ROTH, JAMES ALLEN

EFFECTS OF STEROID HORMONES ON BOVINE POLYMORPHONUCLEAR LEUKOCYTE FUNCTION

Iowa State University PH.D. 1981

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ___√___.

1. Glossy photographs or pages ___✓___
2. Colored illustrations, paper or print _____
3. Photographs with dark background ___✓___
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages _____
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Other _____

Effects of steroid hormones on bovine polymorphonuclear leukocyte function

by

James Allen Roth

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Veterinary Microbiology and Preventive Medicine

Major: Veterinary Microbiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1981

TABLE OF CONTENTS

GENERAL INTRODUCTION

This dissertation deals with the effects of the glucocorticoids, dexamethasone and cortisol, and the female sex hormones, estradiol and progesterone, on the bovine immune system. These hormones are all classified as steroid hormones because of their structural similarity to cholesterol. They have a number of biologic actions and are very important to normal physiologic function. The glucocorticoids play an important role in the regulation of blood glucose levels through their effects on intermediary metabolism (43). Estradiol and progesterone, of course, are essential for reproductive function in the female.

The glucocorticoids and female sex hormones have been associated with altered resistance of cattle to infectious disease for many years. Stress-induced increases in plasma cortisol and the administration of synthetic glucocorticoids have been associated with decreased resistance to infectious disease (62,72). Progesterone has been associated with increased susceptibility of the uterus to bacterial infection, while estrogens have been shown to cause the uterus to be relatively resistant to infection (10,121).

The steroid hormones play an important role in bovine medicine. Modern husbandry practices often lead to alterations in endogenous glucocorticoid hormone levels. Stress associated with weaning (35,56), surgical procedures (69), transportation (35,56,69,128), "handling" (35), and forced exercise (2) has been shown to cause increased

blood cortisol concentrations. Synthetic glucocorticoids are sometimes administered to cattle for the treatment of ketosis, mastitis, respiratory tract disease, udder edema, inflammatory musculo-skeletal conditions, and for the induction of parturition (9,78). The concentrations of estradiol and progesterone in the blood vary during the estrous cycle of the cow. Estrogenic steroids are administered to cattle to correct anestrus, treat pyometra, stimulate uterine expulsion of retained placentas, and induce abortion (151). Progesterone and progesterone-like compounds are used in cattle to maintain pregnancy, treat cystic ovaries, and suppress estrus (151).

Since the endogenous and exogenous levels of these steroid hormones are often increased in cattle, it is important to know their effect on the bovine immune system. The purpose of the experimentation reported here was to determine the specific effects of pharmacologic and physiologic levels of glucocorticoids, estradiol, and progesterone on neutrophil and lymphocyte function in cattle. The effect of the pharmacologic administration of glucocorticoids on neutrophil function was determined by administering dexamethasone to steers and is reported in the section entitled "Effects of _in vivo_ dexamethasone administration on _in vitro_ bovine polymorphonuclear leukocyte function." During the course of this experimentation it became apparent that it would be necessary to separate bovine eosinophils from neutrophils and to evaluate their functions separately. This was necessary because glucocorticoids cause a marked eosinopenia; it could not be determined

if the effect of dexamethasone was due to an effect on neutrophil function or simply was due to a decreased proportion of eosinophils present. The method for separating the two cell types is reported in the section entitled "Isolation of neutrophils and eosinophils from the peripheral blood of cattle and comparison of their functional activities." The administration of dexamethasone had profound effects on neutrophil function and other workers had reported that dexamethasone depressed lymphocyte function (39,95). The next step was to determine if high physiologic levels of the natural glucocorticoid, hydrocortisone (cortisol) had any effect on neutrophil or lymphocyte function. This was accomplished by administering adrenocorticotropic hormone to steers and subsequently evaluating the functional activity of these cells. The results of this experimentation are reported in the section entitled "Effects of adrenocorticotropin administration on bovine polymorphonuclear leukocyte function and lymphocyte blastogenesis."

The effect of pharmacologic levels of estradiol and progesterone on lymphocyte and neutrophil function was evaluated by administering estradiol cypionate and progesterone to steers. The results of this experimentation are reported in the section entitled "Effect of estradiol and progesterone on lymphocyte and neutrophil function in steers." The effect of physiologic levels of estradiol and progesterone on bovine neutrophil function was determined by evaluating neutrophil function during the normal estrous cycle of cows and correlating the results with the concentration of estradiol and progesterone present

in the serum. These results are reported in the section entitled "Association of elevated estradiol and progesterone blood levels with altered bovine polymorphonuclear leukocyte function."

The initial section of this dissertation is a review of the effects of glucocorticoids on the bovine immune system, and includes the pertinent findings from our research and that of other workers.

THE EFFECTS OF GLUCOCORTICOIDS ON THE BOVINE IMMUNE SYSTEM: A REVIEW[1]

Introduction

The natural and synthetic glucocorticoids play an important role in the practice of bovine medicine. Physiologic elevation of glucocorticoids (cortisol and corticosterone) in association with stress is an important component in the pathogenesis of infectious diseases, including "shipping fever" the most costly disease of feedlot cattle (60). Synthetic glucocorticoids are pharmacologic agents used in cattle for the treatment of ketosis, mastitis, respiratory tract disease, udder edema, inflammatory musculoskeletal conditions, and for the induction of parturition (9,78). The administration of synthetic glucocorticoids has been associated with exacerbation of infectious disease processes; therefore, the concurrent administration of efficacious antibacterial agents is often recommended. This clinical evidence of exacerbation of infectious diseases by glucocorticoids has lead to the conception that the glucocorticoids impair the host defense mechanisms.

There are two broad categories of defense mechanisms which are important in controlling infectious agents, "native" immune mechanisms and "acquired" immune mechanisms. The native immune mechanisms

---

[1]Submitted for publication to the Journal of the American Veterinary Medical Association by J. A. Roth and M. L. Kaeberle.

are naturally present in normal animals without induction by vaccination or previous exposure. Factors which play an active role in native immunity are certain serum components such as complement, lysozyme, and beta lysins, and the phagocytic activity of neutrophils and macrophages. These mechanisms are very important in the early stages of infection by an organism to which the animal has not previously been exposed. Acquired immunity is generally more effective than native immunity, but it is not present when an animal first encounters an organism, and it does not reach effective levels until several days after the initiation of infection. Acquired immune mechanisms which develop in response to antigens can be classified into two categories; humoral immunity which is mediated by antibody, and cell-mediated immunity. Lymphocytes are very important in acquired immunity. The majority of circulating lymphocytes are of two main types; B lymphocytes, some of which differentiate into plasma cells and produce antibody, and T lymphocytes which are responsible for cell-mediated immunity. The role of T lymphocytes in cell-mediated immunity is quite complex. There are a number of subpopulations of T lymphocytes, such as T helper cells and T suppressor cells which modulate B lymphocyte activity and cytotoxic T cells which can kill certain viral-infected and tumor cells. T lymphocytes also may release lymphokines which have a number of biologic effects such as enhancement of the inflammatory response and enhancement of the phagocytosis and destruction of microorganisms by macrophages. While macrophages and neutrophils are

phagocytically active in the absence of biologic amplifiers such as antibody and lymphokines, these lymphocyte products greatly enhance phagocytic cell function or activity.

Host defense against infectious disease is a complex process involving the interaction of many cell types and humoral components. Glucocorticoids may potentially alter host resistance to infectious disease by interfering with any of these mechanisms. In recent years, there has been a significant amount of experimentation on the effects of glucocorticoids on both the human and bovine immune systems. This paper will attempt to summarize this research. The research which has been conducted in the bovine will be emphasized with references to findings in other species to clarify certain points or where voids exist in the bovine research.

Clinical Evidence for Immunosuppression by Glucocorticoids

Stress has long been associated with the occurrence of infectious disease. An important response of the body to stress is the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. ACTH stimulates the adrenal cortex to increase the synthesis and secretion of cortisol (hydrocortisone) resulting in elevated blood cortisol levels (139). Reports of stressful conditions which contribute to increased blood cortisol concentrations in cattle have recently been reviewed (139). Some of these conditions are: weaning (35,56),

transportation (35,56,69,128), "handling" (35), castration and dehorning (69), parturition (54,64), forced exercise (2), endotoxin-induced mastitis (105), neonatal diarrhea (20,86), and certain acutely painful conditions (139). Also, newborn calves normally have a high blood cortisol concentration (20,70,80). A few days before parturition the fetal serum cortisol level begins to rise (33), reaches a very high level at the time of parturition, and progressively declines thereafter until about 20 days of age (20,70,80). The increase in the fetal blood cortisol level near term is apparently important in the initiation of parturition in the bovine.

Probably the best example of stress-associated infectious disease in cattle is "shipping fever" or the "bovine respiratory disease complex." This association is clearly indicated by the observation that most fatalities from shipping fever pneumonia occur during the first 45 days in the feedlot (68). A wide variety of infectious agents have been associated with shipping fever pneumonia but no single agent is consistently present or capable of reproducing the syndrome. A working hypothesis for the pathogenesis of shipping fever was advanced in 1957 by Hoerlein and Marsh (62) and has been supported by subsequent research (60). The hypothesis proposes that stressors encountered by the animal reduce its resistance mechanisms sufficiently to allow viral and other non-bacterial infections to occur. These infectious agents further reduce the host's resistance, thus allowing bacterial infection of the respiratory tract to occur. Many of the stresses associated with

normal management practices are known to cause increased plasma cortisol concentrations.

Dexamethasone is a synthetic glucocorticoid that is commonly administered to cattle. It is approximately 30 times more potent than cortisol as an anti-inflammatory agent (43). Dexamethasone administration has been associated with adverse reactions in cattle including: the recrudescence of latent infections with either the virulent (39,40, 96,109,130) or the temperature sensitive (109), infectious bovine rhinotracheitis (IBR) virus, the recrudescence of the bovine mammillitis strain of bovid herpesvirus 2 (85), the conversion of a mild coccidial infection into an acute or peracute disease (101,143), exacerbation of Anaplasma marginale (79) and Babesia argentina parasitemia (22), and predisposition to a fatal viremia in bovine viral diarrhea (BVD) virus infected calves (131). One clinical application of dexamethasone has been in the treatment of cattle suffering from bronchial pneumonia in the belief that it may have a beneficial effect by reducing the inflammatory response in the lungs. However experimentation has indicated that when dexamethasone administration was combined with antibacterial and antihistamine therapy for the treatment of bronchial pneumonia in cattle, the result was a poorer response to treatment, more relapses, and increased death loss (27).

Effects of Glucocorticoids on Humoral Immunity

Humoral immunity is mediated by antibodies which are produced by plasma cells. When a virgin B lymphocyte contacts an antigen that it recognizes by a specific receptor, the cell undergoes a process of differentiation accompanied by proliferation. Some of the progeny cells become plasma cells and produce antibody specific for the stimulating antigen. Other progeny cells become long-lived memory cells and are responsible for the anamnestic response or "booster effect" upon subsequent exposure to antigen. Antibodies defend the host against infection by a number of mechanisms including the activation of complement, facilitation of phagocytosis, neutralization of toxins and neutralization of viruses. Glucocorticoids may potentially cause decreased serum antibody concentrations by suppressing antibody production, increasing antibody catabolism, or a combination of these mechanisms (45). A general physiologic effect of glucocorticoids is to increase the rate of protein catabolism. This would apply to the catabolism of antibody as well. Whether or not a decrease in antibody concentration occurs depends upon the type of glucocorticoid administered, the dosage, and the duration of treatment. For example, methylprednisolone, when given daily to human patients at a high dosage for 3 to 5 days caused a significant reduction in the serum concentration of immunoglobulin G (IgG) when measured 2 to 4 weeks later (18). However, prednisone administered to patients on an alternate day

schedule produced no effect on antibody levels (148).

