the Brush Border Surface of the Newborn Pig Intestine. In, *Maternofoetal Transmission of Immunoglobulins*. Ed. W. A. Hemmings, Cambridge University Press, Cambridge, (1975): 381-395.

Smith, T.: The Immunological Significance of Colostrum. I. The Relation Between Colostrum, Serum, and the Milk of Cows Normal and Immunized Towards *B. coli*. J. Exp. Med., 51, (1930): 473-481.

Smith, T., and Little, R. B.: The Significance of Colostrum to the Newborn Calf. J. Exp. Med., 36, (1922a): 181-198.

Smith, T., and Little, R. B.: Cow Serum as a Substitute for Colostrum in Newborn Calves. J. Exp. Med., 36, (1922b): 453-468.

Smith, T., and Little, R. B.: The Absorption of Specific Agglutinins in Homologous Serum Fed to Calves During the Early Hours of Life. J. Exp. Med., 37, (1923): 671-683.

Smith, T. and Orcutt, M. L.: The Bacteriology of the Intestinal Tract of Young Calves with Special Reference to the Early Diarrhea ("Scours"). J. Exp. Med., 41, (1925): 89-106.

Smith, V. G., Convey, E. M., and Edgerton, L. A.: Bovine Serum Corticoid Response to Milking and Exteroceptive Stimuli. J. Dairy Sci., 55, (1972): 1170-1173.

Smith, V. G., Edgerton, L. A., Hafs, H. D., and Convey, E. M.: Bovine Serum Estrogens, Progestins and Glucocorticoids During Late Pregnancy, Parturition and Early Lactation. J. Anim. Sci., 36, (1973): 391-396.

Smith, V. R. and Erwin, E. S.: Absorption of Colostrum Globulins Introduced Directly into the Duodenum. J. Dairy Sci., 42, (1959): 364-365.

Smith, V. R., Reed, R. E., and Erwin, E. S.: Relation of Physiological Age to Intestinal Permeability in the Bovine. J. Dairy Sci., 47, (1964): 923-924.

South, M. A., Cooper, M. D., Wollheim, F. A., Hong, R., and Good, R. A.: The IgA System. I. Studies of the Transport and Immunochemistry of IgA in the Saliva. J. Exp. Med., 123, (1966): 615-627.

Speicher, J. A. and Hepp, R. E.: Factors Associated with Calf Mortality in Michigan Dairy Herds. J. Am. Vet. Med. Assoc., 162, (March 15, 1973): 463-466.

Staley, T. E., Corley, L. D., Bush, L. J., and Jones, E. W.: The Ultrastructure of Neonatal Calf Intestine and Absorption of Heterologous Proteins. Anat. Rec., 172, (1972): 559-580.

Stormont, C.: The Role of Maternal Effects in Animal Breeding: I. Passive Immunity in Newborn Animals. J. Anim. Sci., 35, (1972): 1275-1279.

Stott, G. H., Wiersma, F., Menefee, B. E., and Radwanski, F. R.: Influence of Environment on Passive Immunity in Calves. J. Dairy Sci., 59, (1976): 1306-1311.

Strott, C. A., West, C. D., Nakagawa, K., Kondo, T., and Tyler, F. H.: Plasma 11-Deoxycorticosteroid and ACTH Response to Metyrapone (Plasma Metyrapone Test). J. Clin. Endocr., 29, (1969): 6-11.

Sugimoto, M., Kojima, A., and Endo, H.: Role of Glucocorticoids in Terminal Differentiation and Tissue-Specific Function (A Review). Develop. Growth Differentiation, 18, (1976): 319-327.

Sunshine, P., Herbst, J. J., Koldovsky, O., and Kretchmer, N.: Adaptation of the Gastrointestinal Tract to Extra-Uterine Life. Ann. N.Y. Acad. Sci., 176, (1971): 16-29.

Takahara, J., Ogawa, N., and Ofuji, T.: Extra-Adrenal Action of Metyrapone Upon Human Growth Hormone Secretion in Man. Endocr. Jap., 19, (1972): 197-201.

Tucker, H. A., Convey, E. M., and Koprowski, J. A.: Milking-Induced Release of Endogenous Prolactin in Cows Infused with Exogenous Prolactin. Proc. Soc. Exp. Biol. Med., 142, (1973): 72-75.