Glucocorticoids do have an effect on antibody levels in cattle under certain conditions. May et al. (87) reported that severe heat stress or the injection of 3 doses of "supercortisol" (1.0 mg/kg) at 48 hour intervals impaired the O agglutination antibody response of 6-month-old calves following a single or repeated vaccination with Salmonella dublin. Cummins and Rosenquist (36) reported that hydrocortisone administered to cattle every 8 hours for 3 days did not significantly affect the neutralizing antibody response to IBR virus challenge administered during the first day of hydrocortisone therapy. Gwazdauskas et al. (56) conducted an experiment which compared the ability of calves to produce antibody to porcine and equine red blood cells administered 1 week prior to, or at the time of, weaning. They determined that the stress of weaning caused increased plasma glucocorticoid concentrations, and that antibody production was greater when antigen was given one week prior to weaning than when antigen was administered the day of, or the day after, weaning. These findings suggest that the stress of weaning caused increased plasma cortisol levels which impaired the animal's ability to produce antibody. Therefore, the physiologic state of the animal should be considered when designing vaccination programs. In order to get a maximal antibody response, cattle should be vaccinated when they are under a minimum amount of stress.

Effect of Glucocorticoids on the Transfer of Passive Immunity to the Calf

Immunoglobulins are not transferred across the placenta in the cow. Therefore, in order for the calf to receive passive immunity, which is essential for its survival, immunoglobulins must be present in the colostrum, the calf must receive the colostrum during the first 24 to 36 hours of life, and the immunoglobulins in the colostrum must be transported intact through the epithelial lining of the gut into the calf's blood stream. Because blood cortisol levels are quite high in the calf at birth (20,70,80) and, since glucocorticoids are often used to induce parturition in the cow, it is important to know if glucocorticoids have an affect on colostral immunoglobulin levels or the absorption of immunoglobulin from the gut of the calf.

Brandon et al. (15) reported that the administration of dexamethasone trimethylacetate (a long acting synthetic glucocorticoid) to cows 6 to 8 weeks before the expected date of calving resulted in parturition 9 to 19 days later and a decreased transfer of IgG1 from the cow's serum into the colostrum. In contrast, when Hoerlein and Jones (61), used a faster acting glucocorticoid (flumethasone) closer to the time of expected parturition (3 to 22 days before) they observed that calving occurred an average of 41 hours postinjection and there was no effect on colostral IgG levels.

The type of glucocorticoid and the timing of the induced parturition are apparently also important in determining whether the neonatal calf

will have a normal or impaired ability to absorb immunoglobulins from colostrum. Husband et al. (65) used the long acting steroid dexamethasone trimethylacetate to induce parturition in the last 2 months of gestation. Calves were removed from their dams before nursing and were fed 1 liter of mixed colostrum 1 to 2 hours after birth and again 4 hours later. These calves absorbed approximately one half as much colostral immunoglobulin as calves which had undergone a normal gestation and received the same amount of colostrum from the same source. In contrast, when faster acting glucocorticoids (flumethasone or dexamethasone) were used to induce parturition 3 to 22 days before the expected calving date, the neonatal calves did not have an impaired ability to absorb immunoglobulins (61,93,152). An important difference in these studies is that the calves which had impaired absorption of colostral antibody were of a younger gestational age at the time of induced parturition. These results indicate that the type of glucocorticoid used and the timing of the induction of parturition are important determinants of the effect of glucocorticoids on colostral immunoglobulin concentration and the ability of the calf to absorb immunoglobulin from the colostrum.

The decreased absorption of immunoglobulin from the gut of the calf which had been observed by Husband et al. (65) following the induction of parturition was apparently not due to increased glucocorticoid levels in the calf's blood at birth. A high plasma cortisol concentration at birth does not seem to affect the absorption of colostral

immunoglobulin from the gut of the calf (21,146). The administration of ACTH (70,145) or fluoroprednisolone (145) at birth also failed to inhibit the absorption of colostral antibody from the gut.

Glucocorticoid Effects on Interferon Production in Cattle

Interferon is a substance which may play an important role in host resistance to viral infection, particularly during the early stages of infection. Infection with the IBR virus will induce interferon production in cattle (112). Fulton and Rosenquist (47) demonstrated that hydrocortisone decreased *in vitro* interferon production in bovine fetal spleen and peripheral blood leukocyte cultures. However, they used a level of hydrocortisone that was approximately 10^4 times higher than physiologic levels. Cummins and Rosenquist (36,37) subsequently reported that the administration of hydrocortisone to calves at the time of IBR virus inoculation did not decrease, but actually increased serum interferon concentrations. The hydrocortisone-treated calves, however, had higher and more persistent viremias than the control calves in spite of their higher interferon concentrations. These data indicate that elevated glucocorticoid levels do not inhibit interferon production in calves, but by some mechanism it does decrease the animal's resistance to IBR virus infection. The effect of more potent glucocorticoids on interferon production in cattle is not known.

Glucocorticoid Effects on Lymphocyte Blastogenesis

According to the clonal selection theory first advanced by Burnet (17), each lymphocyte can respond to only one antigenic specificity. When lymphocytes come into contact with their respective antigen, a proportion of them will be stimulated to undergo mitosis, cell proliferation, and differentiation and will produce more lymphocytes with the same antigenic specificity (a clone of lymphocytes). This expansion of the clone is important in increasing the number of B cells that will be available to differentiate into plasma cells and produce antibody, and in increasing the number of T cells with a particular antigenic specificity so that a significant cell-mediated immune response can be mounted. Therefore, the ability of lymphocytes to undergo mitosis is very important to the immune response.

A method which is commonly used by researchers to evaluate the ability of lymphocytes to undergo mitosis is referred to as the lymphocyte blastogenesis assay or the lymphocyte transformation assay. The lymphocyte blastogenesis assay can be conducted using either specific antigens or non-specific mitogens. When specific antigens are used, such as IBR virus, only lymphocytes from those animals which have developed an immune response to IBR will demonstrate significant blastogenesis. The test results will then give an estimation of the cell-mediated immune competence of an animal against IBR infection. Mitogens are substances which are generally extracted from plants and

have the peculiar property of stimulating lymphocytes to undergo mitosis. Mitogens are not selective for lymphocytes with a certain antigenic specificity, but they may be relatively specific for certain sub-classes of lymphocytes, such as T cells or B cells.

Research has been conducted on the effects of glucocorticoids on bovine lymphocyte blastogenesis in response to non-specific mitogens. Dexamethasone administration to cattle has been shown to suppress lymphocyte blastogenesis in response to phytohemagglutinin (PHA) (39,95). PHA is generally considered to be a T lymphocyte specific mitogen. Lymphocyte blastogenesis in response to PHA is therefore considered to be an *in vitro* correlate of *in vivo* cell-mediated immune competence. Increased plasma cortisol concentration induced by the administration of ACTH to cattle has also been reported to cause depressed lymphocyte blastogenesis in response to the mitogens PHA and concanavalin A (Con A) (118). The response of lymphocytes from the same cattle to pokeweed mitogen was somewhat suppressed, but not significantly. Con A, like PHA, is considered to be a T lymphocyte specific mitogen. Pokeweed mitogen, in man at least, will stimulate both T and B lymphocytes. These results suggest that increased plasma cortisol in calves has a more profound effect on circulating T lymphocytes than on circulating B lymphocytes. In man, glucocorticoids have been reported to suppress T lymphocyte blastogenesis while not affecting, or even enhancing B lymphocyte function (28,45). The mechanism of the depressed lymphocyte blastogenic response to T cell mitogens observed following the

administration of dexamethasone or ACTH to cattle is not known. It may be due to a direct effect of the steroid on the lymphocytes to inhibit mitosis, to a corticosteroid induced inhibition of T cell growth factor production by mononuclear cells (which has been reported to be important for blastogenesis) (135), or to a redistribution of a proportion of the T lymphocytes out of the circulation and into an extravascular lymphocyte compartment. In man, the administration of glucocorticoid causes the recirculating lymphocytes to leave the intravascular space and enter the tissues, but it does not affect the nonrecirculating lymphocytes. Quantitatively, both T and B lymphocytes are depleted from the blood stream, but there is a relatively greater depletion of T cells since these cells circulate more extensively (108). In cattle, the administration of the potent glucocorticoids, 9 α-fluoroprednisolone or dexamethasone, has been reported to induce a lymphopenia (39,124,157). However, ACTH administration to normal cattle does not cause a lymphopenia (104, 106,118) or only a very transient lymphopenia (91). Wilkie et al. (157) have shown that the dexamethasone-induced lymphopenia does not result in an altered proportion of erythrocyte rosette (T cells) or antibody-complement rosette forming cells (B cells). Therefore, in contrast to the findings in man there is apparently no selective depletion of T lymphocytes from the peripheral circulation in cattle following glucocorticoid administration. Bloom et al. (11) have studied the effect of glucocorticoids on lymphocytes in cattle with

persistent lymphocytosis associated with bovine leukemia virus infection. Most of the increase in peripheral blood lymphocytes in bovine leukemia virus-infected cows with persistent lymphocytosis is due to cells bearing surface markers typical of B lymphocytes (94,155,157). The administration of prednisolone to these animals resulted in an 80% to 90% decrease in the number of peripheral blood lymphocytes. The decrease in total lymphocytes was due almost entirely to a decrease in the expanded B lymphocyte population. The lymphocytes from bovine leukemia virus infected cows have been found to contain specific, high affinity binding sites for glucocorticoids (12). It wasn't determined if these binding sites differ from those found on normal bovine lymphocytes; however, glucocorticoids did not cause a selective depletion of B lymphocytes from the peripheral blood in normal cattle (157).

There are marked species differences in susceptibility to glucocorticoid hormones. Species are generally classified into steroid resistant and steroid susceptible groups based on the relative ease of producing lymphoid depletion by glucocorticoid administration (28). Compared to the steroid sensitive species (hamster, mouse, rat, rabbit) glucocorticoid induced lymphopenia in cattle is not as profound or as easily produced. On this basis, cattle could be considered a steroid resistant species, as has been suggested by other workers (12).

Glucocorticoid Effects on Neutrophil Function

The principal function of the neutrophil is the phagocytosis and destruction of invading microorganisms. The neutrophil is well-equipped to perform this function and has a number of mechanisms for destroying microorganisms (144). In order to be effective, the neutrophil must first come into the vicinity of the invading microorganism. This is achieved by the chemotactic attraction of the neutrophil to the site. Chemotactic factors may be produced directly by certain microorganisms, be generated by the cleavage of certain complement components, or be released by sensitized lymphocytes at the site of infection or inflammation. The chemotactic factors will diffuse away from the site to form a gradient. Neutrophils in the area will migrate along the gradient toward the source of the chemotactic factor and will thus arrive at the site of inflammation. Once neutrophils have arrived at the site of infection, they may begin to ingest the microorganisms if those agents are susceptible to phagocytic activity. Most pathogenic microorganisms must be opsonized before they can be ingested; bacteria are opsonized by the attachment of specific antibody and/or complement to their surface. The opsonization process greatly facilitates ingestion. When a neutrophil comes into contact with an opsonized particle, it will attempt to surround the particle with pseudopodia and internalize it by the process of phagocytosis. The internalized particle will be within a membrane bound vesicle

called a phagosome.

The neutrophil cytoplasm contains two types of membrane bound lysosomes or granules; primary or azurophilic granules and secondary or specific granules. These lysosomes contain numerous hydrolytic enzymes and other sutstances which are important to the bactericidal activity of the neutrophil. Once a particle is ingested and is inside a phagosome the neutrophil will "degranulate"; some of the lysosomes will fuse with the phagosome and release their contents into the phagosome with the ingested particle. The hydrolytic enzymes can function under aerobic or anaerobic conditions to attempt to destroy the ingested microorganisms.

In addition to the hydrolytic enzymes in its granules, the neutrophil has very potent bactericidal mechanisms that can function only under aerobic conditions. These mechanisms are related to the oxidative metabolism of the neutrophil (5). When a neutrophil is stimulated by an opsonized particle there is a rapid increase in its oxygen usage. This burst of oxidative metabolism results in the production of some highly reactive short-lived oxygen species, specifically hydrogen peroxide (H_2O_2), superoxide anion, the hydroxyl radical, and perhaps singlet oxygen. All of these components can damage microbial organisms. The H_2O_2 formed may also react with halide ions in a reaction catalyzed by a myeloperoxidase enzyme which is present in the primary granules. This myeloperoxidase catalyzed reaction is one of the most potent bactericidal mechanisms

of the neutrophil (76), and is also potentially fungicidal and virucidal (8,134). In order for this reaction to occur, degranulation must take place so that myeloperoxidase will be present in the phagolysosome.

In addition to its important role in the phagocytosis and destruction of pathogenic bacteria, the neutrophil may also play an important role in controlling certain viral infections via a mechanism referred to as antibody-dependent cell-mediated cytotoxicity (ADCC) (154). As the name implies, this mechanism requires the presence of antibody which presumably forms a bridge between the neutrophil and the virus-infected target cell. The neutrophil will then attempt to destroy the target cell. The mechanism of this cell destruction is not known but is thought to involve a direct membrane to membrane interaction (52). The neutrophil is the most efficient bovine cell capable of mediating ADCC (51) and is thought to play an important role in the destruction of herpesvirus-infected cells and in the recovery from herpesvirus infections (such as infectious bovine rhinotracheitis) by limiting virus spread (4,119,153).

Neutrophils therefore undergo a number of processes in attempting to control invading microorganisms. These steps are: 1) adherence of neutrophils to vascular epithelium and exit from the blood vessel via diapedesis, 2) random migration and directed migration along a chemotactic gradient, 3) the engulfment and internalization of opsonized

microorganisms, 4) degranulation, 5) the generation of oxygen-free radicals and H_2O_2, 6) the myeloperoxidase catalyzed reaction, and 7) antibody-dependent cell-mediated cytotoxicity. Assays can be conducted for each of these individual processes. If any of these processes are impaired in the neutrophil, one would expect that the neutrophil would not be able to perform its function of controlling microbial infection as efficiently and the animal would be more susceptible to microbial infection.

There is evidence that glucocorticoids can impair several aspects of neutrophil function. In man, glucocorticoids have been shown to decrease the "stickiness" of neutrophils. They are less able to adhere to nylon fibers and presumably to vascular endothelial cells. This results in a decreased margination of neutrophils in the vascular channels and reduced exit of neutrophils from the blood into the tissues (24,30,82,88,89). The administration of glucocorticoids to cattle also causes a decrease in the marginated pool of neutrophils (23), presumably through a decrease in their ability to adhere to vascular endothelial cells. This leads to a reduced ability of neutrophils to arrive at the site of inflammation. The reduced margination of neutrophils is partially responsible for the marked neutrophilia which is caused by glucocorticoid or ACTH administration to cattle (23,57,106). An increased input of neutrophils from the bone marrow storage pool is also partially responsible for the neutrophilia. The increase in neutrophils usually occurs without a

significant increase in band or other immature forms (23).

The administration of dexamethasone (113) or ACTH (118) to cattle will cause a dramatic increase in *in vitro* random migration by neutrophils. A similar increase in neutrophil random migration has been reported following the administration of glucocorticoids to man (140, 142). Those workers have shown that the increase in random migration is not due to a direct effect of the glucocorticoid upon the neutrophil, but rather to a neutrophil migration stimulating factor which is released by mononuclear cells after glucocorticoid administration. They theorize that this peptide factor acts to inhibit microtubule assembly within the neutrophil and that this would lead to increased random migration but decreased chemotactic responsiveness, adhesiveness, and secretion of lysosomal enzymes (141,142). This factor would thus mimic many of the effects observed after the *in vivo* administration of glucocorticoids. It isn't known if the increased random migration observed in neutrophils from steroid treated cattle is due to a direct effect of glucocorticoids on the neutrophil, or if the action is indirect and due to a mononuclear cell produced factor.

A single large dose of dexamethasone given intramuscularly to cattle has been shown to depress the ability of neutrophils to ingest *S. aureus* (113). There are conflicting reports on the effect of corticosteroids on ingestion by human neutrophils. Corticosteroids have been reported to inhibit ingestion by neutrophils (34,66) or to have no effect on ingestion (25,48,84). These discrepancies may be due

to differing techniques for evaluating ingestion or to the use of differing amounts and types of glucocorticoids. Dexamethasone has also been reported to impair the oxidative metabolism of the bovine neutrophil (113). Glucocorticoids have a similar effect on human neutrophils (25,26,46,84). The depression of oxidative metabolism indicates that an important component of the neutrophil's bactericidal mechanism is impaired by dexamethasone administration to cattle.

The ability to mediate ADCC was also depressed in neutrophils taken from dexamethasone-treated cattle (113) and in bovine neutrophils which were treated in vitro with dexamethasone (153). In contrast, dexamethasone administration in man has been reported to enhance neutrophil mediated ADCC (107). The inhibition of the ability of the bovine neutrophil to mediate ADCC may play an important role in allowing infectious bovine rhinotracheitis virus, which has been recrudesced by the administration of dexamethasone, to multiply.

The level and type of glucocorticoid appears to be an important determinant of the effect on neutrophil function. Elevated plasma cortisol levels which were maintained for three days by ACTH administration to cattle had a much less profound effect on neutrophil function than did a single dose of dexamethasone (113). Elevated plasma cortisol caused a marked enhancement of random migration by neutrophils but had no significant effect on S. aureus ingestion, oxidative metabolism, or ADCC by neutrophils. Elevated cortisol levels did inhibit the myeloperoxidase catalyzed reaction which is one of the most potent

bactericidal mechanisms of the neutrophil. The difference between the effects of dexamethasone and cortisol on neutrophil function presumably reflects the fact that dexamethasone is much more potent as an anti-inflammatory agent than cortisol (43). Alternate day therapy with glucocorticoids has been recommended to avoid their immunosuppressive effects. However, a single large dose of dexamethasome will depress certain functions of the bovine neutrophil for more than 48 hours (113). Therefore, giving dexamethasone on alternate days would maintain the animal in an immunosuppressed state. Perhaps the less potent glucocorticoids would not have this prolonged effect.

Glucocorticoid Effects on Eosinophils

The bovine eosinophil is capable of the same phagocytic and metabolic functions as the neutrophil but to a different extent (115). The eosinophil probably does not play an important role in destroying bacteria but is important in the host's defense against the tissue phase of certain parasitic infections (19). Glucocorticoids rapidly cause an eosinopenia in most species including the bovine (57,106,124). The mechanism for the induction of this eosinopenia in the bovine is not known, but has been proposed to be related to histamine levels (125). In rats it has been shown to be due to a redistribution of eosinophils from the blood to the peripheral lymphoid tissues (122). The effect of glucocorticoid administration on eosinophil function is

difficult to study because the resultant eosinopenia makes it nearly impossible to obtain sufficient eosinophils for functional characterization. In man, the administration of prednisone has been shown to inhibit eosinophil adherence properties and chemotactic responsiveness (30).

Glucocorticoid Effects on Monocytes and Macrophages

In man, glucocorticoid administration will cause a monocytopenia, probably due to the redistribution of monocytes from the blood stream to the tissues and to a decreased rate of release of monocytes from the bone marrow (45). The effect of glucocorticoids on bovine monocyte kinetics is not certain. Glucocorticoids have been reported to induce a monocytosis (23,124) or to produce no consistent change in the number of monocytes in the bovine (39,91,104). The basis for this discrepancy may be the observation that bovine lymphocytes and monocytes cannot be reliably differentiated by light microscopic observation of Wright's stained blood smears (58).

In spite of the fact that the cells of the mononuclear phagocyte system play a very important role in host defense, the effect of glucocorticoids on bovine monocyte or macrophage function has not been studied. The glucocorticoid effects on mononuclear phagocyte function in other species has been recently reviewed (45,108). In general, the monocyte is sensitive to glucocorticoids and will have reduced phagocytic and bactericidal capabilities following glucocorticoid administration.

Summary

Glucocorticoids are commonly used as pharmacologic agents in cattle; in addition, a wide variety of stressing factors are known to increase blood levels of naturally occurring glucocorticoids. Therefore, it is important to understand the effects of these hormones on the bovine immune system. Because elevated blood glucocorticoid levels are sometimes associated with exacerbations of infectious diseases, it has been assumed that these hormones impair the host's defense mechanisms. Specific immunosuppressive effects of glucocorticoids which have been reported in the bovine are: lymphopenia, decreased secretion of immunoglobulins into colostrum, decreased absorption of colostral immunoglobulins by calves following induced parturition, impairment of the antibody response, depression of lymphocyte blastogenesis, and inhibition of certain neutrophil functions (ingestion, oxidative metabolism, the myeloperoxidase catalyzed reaction, and antibody-dependent cell-mediated cytotoxicity). The occurrence and severity of the immunosuppression are determined by the frequency of administration, time of administration, blood level, and type of glucocorticoid which is present.

ISOLATION OF NEUTROPHILS AND EOSINOPHILS FROM THE PERIPHERAL BLOOD OF CATTLE AND COMPARISON OF THEIR FUNCTIONAL ACTIVITIES[1]

Summary

A procedure for isolating purified populations of neutrophils and eosinophils from bovine peripheral blood is described. The procedure involves the centrifugation of anticoagulated whole bovine blood, flash lysis of the packed red blood cells, and separation of the eosinophils from the neutrophils by centrifugation on a Ficoll-Hypaque density gradient. The following parameters were evaluated on populations of greater than 90% pure eosinophils and neutrophils: 1) random migration under agarose, 2) ingestion of ^{125}I-labeled Staphylococcus aureus, 3) nitroblue tetrazolium reduction, 4) iodination, or the conversion of ^{125}I to a trichloroacetic acid precipitable form, 5) antibody-dependent cell-mediated cytotoxicity, and 6) the amount of intracellular peroxidase. Neutrophils were significantly more active than eosinophils in the ingestion of S. aureus and in antibody-dependent cell-mediated cytotoxicity. Eosinophils were much more active than neutrophils in the resting and stimulated iodination test, and they contained higher levels

---

[1]J. A. Roth and M. L. Kaeberle. 1981. J. Immunol. Methods, accepted for publication.

of peroxidase. There was no difference between the two cell types in their ability to reduce nitroblue tetrazolium or migrate under agarose. The effect of Ficoll-Hypaque on granulocyte function was determined. Previous contact with Ficoll-Hypaque significantly reduced random migration but other function tests were unaffected.

Introduction

In recent years, the role of the eosinophil in host defense and the inflammatory response has been the subject of an increasing amount of research. This research has been hampered by the difficulty in obtaining large quantities of highly purified populations of eosinophils and neutrophils from the peripheral blood of normal animals (41,7). Purified eosinophils have been obtained from patients with eosinophilia and from peritoneal exudates containing a naturally occurring or artificially induced high percentage of eosinophils. Purified preparations of eosinophils have also been isolated from the bovine mammary gland following the instillation of an Ascaris suum extract (50). This research has provided important information, but some authors express concern that the eosinophils from these sources may not be normal (41,42,53,77,149).

Methods have been reported for isolating eosinophils and neutrophils in a highly purified form from normal human peripheral blood (53,149).

These techniques have allowed the investigators to evaluate the function of presumably normal eosinophils. A similar technique for isolating purified neutrophils and eosinophils from the peripheral blood of an experimental animal would enable the determination of eosinophil function during experimental manipulations which cannot readily be performed in man.

In this paper, we describe a technique utilizing Ficoll-Hypaque density gradient centrifugation for isolating highly purified populations of neutrophils and eosinophils from the peripheral blood of normal cattle. Both cell types can be isolated from the same blood sample and undergo nearly identical processing steps so that their function can be accurately compared. We evaluated the effect of the Ficoll-Hypaque isolation procedure on the function of the granulocytes, and we compared several aspects of neutrophil and eosinophil function.

Materials and Methods

Animals

Apparently healthy non-pregnant adult cows of various bovine breeds were used as cell donors. Animals with a high normal number of eosinophils in the peripheral blood were selected to optimize cell yield.

Ficoll-Hypaque

Ficoll-Hypaque was prepared by mixing 100 ml of a 10% solution of Ficoll[1] in water with 90 ml of Hypaque[2] and 55 ml of water. The specific gravity was then adjusted to 1.135 g/cm^3 at 25 C by adding additional water or Hypaque.

Isolation of eosinophils and neutrophils

Blood (200 ml) was collected into 20 ml of acid-citrate-dextrose solution (2X concentration of standard formula A) by jugular venapuncture. The blood was centrifuged at 1,000 X g for 20 minutes, and the plasma and buffy coat layer (which contained predominantly mononuclear cells) were discarded. The remaining packed cell volume contained both erythrocytes and granulocytes. The erythrocytes were lysed by the addition of 2 volumes of cold (4 C) phosphate buffered (0.0132 M, pH 7.2) distilled water followed 50 seconds later by the addition of one volume of cold (4 C) phosphate buffered (0.0132 M, pH 7.2) 2.7% NaCl. The lysate was centrifuged at 300 X g for 10 minutes and the pelleted cells resuspended in 10 ml of 0.015 M phosphate buffered saline solution, pH 7.2 (PBSS). The resuspended cells were layered over 4 ml of 1.135 specific gravity Ficoll-Hypaque in a 20 x 125 mm test tube and centrifuged at 500 X g for 45 minutes. The eosinophils formed a band at the

---

[1]Type 400, Sigma Chemical Co., St. Louis, MO.