Vaerman, J. P.: Studies on IgA Immunoglobulins in Man and Animals. Ph.D. Thesis, Universite Catholique de Louvain, 1970. Cited by Porter, P.: The Functional Role of Secretory Antibody Systems in Early Development of the Pig and Calf. Proc. Nutr. Soc., 32, (1973a): 217-222.

Waldemann, T. A. and Jones, E. A.: The Role of IgG-Specific Cell Surface Receptors in IgG Transport and Catabolism. In, *Maternofoetal Transmission of Immunoglobulins*. Ed. W. A. Hemmings, Cambridge University Press, Cambridge, (1975): 123-135.

Walker, W. A. and Isselbacher, K. J.: Uptake and Transport of Macromolecules by the Intestine. Possible Role in Clinical Disorders. Gastroenterology, 67, (1974): 531-550.

Walker, W. A., Isselbacher, K. J., and Bloch, K. J.: Intestinal Uptake of Macromolecules: Effect of Oral Immunization. Science, 1977, (1972): 608-610.

Walker, W. A., Isselbacher, K. J., and Bloch, K. J.: Intestinal Uptake of Macromolecules. II. Effect of Parenteral Immunization. J. Immunol., 111, (1973): 221-226.

Wamukoya, J. P. O. and Conner, G. H.: Local Immune Responses in the Bovine Fetus Vaccinated *in utero* with *Escherichia coli* Antigen. Am. J. Vet. Res., 37, (Feb., 1976): 159-163.

Watson, D. L. and Lascelles, A. K.: Mechanisms of Transfer of Immunoglobulins into Mammary Secretion of Ewes. Aust. J. Exp. Biol. Med. Sci., 51, (1973): 247-254.

Weber, G., Srivastava, S. K., and Singhal, R. L.: Correlation of Glucocorticoid-Induced Synthesis of Hepatic Gluconeogenic Enzymes with Amino Acid Level and RNA Metabolism. Life Sci., 3, (1964): 829-837.

Wild, A. E.: Role of the Cell Surface in Selection During Transport of Proteins from Mother to Foetus and Newly Born. Phil. Trans. Roy. Soc. Lond., B, 271, (1975): 395-410.

Wilson, M. R., Duncan, J. R., Heistand, F., and Brown, P.: The Influence of Preparturient Intra-Mammary Vaccination on Immunoglobulin Levels in Bovine Mammary Secretions. Immunology, 23, (1972): 313-320.

Wolf, R. L., Mendlowitz, M., Soffer, L. J., Roboz, J., and Gitlow, S. E.: Metabolism of Corticotropin in Man. Proc. Soc. Exp. Biol. Med., 119, (1965): 244-248.

Woode, G. N., Jones, J., and Bridger, J.: Levels of Colostral Antibodies Against Neonatal Calf Diarrhoea Virus. Vet. Rec., 97, (1975): 148-149.

APPENDIX

Table A1. Health history of calves in experiment 7

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Group	S	(A)	S	M	S	(A)	A	M	M	S	A	M	M	A	A	S	A	M	S	A	A	M	S
Birth	H N	C As	C N	C N	C N	H As	C As	H As	H As	H As	H N	H N	H As	C N	C As	H N	C As	C As	C N	C As	C N	C As	C As
1.5-2h	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
6h	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	T	T	F	T	F
12h	F	F	F	F	F	F	F	F	T D Ab	F	F	F	F	F	F	F	F	F	T	T	F	T	F
18h	F	F	F	F	F	F	F	F	T D Ab	F	F	F	F	F	F	F	F	F	T	T Ab	F	T Ab	F
24h	F	(F) D Dp	F	F	F	F	F	F	T D Ab	F	F	T D Ab	F D Ab	T D Ab	F	F	F	F	T	T Ab	F	T D Ab	F
30h	F	(F) D Dp	F	F	T D Ab	F	F	F	T D Ab Dp	F	T D Ab	T D Ab	T D Ab	T D Ab	F D	F	F	F	T	F	T Ab	T D Ab	F D Ab
36h	D	D Dp	D	D	D	D Dp	D Ab	D Ab	D Ab Dp	F	D Ab	D Ab	D Ab R	D Ab	D Ab	D Ab		D Ab		D Ab Dp	D Ab	D Ab Dp	D Ab
42h	D	Died	F D	F	F D Ab	Died	F D R	F D Ab	T D Ab Dp	D Ab	F D Ab R	D Ab R	F Ab R	F D Ab R	D Ab R	F D Ab R	F	F D Ab R		D D	F D Ab	D Ab Dp	F Ab R
48h	D		F D	F	D		D R	D Ab R	D Dp	F D Ab R	D Ab R	D Ab R	R	F Ab R	F Ab	R		R	F	F D Ab R	Ab R	T D Ab Dp	Ab R
49-72h	Died		F		F R		R	R	Died	R	F R	R	F R	R	R	R	F	R		F R	F R	Died	R