[2]Hypaque sodium 50%, Winthrop Laboratories, New York, NY.

interface between the Ficoll-Hypaque and the PBSS, and the neutrophils formed a button in the bottom of the tube. The individual cell populations were removed, washed twice in PBSS, counted, and resuspended in PBSS to a concentration of 5.0×10^7 granulocytes/ml. Only those cell preparations in which greater than 90% pure populations of eosinophils and neutrophils could be obtained from the same blood sample were used.

Determination of the effect of Ficoll-Hypaque on granulocyte function

The granulocyte-rich cell preparation obtained following the lysis of the red blood cells as described above were used to evaluate the effect of Ficoll-Hypaque on granulocyte function. An aliquot of granulocytes was pelleted by centrifugation at 600 X g for 10 minutes, then resuspended in either PBSS or Ficoll-Hypaque and allowed to stand at room temperature for 45 minutes. The cells were then washed twice by centrifugation in PBSS prior to use in the granulocyte function tests.

Random migration under agarose

Evaluation of random migration under agarose was performed as previously described (114). Agar consisted of bicarbonate buffered Minimum Essential Medium with Earle's salts[1] containing 0.8% agarose,

---

[1]Grand Island Biological Co., Grand Island, NY.

10% fetal calf serum, and 1% Antibiotic Antimycotic[1] in 60 x 15 mm tissue culture grade petri plates.[2] Cells were placed into wells cut into the agar, and the petri plates were placed into an incubator (37 C) with a humidified 5% CO_2 atmosphere. Eighteen hours later the cells were fixed with formalin, the agar removed from the petri plate, and the cells adherent to the plastic were stained with modified Wrights' stain. The area of migration was determined and expressed in mm^2.

S. aureus ingestion

Heat killed ^{125}I-iododeoxyuridine-labeled S. aureus cells were used in the ingestion assay at a bacteria to granulocyte ratio of 60:1 as previously described (114). Granulocytes (2.5×10^6), bovine anti-S. aureus serum, and ^{125}I-labeled S. aureus in a total volume of 0.5 ml Earle's balanced salt solution were incubated together for 10 minutes at 37 C. Lysostaphin was then added and the incubation continued for 30 minutes. The cells were washed twice with PBSS, and the granulocyte associated radioactivity was determined in a gamma counter. The results are expressed as the percentage of the total S. aureus ingested.

---

[1]Grand Island Biological Co., Grand Island, NY.

[2]#1007 Falcon, Oxnard, CA.

Nitroblue tetrazolium reduction

The quantitative nitroblue tetrazolium (NBT) reduction assay was performed as previously described (114), by adding 5.0 x 10^6 granulocytes to a suspension of opsonized zymosan in 1.0 ml of Earle's balanced salt solution containing 0.4 mg of NBT. After a 5.0 minute incubation in a 37 C water bath, 5.0 ml of cold N-ethylmaleimide was added to stop the reaction. The purple formazan formed by the reduction of NBT was pelleted by centrifugation, resuspended in 5.0 ml of pyridine by sonication and heated in a 100 C water bath for 10 minutes. The pyridine was then clarified by centrifugation, and the optical density at 580 nm was determined. The results are expressed as O.D./5.0 x 10^6 granulocytes/5 minutes in 5.0 ml of pyridine.

Iodination

The iodination procedure was performed as previously described (114). The standard reaction mixture for the determination of stimulated iodination contained 2.5 x 10^6 granulocytes, 0.05 μCi ^{125}I, 40 nmole NaI, and 0.5 mg of opsonized zymosan in 0.5 ml of Earle's balanced salt solution. For the determination of resting iodination, the opsonized zymosan was omitted. Twenty minutes after the PMNs were added, the reaction was stopped by adding 2.0 ml of cold (4 C) 10% trichloroacetic acid. The resulting precipitate was washed one additional time in 10% trichloroacetic acid, and the amount of radioactivity in the precipitate was determined in a gamma counter. The results are expressed as nmole NaI/10^7 granulocytes/hr.

Peroxidase assay

Cells for the peroxidase assay were suspended in PBSS at a concentration of 5.0×10^7 granulocytes/ml. The cell suspensions were freeze thawed 5 times and sonicated to disrupt the cell membranes. Peroxidase activity in the crude lysate was determined using a spectophotometric procedure described by Kimball et al. (74). An aliquot of crude lysate was added to a solution containing one part 0.1 percent O-tolidine in ethanol, one part 1.5 μM H_2O_2, one part 10 percent Triton-X-100[1] in water, and 10 parts 0.1 M citrate buffer pH 5.0. The reaction mixtures were incubated at room temperature for 6.0 minutes, and the chromogen was developed by adding 0.1 ml of 50 percent NaOH. Horseradish peroxidase was used as a standard. Absorbance was determined at 435 nm, and results expressed as microgram equivalents of horseradish peroxidase per 5.0×10^7 cells.

Antibody-dependent cell-mediated cytotoxicity

The antibody-dependent cell-mediated cytotoxicity (ADCC) assay was performed by a modification of the method of Wardley et al. (154), utilizing ^{51}Cr labeled chicken red blood cells (CRBCs) as target cells. Briefly, heparinized chicken blood was collected, the CRBCs were washed in PBS, and 1.25×10^7 CRBCs were suspended in Medium 199[2] containing

---

[1]Packard Inst. Co., Inc., Downers Grove, IL.

[2]Grand Island Biological Co., Grand Island, NY.

200 µCi of ^{51}Cr.[1] After a one hour incubation at 37 C, the CRBCs were washed twice and then suspended in Medium 199 at a cell concentration of 1.25 x 10^6ml. The reaction mixture contained 2.5 x 10^5 CRBCs and 2.5 x 10^6 granulocytes (effector: target cell ratio of 10:1) in 0.5 ml of Medium 199 containing 10% bovine anti-CRBC serum. The assay was conducted in duplicate in 12 x 75 mm plastic test tubes. Triton X controls, antibody controls, and granulocyte controls were included. After a three hour incubation at 37 C in a humidified 5% CO_2 atmosphere, the reaction tubes were centrifuged at 500 X g for 10 minutes and an aliquot of supernatant solution was removed for gamma counting. The results were expressed as percent of specific release and were calculated using the following formula:

$$\text{Percent specific release} = \frac{\text{(mean CPM test)} - \text{(mean CPM control)}}{\text{(mean Triton-X treated CPM)} - \text{(mean CPM control)}} \times 100$$

Results

Granulocyte isolation procedure

Large numbers of highly purified neutrophils and eosinophils were isolated from the blood of selected normal animals (Tables 1 and 2,

---

[1] Na_2CrO_4, New England Nuclear, Boston, MA.

Figure 1). The cells did not have a tendency to clump and were metabolically active. An average of 81.2% of the neutrophils and 49.9% of the eosinophils in the peripheral blood were recovered using this technique (Table 1). Certain blood samples did not yield both types of granulocytes in greater than 90% purity (Figure 2). Those cells were not included in this study.

Relative functional activities of neutrophils and eosinophils

Both neutrophils and eosinophils were active in all of the function tests (Table 3). There was no significant difference in the area of random migration under agarose by neutrophils and eosinophils or in their ability to reduce NBT. Eosinophils were not nearly as efficient as neutrophils at ingesting S. aureus (9.2% ingestion for eosinophils versus 25.2% ingestion for neutrophils) or at mediating ADCC of chicken red blood cells (41.5% specific release of ^{51}Cr for eosinophils versus 80.0% for neutrophils). However, the eosinophils were much more active than neutrophils in the stimulated iodination reaction (93.9 nmole NaI/10^7 granulocytes/hr for eosinophils versus 18.4 nmole NaI/10^7 granulocytes/hr for neutrophils) and in the unstimulated iodination reaction (16.6 nmole NaI/10^7 granulocytes/hr for eosinophils versus 0.9 nmole NaI/10^7 granulocytes/hr for neutrophils). Eosinophils also contained considerably more peroxidase activity than neutrophils (2.97 µg equivalents of horseradish peroxidase per 5.0×10^7 granulocytes for eosinophils versus 0.67 µg equivalents of horseradish peroxidase per 5.0×10^7 granulocytes for neutrophils).

Table 1. Relative numbers of neutrophils and eosinophils in the peripheral blood of cattle and percent of these cells recovered by the isolation procedure

	Neutrophils	Eosinophils
Total in peripheral blood ($cells/mm^3$)	1,456 ± 464[a]	1,259 ± 416
Normal range for cattle ($cells/mm^3$)[b]	600 - 4,000	0 - 2,400
Number of cells recovered per mm^3 of peripheral blood	1,183 ± 321	628 ± 482
Percent recovery	81.2 ± 22.1	49.9 ± 38.7

[a]Mean ± SEM; n = 10.

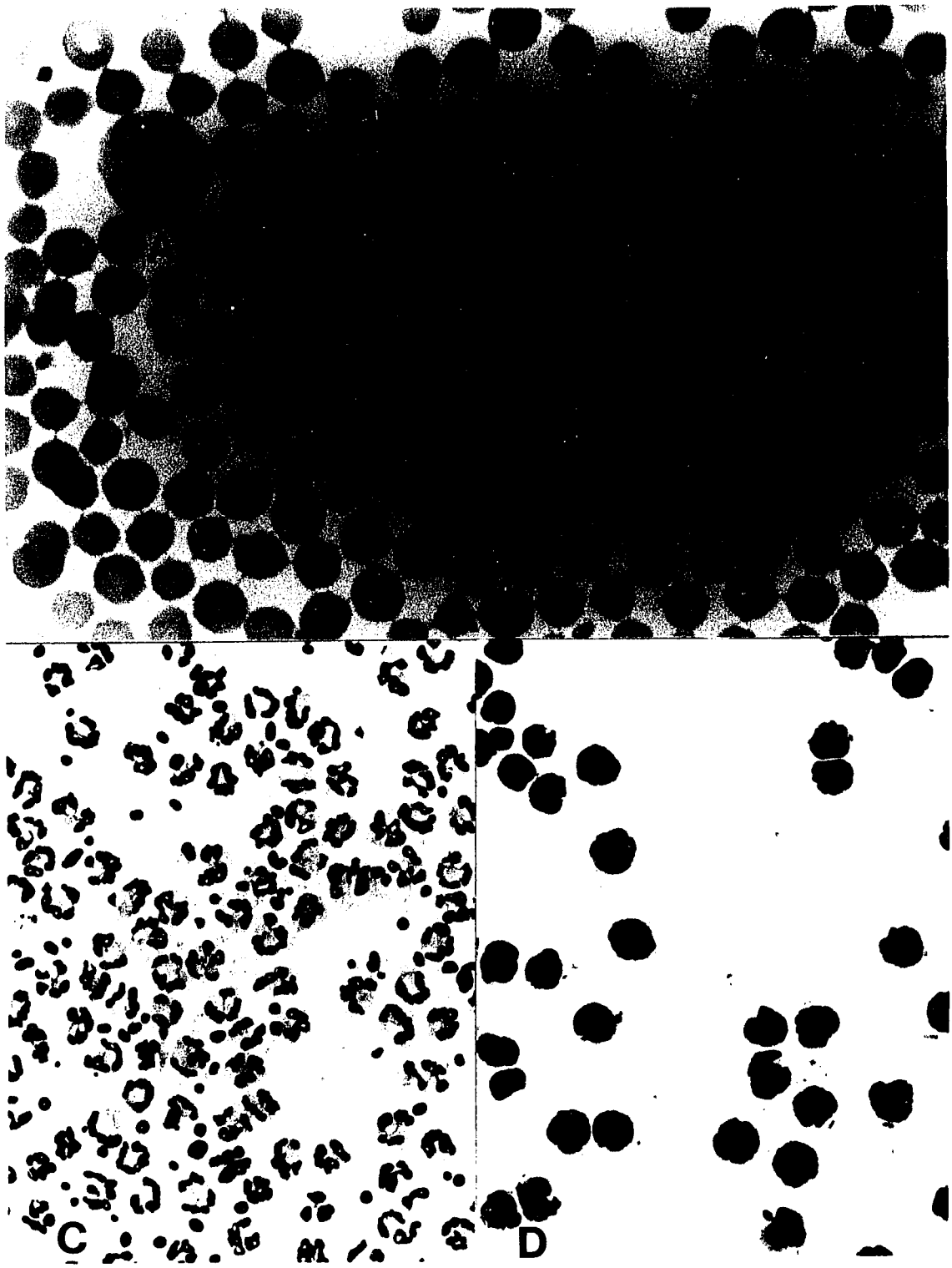
[b]Schalm et al. (125).

Table 2. Cellular composition of the neutrophilic and eosinophilic leukocyte preparations. Cell preparations from blood samples that did not yield relatively pure populations (> 90% of the total granulocytes) of both neutrophils and eosinophils are not included

	Cellular composition of the neutrophil preparation		Cellular composition of the eosinophil preparation	
	Percent of total leukocytes	Percent of total granulocytes	Percent of total leukocytes	Percent of total granulocytes
Mononuclear cells	1.8 ± 1.8[a]		10.4 ± 11.9	
Neutrophils	95.5 ± 2.8	97.2 ± 1.8	2.7 ± 2.3	3.0 ± 1.8
Eosinophils	2.7 ± 1.8	2.8 ± 2.9	86.9 ± 13.1	97.0 ± 2.9

[a]Mean ± SEM; n = 21.

Figure 1. Wright's stained smear of bovine peripheral blood showing a neutrophil (A) and eosinophil (B). X 1800. Purified bovine neutrophils (C) and eosinophils (D). X 690


C
D

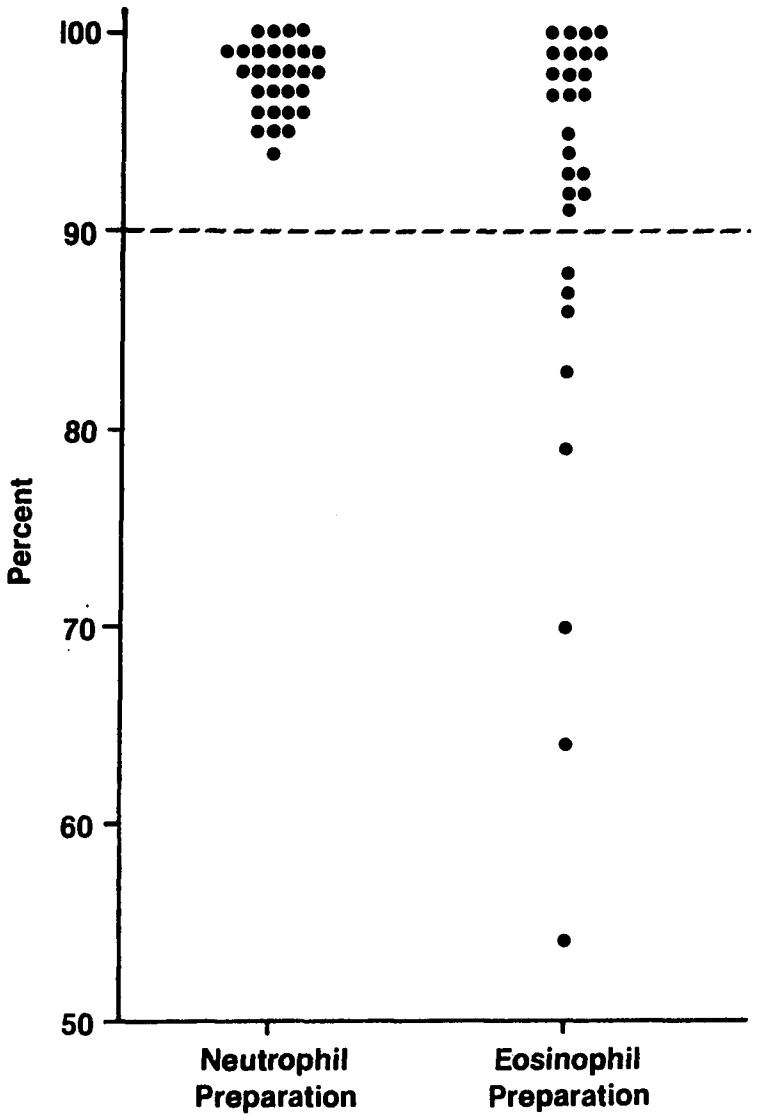


Figure 2. Purity of the neutrophil and eosinophil cell preparations from 29 blood samples expressed as a percent of the total granulocytes present

Table 3. Activity of neutrophils and eosinophils in the various granulocyte function tests

	(n)	Neutrophils	Eosinophils
Random migration (mm^2)	(10)	9.9 ± 1.0[a]	10.2 ± 1.2
S. aureus ingestion (percent)	(21)	25.2 ± 1.7***	9.2 ± 1.3***
NBT reduction (O.D./5.0 x 10^6 granulocytes/5 min)	(21)	0.29 ± .03	0.30 ± .03
Stimulated iodination (nmole NaI/10^7 granulocytes/hr)	(20)	18.4 ± 1.1***	93.9 ± 7.0***
Resting iodination (nmole NaI/10^7 granulocytes/hr)	(20)	0.9 ± 0.2***	16.6 ± 2.5***
ADCC (percent)	(19)	80.0 ± 3.8***	41.0 ± 4.8***
Peroxidase (µg equivalents of horseradish peroxidase/ 5.0 x 10^7 granulocytes)	(6)	0.67 ± 0.15***	2.97 ± 0.51***

[a]Mean ± SEM.

***The values for these parameters differ significantly between the two cell types ($P < 0.001$).

Effect of Ficoll-Hypaque on granulocyte function

Granulocytes which had been suspended in Ficoll-Hypaque for 45 minutes had a dramatically impaired ability to migrate under agarose when compared to cells which had been suspended in saline (Table 4). The Ficoll-Hypaque treated cells had a mean (± SEM) migration area of 22.4 (± 2.9) mm^2 and the saline treated cells had a mean (± SEM) migration area of 67.8 (± 3.7) mm^2. The other granulocyte function tests were not significantly altered by exposing the cells to Ficoll-Hypaque (Table 4).

Discussion

The cell isolation procedure described enables one to obtain high numbers of purified eosinophils and neutrophils from normal cattle. Both cell types can be obtained from the same blood sample and they undergo nearly identical isolation procedures so that the function of the two cell types can be accurately compared. One advantage of using cattle is that they can be given a drug or infectious agent then large numbers of cells can be obtained on a daily basis for a prolonged period of time. This allows the investigator to evaluate granulocyte function frequently during the course of an experiment. The granulocytes come from normal animals and have not been altered by treatment with chemotactic agents. The bovine neutrophils pass through the Ficoll-Hypaque whereas the eosinophils do not. Bovine neutrophils, therefore,

Table 4. Activity of a mixed granulocyte population in the various granulocyte function tests after being suspended in Ficoll-Hypaque or saline for 45 minutes then washed twice. The cell preparations contained an average of 70.0% neutrophils, 24.9% eosinophils and 5.1% mononuclear cells

	Saline Treated	Ficoll-Hypaque Treated
Random migration (mm^2)	67.8 ± 3.7***[a]	22.4 ± 2.9***
S. aureus ingestion (percent)	25.4 ± 7.4	28.5 ± 5.6
NBT reduction (O.D./5.0 x 10^6 PMNs 5 min.)	0.21 ± .04	0.20 ± .02
Iodination (nmole NaI/10^7 PMNs/hr)	39.6 ± 6.5	36.6 ± 6.0
ADCC (percent)	79.6 ± 3.8	76.6 ± 5.0

[a]Mean ± SEM; n = 9.

***The values for these parameters differ significantly between the two cell types ($P < 0.001$).

have a higher density than bovine eosinophils. This is similar to the situation in the rat (123) but is unlike the situation in man where eosinophils have a higher density than neutrophils (149).

The Ficoll-Hypaque used to separate the neutrophils and eosinophils was found to markedly impair random migration under agarose. The reason for this is not known. It apparently is not due to a general toxic effect because the other granulocyte function tests were unaffected. Those data suggest that Ficoll-Hypaque purified cells may not give reliable results when used in assays requiring cell migration such as assays for random migration, chemotaxis, or migration inhibition factor. The Ficoll-Hypaque treatment may alter or mask the effect which is being studied. It is not known if this is a general property of Ficoll-Hypaque purified cells or if it only occurs with the particular formulation of Ficoll-Hypaque used in this study. In this study, no difference was found in the ability of purified neutrophils and eosinophils to migrate under agarose. However, these data must be interpreted with caution because of the observed inhibitory effect of Ficoll-Hypaque on granulocyte migration under agarose.

Bovine eosinophils did not ingest S. aureus nearly as efficiently as neutrophils. The neutrophils ingested an average of 25.2% of the suspension of S. aureus in 10 minutes (approximately 15 bacteria ingested per neutrophil), whereas the eosinophils ingested an average of only 9.2% of the S. aureus (approximately 6 bacteria ingested per eosinophil). This is in agreement with reports of inefficient phagocytosis of

particulate material by human eosinophils (32,77,92), and with the concept that eosinophils are important in the phagocytosis of immune complexes but do not play an important role in the phagocytosis and destruction of bacterial organisms (19). Sanderson and Thomas (123) have shown that the rat eosinophil engulfs and regurgitates antibody-coated CRBCs several times in an hour whereas the rat neutrophil will engulf an antibody coated CRBC and remain quiescent until the remains of the cell are expelled. The S. aureus ingestion assay as performed here would only detect those organisms which were retained within the phagocytic cell. Any S. aureus which are regurgitated would be destroyed by lysostaphin. If the bovine eosinophil does actively engulf and regurgitate S. aureus, the relatively poor ingestion rate for eosinophils may not reflect inefficient ingestion but may be due to active regurgitation of S. aureus which is subsequently destroyed by lysostaphin.

Bovine neutrophils and eosinophils reduced NBT equally well. NBT is directly reduced by superoxide anion which is formed during the burst of oxidative metabolism associated with phagocytosis (158). These data, therefore, indicate that there is no quantitative difference in the burst of oxidative metabolism associated with phagocytosis by bovine neutrophils and eosinophils. This is in contrast to the reports with human eosinophils in which other investigators have found human eosinophils to have a higher rate of oxidative metabolism than human neutrophils following phagocytosis (6,77,92).

The bovine eosinophils had a much higher resting and stimulated iodination activity than the bovine neutrophils. There are conflicting reports regarding the resting and stimulated iodination activity of human eosinophils. Using purified eosinophils from patients with peripheral blood eosinophilia, Bujak and Root (16) reported that human eosinophils had a high resting iodination activity which wasn't increased further upon stimulation. In contrast, Klebanoff et al. (77) studied eosinophils from the ascitic fluid of a patient with eosinophilic gastroenteritis and reported that human eosinophils had a low resting iodination activity which increased dramatically upon stimulation. The conflicting results in these two reports may be due to differences in technique or more likely to the different sources of the eosinophils. Iodination, or the conversion of iodide to a trichloroacetic acid precipitable form is a measure of the peroxidase-H_2O_2-iodide antibacterial system which results in protein iodination and bacterial killing. It may seem paradoxical that the burst of oxidative metabolism (which generates H_2O_2) of bovine neutrophils and eosinophils is equivalent, but the eosinophil has a much more active peroxidase-H_2O_2-iodide antibacterial system. However, we have previously demonstrated that the oxidative metabolism is not the limiting factor in the iodination reaction by bovine granulocytes (114). It appears that the availability of peroxidase to catalyze the reaction is the limiting factor. The results reported here which demonstrate that the bovine eosinophil contains 4 to 5 times more peroxidase and has an

iodination value 4 to 5 times higher than the bovine neutrophil while having equivalent NBT reduction supports this conclusion. Therefore, the iodination reaction as performed here appears to be a sensitive assay for peroxidase activity but not oxidative metabolism.