S = saline/gelatin control; A = ACTH treatment; M = metyrapone treatment; C = cow; H = heifer; N = natural delivery; As = assisted delivery; F = fed well, strong, sucking; T = stomach tube required since poor, or no, sucking; D = diarrhea; Ab = treatment with antibiotics intravenously; Dp = depressed condition; R = recovering.

Table A2. Serum glucocorticoid concentrations in the control calves

CON-TROL	0h	1/4h	1/2h	1h	1.5h	2h	2.5h	3h	6h	12h	18h	24h	30h	36h	42h	48h
H 1	---	---	134.1	111.3*†	113.1	96.4	---	76.4	39.6	31.5	24.5	23.6	47.1	58.8	63.4	54.7
C 3	---	---	---	74.8	75.3*	80.3†	49.8	44.9	74.7	34.6	34.4	36.9	20.0	28.2	32.4	39.2
C 5	---	109.1	144.7*	114.0†	140.2	121.4	94.4	94.7	70.6	52.4	83.8‡	85.8‡	119.8‡	129.8‡	120.5‡	122.5‡
H 10	104.0	117.5	85.8*†	77.9	67.6	60.1	56.3	38.9	42.6	36.0	20.3	27.1	25.9	32.6	27.8	23.2
H 16	141.1	151.6	158.1*†	160.2	138.4	119.6	107.3	98.4	57.9	55.2	45.1	44.3	25.7	29.9	32.0	25.6
C 19	---	141.1	147.4†	132.8*	98.4	79.0	61.2	45.8	78.2	66.9	40.8	61.2	67.9	67.0	53.7	56.8
C 23	172.2	187.4*	190.1†	206.5	184.6	154.0	133.0	123.9	84.0	48.4	55.8	54.5	91.2‡	33.8	48.6	52.2
Mean ± SE	139.1 19.7	141.3 13.8	143.4 13.9	125.4 17.6	116.8 15.5	101.5 12.1	83.7 13.6	74.7 12.3	63.9 6.6	46.4 4.9	36.8 5.4	41.3 6.1	37.3 8.9	41.7 6.8	43.0 5.8	42.0 6.1

*Cannula inserted in jugular vein before this sample was taken.

†First injection was given before this sample.

‡These values were not used in calculating the mean.

Table A3. Serum glucocorticoid concentrations in the ACTH-treated calves

ACTH	0h	1/4h	1/2h	1h	1/2h	2h	2.5h	3 h	6h	12h	18h	24h	30h	36h	42h	48h
C 7	83.6	89.7†	100.1	98.1	106.0	116.2	119.8	113.1	97.9	75.4	59.0	68.1	53.4	47.9	56.2	40.6
H 11	---	108.9	106.2†	115.2*	102.6	100.7	91.5	85.5	87.3	56.3	48.6	44.6	43.8	36.5	40.5	36.6
C 14	---	91.1	122.4†	117.1*	135.2	141.4	117.1	103.5	97.6	87.2	81.1	84.4	58.9	53.7	36.3	36.2
C 15	99.5	108.8†	107.6	117.3	127.2	114.7	104.6	109.8	106.9	84.9	74.4	69.5	86.8	88.9	84.9	80.6
C 17	---	108.0	142.4*†	173.4	156.5	152.3	158.5	138.7	146.4	122.4	104.2	90.9	75.9	81.7	37.9	39.9
C 20	---	---	126.4	149.8*†	180.4	168.1	172.3	166.4	169.2	142.8	104.8	88.6	85.5	66.1	67.7	52.4
C 21	---	130.9	149.0*†	186.0	197.4	197.2	202.6	209.0	160.5	99.2	78.6	85.7	75.6	65.6	36.2	43.4
Mean	91.6	106.2	122.0	136.7	143.6	141.5	138.1	132.3	123.7	95.5	78.7	76.0	68.6	62.9	51.4	47.1
± SE	8.0	6.1	7.1	12.6	13.7	12.9	15.3	16.2	12.8	11.0	7.9	6.2	6.3	7.0	7.2	6.0

*Cannula inserted in jugular vein before this sample was taken. ken.