The eosinophil-rich cell preparations were only about one half as effective as the neutrophil-rich cell preparations at releasing ^{51}Cr from antibody coated chicken red blood cells. This is similar to the results reported for ADCC by purified human eosinophils and neutrophils when either CRBCs (123) or schistosomula of Schistosoma mansoni (149) were used as target cells. In the latter experiment, even though the eosinophil did not release ^{51}Cr as efficiently as the neutrophils, they were shown to adhere more strongly to the schistosomula and to induce greater microscopically detectable damage.

The differences in activity observed between the two purified cell populations was not thought to be due to selective damage to one cell type during the isolation procedure. Both types of cell preparations went through identical cell separation procedures; the only difference for the two cell types was their location in the Ficoll-Hypaque solution following centrifugation. Another reason for discounting the possibility of selective damage to one cell type is that the neutrophil was more active than the eosinophil at ingestion and ADCC while the eosinophil was more active at iodination and contained more peroxidase than the neutrophil.

The cell isolation procedure described here will allow the use of the bovine as a model animal for use in further research into the role of the eosinophil in host defense and the inflammatory response. The bovine is well-suited for this sort of experimentation because a pharmacologic or infectious agent may be administered and a large amount of blood may be collected at frequent intervals to monitor the effects on eosinophil and neutrophil function.

EFFECTS OF *IN VIVO* DEXAMETHASONE ADMINISTRATION ON *IN VITRO* BOVINE POLYMORPHONUCLEAR LEUKOCYTE FUNCTION[1]

Summary

Polymorphonuclear leukocyte function was evaluated *in vitro* following the *in vivo* administration of a single dose of dexamethasone to cattle. Purified PMNs from dexamethasone-treated cattle displayed enhanced random migration under agarose but impaired ingestion of *Staphylococcus aureus*, nitroblue tetrazolium reduction, chemiluminescence, iodination, and antibody-dependent cell-mediated cytotoxicity. The depression of iodination may have been related to a drop in the proportion of eosinophils present in the PMN preparations following dexamethasone administration.

Introduction

Glucocorticoids, when administered to man or animals, have been observed to decrease the inflammatory response and to predispose to bacterial infection (38). Polymorphonuclear leukocytes (PMNs) play an important role in both the inflammatory response and in controlling bacterial infection. For this reason, numerous investigations

---

[1]Submitted for publication to *Infection and Immunity* by J. A. Roth and M. L. Kaeberle.

have been conducted on the effects of glucocorticoids on PMN function. There are many reports of defects in PMN function following *in vitro* or *in vivo* exposure to glucocorticoids. However, it is difficult to reach a conclusion on the overall effects of glucocorticoid therapy on PMN function due to conflicting results and variations in experimental techniques. One potential reason for conflicting results when PMNs are exposed *in vitro* to glucocorticoids is that, in some experiments, the succinate form of glucocorticoids were used (34,66,84). The succinate must be split off in the liver by an esterase before the drug is active. Spontaneous cleavage of the succinate under *in vitro* conditions occurs slowly. With extremely high concentrations of the succinate compound, enough free glucocorticoid may be present to affect PMN function (25,90,102). Another possible explanation for the presence of conflicting results when comparing the *in vitro* and *in vivo* exposure of PMNs to glucocorticoids is that *in vivo* administration of glucocorticoids may have indirect effects on PMN function by altering the activity of other mediators of inflammation. For example, betamethasone has been shown to block prostacyclin synthesis by vascular endothelial cells (83). Prostacyclins may have potent effects on PMN function (156). For these reasons, it is desirable to design experiments using *in vivo* treatment of healthy individuals with glucocorticoids in order to obtain information relative to the effects of therapeutic glucocorticoid administration on PMN function.

Some of the reports of *in vivo* treatment with glucocorticoids have compared PMNs from patients who are on glucocorticoid therapy for an

underlying pathologic process to PMNs obtained from normal individuals (25,26,140). It is difficult to be certain if the defects in PMN function observed are due to glucocorticoid treatment or to the underlying pathologic process. There are reports comparing the function of PMNs from normal subjects who have voluntarily received a therapeutic dosage of glucocorticoid to that of normal controls (30,88). This has yielded important information, but only one or two aspects of PMN function have been evaluated.

Glucocorticoids are extensively used therapeutically in cattle, but their effects on the immune system are not well-characterized. Dexamethasone is a potent glucocorticoid which is commonly administered to cattle for the induction of parturition, the alleviation of physiologic udder edema, the reduction of musculoskeletal inflammation, and the treatment of ketosis (78). Dexamethasone administration to cattle has been observed to have serious detrimental consequences such as the recrudescence of infectious bovine rhinotracheitis virus (39,40,109), the predisposition to a fatal viremia in bovine viral diarrhea virus-infected calves (131), and a more severe disease course when administered to cattle suffering from bronchial pneumonia (27). The observed potentiation of infectious processes indicates that dexamethasone suppresses the bovine immune system. Dexamethasone has previously been shown to suppress mitogen induced bovine lymphocyte blastogenesis (39,95) which is considered an *in vitro* correlate of *in vivo* cell-mediated immunity.

The purpose of the experimentation reported here was to determine the effects of a pharmacologic dose of dexamethasone on bovine PMN function. Six different functional parameters were evaluated on PMNs which were obtained from control and dexamethasone-treated cattle. All six tests were performed within a few hours of the time that the blood sample was drawn so that the results could be accurately compared.

Materials and Methods

Animals and dexamethasone treatment

Apparently healthy adult Holstein-Fresian steers and bulls were used. Five animals served as controls and seven animals were injected intramuscularly with 40 mg of dexamethasone (0.058 to 0.088 mg/kg).[1] Blood samples were obtained prior to and at two hours and 1,2,3,7,9,10, and 13 days following dexamethasone administration for the evaluation of PMN function.

PMN isolation

PMNs were isolated as previously described (114). Briefly, peripheral blood was collected into acid-citrate-dextrose solution,

---

[1]Azium, Schering Corp. Kenilworth, NJ.

centrifuged, and the plasma and buffy coat layer were discarded. The packed red blood cell layer was treated with two volumes of cold, phosphate-buffered distilled water for 50 seconds; isotonicity was restored by adding one volume of cold, phosphate-buffered 2.7% NaCl. The remaining cells were washed with 0.015 M phosphate-buffered saline solution, pH 7.2 (PBSS) by centrifugation and were suspended in PBS to a concentration of 5.0×10^7 PMNs (neutrophils plus eosinophils) per ml. The cells were held at room temperature and were used in all six PMN function tests within three hours of the time they were standardized. The cell isolation procedure generally yielded a PMN preparation of greater than 90% purity.

Migration under agarose

The ability of the PMNs to migrate under agarose was evaluated as previously described (114). The test was performed in 60 x 15 mm tissue culture petri plates containing 5.0 ml of agar gel consisting of 0.8% agarose,[1] 10% fetal calf serum, and 1% Antibiotic-Antimycotic[2] in minimum essential medium. Wells 2.0 mm in diameter were punched in the agarose and were filled with the PMN suspension. The plates were incubated at 37 C in a 5% CO_2 enriched atmosphere overnight. The cells were then

---

[1]#57035 Gallard Schlessinger, Chemical Mfg. Corp., Carle Place, NY.

[2]GIBCO Laboratories, Grand Island, NY.

fixed with formaldehyde and stained with modified Wrights' stain. The area of migration was determined by calculating the total area of migration and subtracting the area of the inner well. The test was conducted in duplicate, and the results are expressed as the square mm of migration.

Ingestion of Staphylococcus aureus

The ingestion of ^{125}I-labeled S. aureus by PMNs was evaluated as previously described (114). The S. aureus were labeled by growth in the presence of [^{125}I] iododeoxyuridine[1] and 5-fluorodeoxyuridine.[2] The ^{125}I-labeled S. aureus was washed by centrifugation in PBSS, and killed by heating to 60 C for one hour, washed again in PBSS, and resuspended to a concentration such that a 1:10 dilution in PBSS had an optical density of 0.4 at λ = 600 nm. The ingestion assay mixture contained 100 µl of ^{125}I-labeled S. aureus, 50 µl of PMN suspension (2.5 x 10^6 PMNs, bacteria to PMN ratio = 60:1), 50 µl of a 1:10 dilution of bovine anti-S. aureus serum, and 0.3 ml of Earle's balanced salt solution (EBSS).[3] The reactants were combined and incubated at

---

[1]New England Nuclear, Boston, MA.

[2]Sigma Chemical Co., St. Louis, MO.

[3]GIBCO Laboratories, Grand Island, NY.

37 C. After ten minutes, 0.5 IU of lysostaphin[1] was added and the incubation was continued for an additional 30 minutes to destroy the uningested S. aureus. The PMNs were then washed twice in PBSS by centrifugation, and the amount of radioactivity associated with the PMNs was determined in a gamma counter. The test was conducted in duplicate, and the results are expressed as the percent of the total S. aureus ingested.

Nitroblue tetrazolium reduction

The quantitative nitroblue tetrazolium (NBT) reduction test was performed as previously described (114). The reaction mixture contained 0.4 mg of NBT,[1] 1.0 mg of preopsonized zymosan, and 5.0 x 10^6 PMNs in 1.0 ml of EBSS. The reactants were combined and incubated at 37 C. After 5.0 minutes, 5.0 ml of cold 1 mM N-Ethylmaleimide[1] in PBSS was added to stop the reaction. The purple formazan formed by the reduction of NBT was pelleted by centrifugation, resuspended in 5.0 ml of pyridine by sonication and placed in a boiling water bath for ten minutes. The pyridine was then clarified by centrifugation and the optical density at λ = 580 nm was determined. The test was conducted in triplicate, and the results are reported as the optical density per 5.0 x 10^6 PMNs per 5.0 minutes in 5.0 ml of pyridine.

---

[1]Sigma Chemical Co., St. Louis, MO.

Iodination

The iodination procedure was performed as previously described (114). The standard reaction mixture contained 40 nmole NaI, 0.05 μCi [^{125}I],[1] 2.5 x 10^6 PMNs, and 0.5 mg of preopsonized zymosan in a total volume of 0.5 ml EBSS. The reactants were combined in 12 x 75 mm polystyrene snap cap test tubes[2] and incubated for twenty minutes at 37 C with end-over-end tumbling. The reaction was terminated by the addition of 2.0 ml of cold 10% trichloroacetic acid. The resulting precipitate was washed one additional time in 10% trichloroacetic acid, and the amount of radioactivity in the precipitate was determined in a gamma counter. The test was conducted in duplicate, and the results are expressed as nmole NaI/10^7 PMNs/hr.

Chemiluminescence

Chemiluminescence was measured as previously described (114) in a liquid scintillation spectrometer[3] at ambient temperature with one photomultiplier tube switched off. Test vials contained 5.0 mg of preopsonized zymosan and 1.0 x 10^7 PMNs in a total volume of 10.0 ml of

---

[1]Carrier free in 0.1 M NaOH, New England Nuclear, Boston, MA.

[2]#2058 Falcon, Oxnard, CA.

[3]Model DPM 100, Beckman Instruments, Inc., Irvine, CA.

bicarbonate buffered (0.026 M, pH 7.2) Geys balanced salt solution with Ca^{++} and Mg^{++} and without phenol red.[1] Each vial was counted for one minute at approximately ten minute intervals. Counting was continued for at least 70 minutes. The test was conducted in duplicate, and the results were converted from counts per minute to net counts per hour as previously described (114).

Antibody-dependent cell-mediated cytotoxicity (ADCC)

The ADCC reaction was conducted using ^{51}Cr-labeled chicken red blood cells as target cells. The standard reaction mixture contained 2.5×10^6 PMNs, 2.5×10^5 ^{51}Cr-labeled chicken red blood cells and 50 µl of bovine anti-chicken red blood cell antiserum in a total volume 0.5 ml of Medium 199.[1] The test was conducted in duplicate and Triton-X controls, antibody controls, and granulocyte controls were also set up. The reactants were combined and incubated at 37 C for 3.0 hours, the tubes were centrifuged, and an aliquot of supernatant solution was removed for gamma counting. The results are expressed as the percent of ^{51}Cr specifically released.

---

[1]GIBCO Laboratories, Grand Island, NY.

Results

Dexamethasone treatment caused a marked increase in random migration by bovine PMNs (Figure 3). The average area of random migration was slightly increased at two hours postinjection and had doubled by 24 hours postinjection. Random migration by PMNs from dexamethasone-treated animals remained elevated for three days before returning to normal.