†First injection was given before this sample.

Table A4. Serum glucocorticoid concentrations in the metyrapone-treated calves

MET	0h	1/4h	1/2h	1h	1.5h	2h	2.5h	3h	6h	12h	18h	24h	30h	36h	42h	48h
C 4	166.7	144.9†	---	75.3*	71.3	61.8	56.2	51.6	45.8	47.4	55.1	43.5	47.7	43.0	28.6	53.1
H 8	---	108.4	88.7*†	71.1	48.6	37.5	32.4	31.6	12.0	18.0	16.0	16.8	18.8	39.2	37.4	29.8
H 9	175.1	150.2*†	154.9	75.0	68.3	57.8	42.0	45.0	27.6	31.3	39.8	37.4	35.8	36.5	42.0	33.2
H 12	161.8	168.2	184.3*†	121.4*	91.5	65.4	59.6	49.2	18.9	28.3	26.9	36.3	23.6	23.8	19.4	18.6
H 13	128.2	---	102.0†	55.9*	41.6	21.3	20.2	16.7	12.3	13.4	12.7	14.0	15.6	9.3	15.7	19.9
C 18	66.0	86.2†	67.4*	42.1	43.8	32.6	36.4	23.5	20.5	22.6	18.4	20.7	24.2	30.9	25.0	41.6
C 22	132.7	94.8*†	88.4	71.2	63.0	52.8	43.7	36.5	14.7	8.0	7.6	10.6	6.8	4.7	8.5	8.9
Mean	138.4	125.5	114.3	73.1	61.2	47.0	41.5	36.3	21.7	24.1	25.2	25.6	24.6	26.8	25.2	29.3
± SE	16.4	13.6	18.5	9.3	6.8	6.3	5.1	5.0	4.5	4.9	6.4	5.0	5.1	5.6	4.5	5.7

*Cannula inserted in jugular vein before this sample was taken.

†First injection was given before this sample.

Table A5. Serum IgG concentrations (mg/ml) of the control group calves

Calf no.	Time after Birth (hours)					
	1.5	3	6	12	24	48
1	0	0	3.34	15.61	22.74	30.88
3	0	0.60	5.44	9.32	13.78	11.39
5	0	0	5.60	14.46	15.17	17.83
10	0.89	1.09	3.40	11.19	18.78	16.03
16	0.87	1.05	5.80	16.15	16.96	16.06
19	0	1.06	2.75	6.65	9.70	11.03
23	0	0.75	2.05	7.62	11.40	10.56

Table A6. Serum IgG concentrations (mg/ml) of the ACTH group calves

Calf no.	Time after Birth (hours)					
	1.5	3	6	12	24	48
7	0	0.87	3.48	8.39	15.32	17.15
11	0	1.21	6.44	12.80	19.92	18.46
14	0	0.92	5.60	13.79	15.17	15.55
15	0	1.00	3.23	8.16	16.96	19.81
17	0	0.76	1.48	5.91	10.99	13.29
20	0	0	2.75	12.10	17.72	17.84
21	0	1.13	4.82	9.95	15.07	16.48

Table A7. Serum IgG concentrations (mg/ml) of the metyrapone group calves

Calf no.	Time after Birth (hours)					
	1.5	3	6	12	24	48
4	0.73	1.02	4.42	6.77	9.28	10.39
8	0	1.15	5.04	12.73	17.60	17.66
9	0	0	0.98	6.01	8.86	8.21
12	0	0.86	4.56	11.05	12.80	12.06
13	0	0.73	5.88	10.38	14.46	13.91
18	0	1.01	3.18	10.99	13.96	13.29
22	0	0	2.10	6.69	11.49	8.22

MICHIGAN STATE UNIV. LIBRARIES
31293006689883