The ability of PMNs from dexamethasone-treated animals to ingest S. aureus was markedly depressed (Figure 4). The depression of ingestion was detectable at two hours postinjection, was maximal at 24 hours, and was still present at 48 hours postinjection. By three days postinjection, the ability to ingest S. aureus had returned to normal.

Dexamethasone treatment markedly suppressed NBT reduction by PMNs (Figure 5). The suppression was apparent by two hours postinjection and remained for several days. Chemiluminescence, another parameter of the oxidative metabolism of the PMN, showed a similar pattern of suppression following dexamethasone treatment (Figure 6).

Iodination by PMNs isolated from dexamethasone treated cattle was somewhat depressed by two hours postinjection (Figure 7). By 24 hours postinjection, the iodination value was less than 50% of normal. The iodination value remained at approximately 50% of normal for the entire 13 day period following a single injection of dexamethasone.

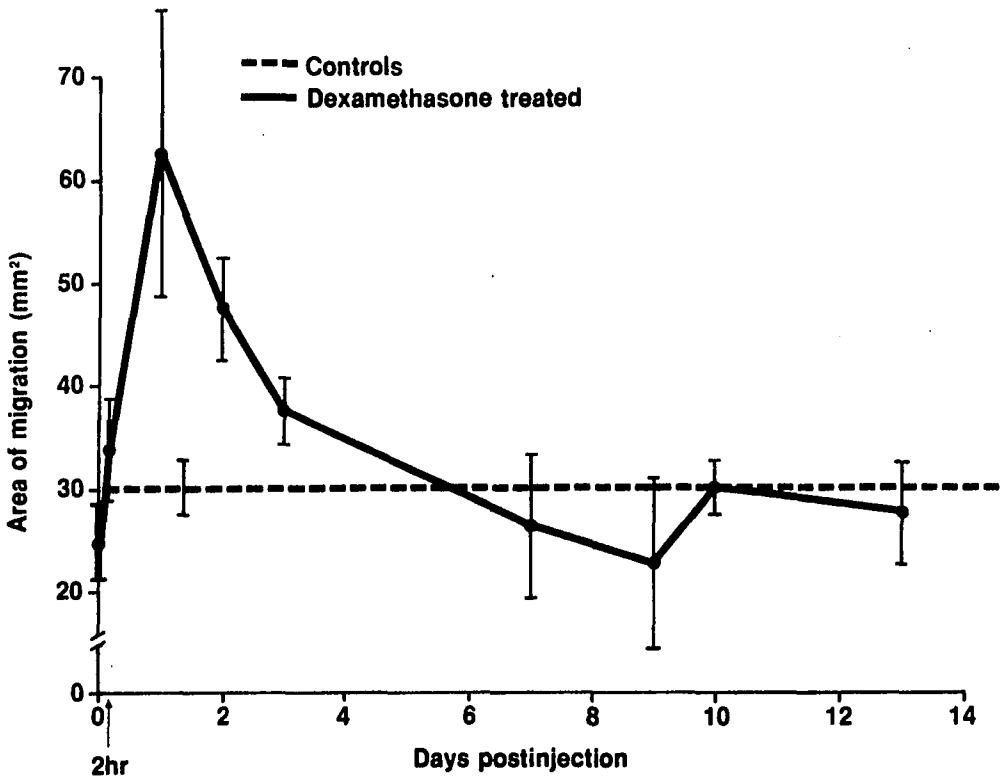


Figure 3. Effect of in vivo dexamethasone administration on the area of random migration by bovine PMNs under agarose. The results are means ± SEM. (n = 49 for the controls; n = 7 for the dexamethasone group)

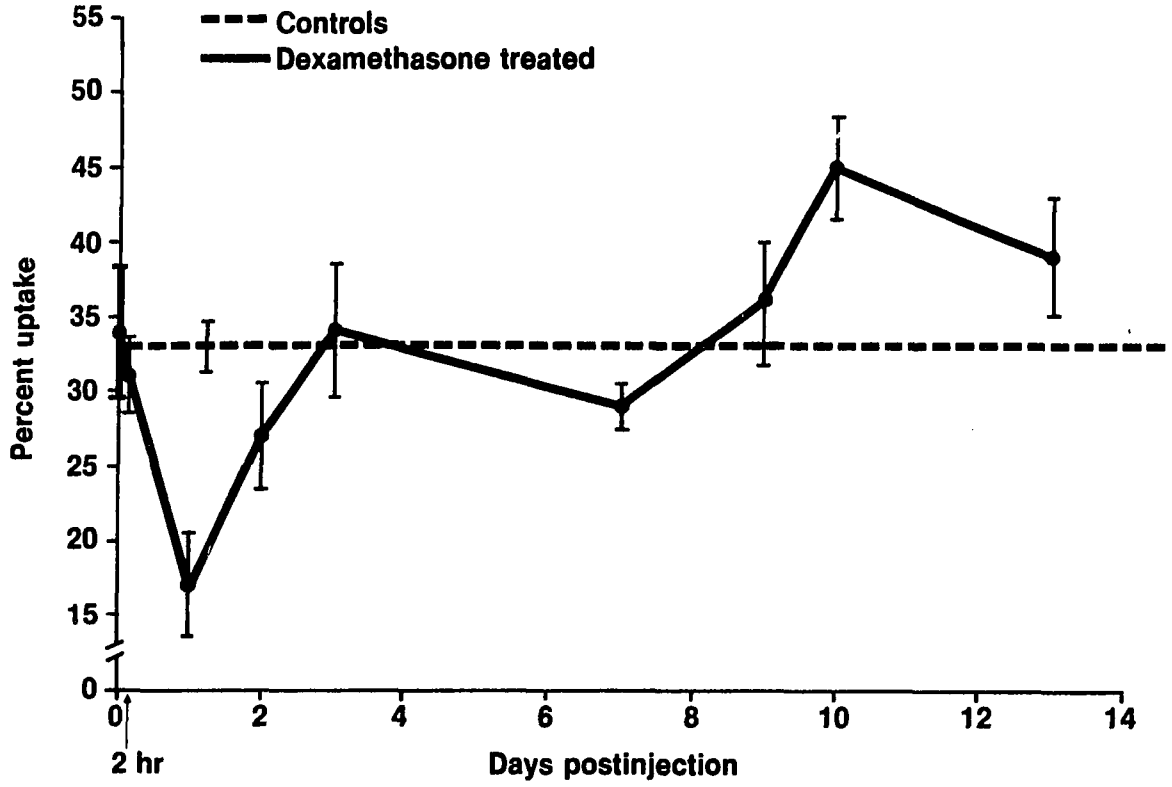


Figure 4. Effect of in vivo dexamethasone administration on the ingestion of ^{125}I-labeled S. aureus by bovine PMNs. The results are means ± SEM (n = 47 for the controls; n = 7 for the dexamethasone group)

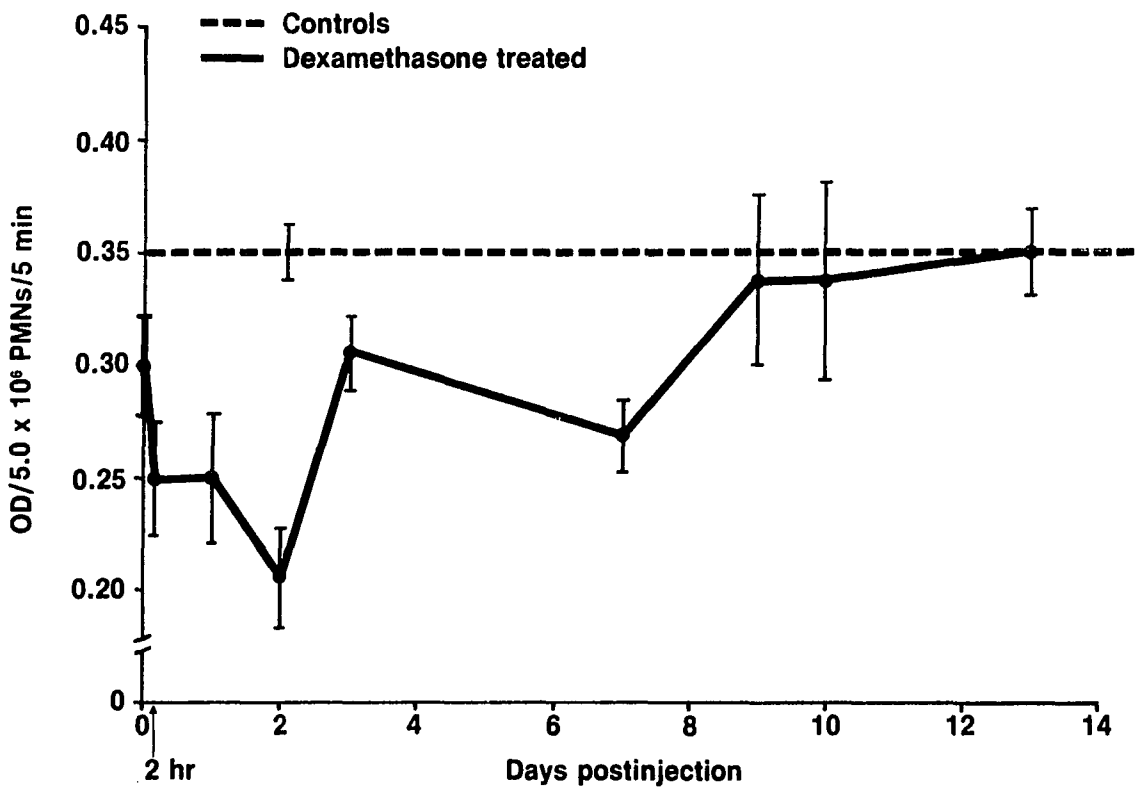


Figure 5. Effect of in vivo dexamethasone administration on the reduction of nitroblue tetrazolium by bovine PMNs. The results are means ± SEM (n = 48 for the controls; n = 7 for the dexamethasone group)

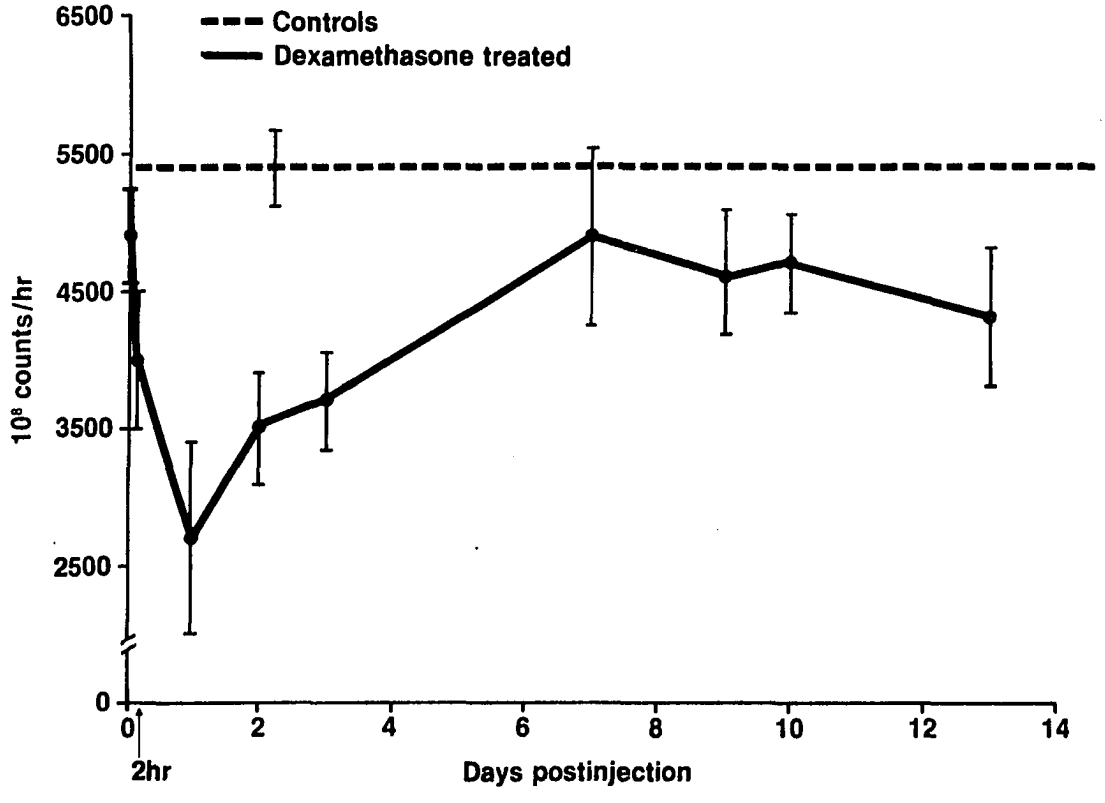


Figure 6. Effect of in vivo dexamethasone administration on chemiluminescence by bovine PMNs. The results are means ± SEM (n = 47 for the controls; n = 7 for the dexamethasone group)

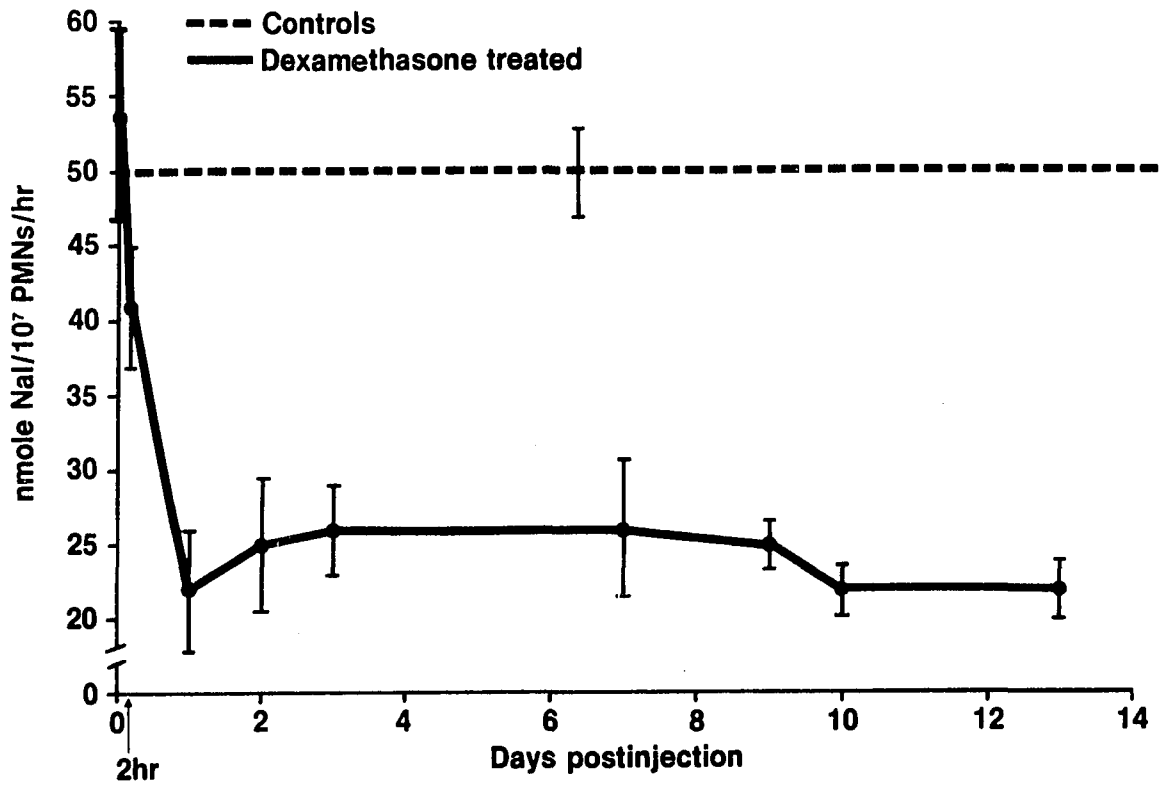


Figure 7. Effect of in vivo dexamethasone administration on iodination by bovine PMNs. The results are means ± SEM (n = 50 for the controls; n = 7 for the dexamethasone group)

Antibody-dependent cell-mediated cytotoxicity by PMNs was somewhat depressed at two hours and was markedly depressed at 24 hours post-injection (Figure 8). By 48 hours postinjection, it had returned to near normal levels.

The percentage of eosinophils in the PMN preparations was markedly reduced following dexamethasone administration and remained low for the entire 13 day period (Figure 9).

Discussion

A single pharmacologic dose of dexamethasone was found to have a profound effect on PMN function when administered to normal cattle. Dexamethasone administration caused an enhancement of random migration (chemokinesis) by the PMNs but a depression of ingestion, oxidative metabolism, the myeloperoxidase-H_2O_2-halide antibacterial system, and antibody-dependent cell-mediated cytotoxicity by PMNs.

The stimulation of random migration observed after dexamethasone administration is similar to results reported for PMNs from human patients being treated with prednisolone for various pathologic conditions. Stevenson (140) demonstrated that the enhancement of migration was not due to a direct effect of the steroid on the PMN but rather was dependent upon the presence of mononuclear leukocytes in the migrating cell population. Stevenson suggested that the steroid reacts with mononuclear leukocytes to produce a PMN migration stimulator. The

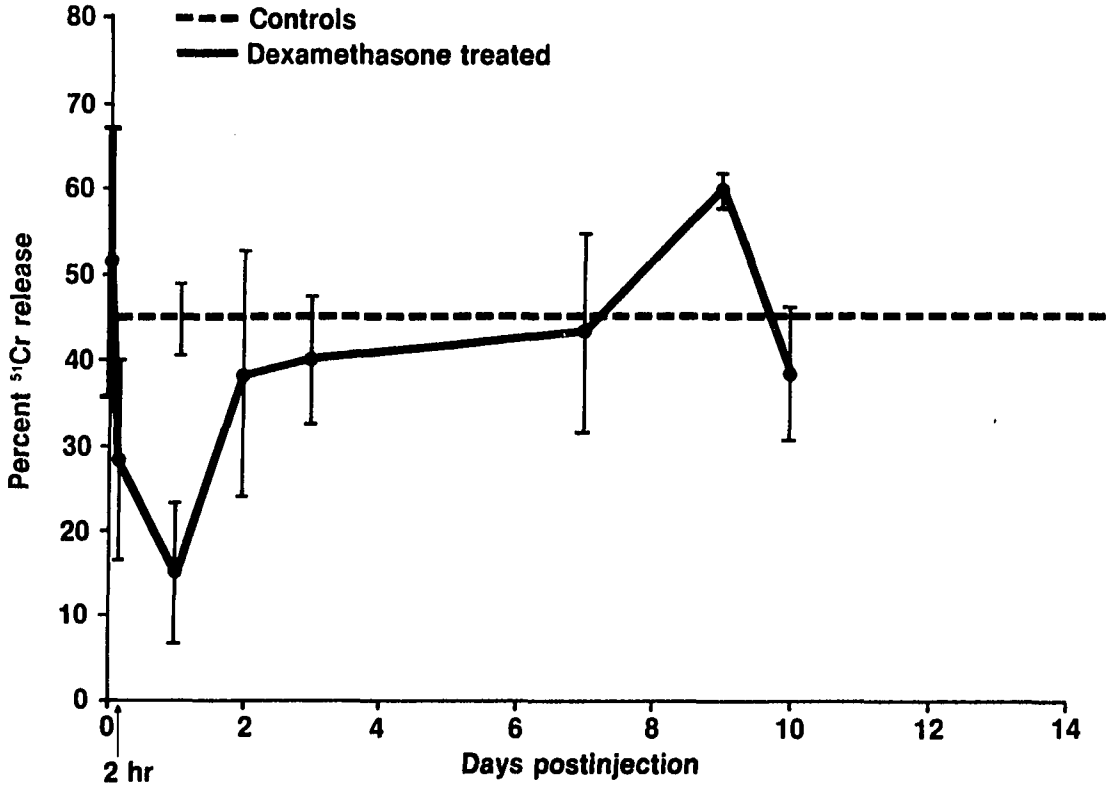


Figure 8. Effect of in vivo dexamethasone administration on antibody-dependent cell-mediated cytotoxicity by bovine PMNs. The results are means ± SEM (n = 39 for the controls; n = 5 for the dexamethasone group)

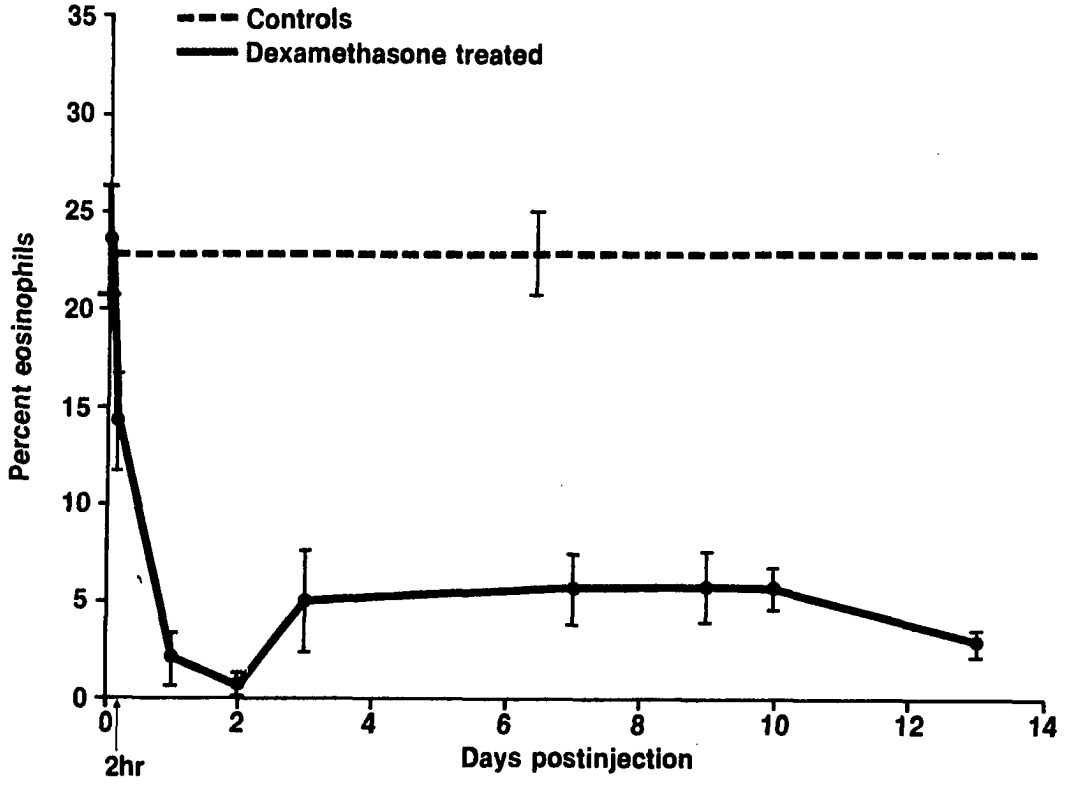


Figure 9. Effect of in vivo dexamethasone administration on the percent of cells in the purified PMN preparation which are eosinophils. The results are means ± SEM (n = 50 for the controls; n = 7 for the dexamethasone group)

leukocyte preparations used in our study generally contained less than 10% mononuclear cells. It is not known if this is a sufficient number of mononuclear leukocytes to affect the bovine PMN migration or if the dexamethasone had a direct stimulatory effect on PMN migration.

There are conflicting reports on the effect of corticosteroids on ingestion by human PMNs. Corticosteroids have been reported to inhibit ingestion by PMNs (34,66) and to have no effect on ingestion (25,84). These discrepancies may be due to differing techniques for evaluating ingestion or to differing amounts and types of corticosteroids used. None of these reports involved the in vivo administration of a pharmacologic dose of corticosteroid to a normal individual. Our results indicate that a single pharmacologic dose of dexamethasone administered in vivo will inhibit ingestion by bovine PMNs.

Our results also demonstrate that dexamethasone administration inhibits NBT reduction and chemiluminescence by bovine PMNs. Inhibition of NBT reduction has been reported previously following the in vitro treatment of human PMNs with hydrocortisone (25,84) and in PMNs from patients with pathologic processes who were being treated with corticosteroids (25,26). Inhibition of PMN NBT reduction and chemiluminescence indicates that dexamethasone administration inhibits the oxidative metabolism of the neutrophil. The oxidative metabolism is a very important aspect of the PMNs bactericidal activity. When a PMN encounters a phagocytosable particle under aerobic conditions, an oxidase enzyme located in the cell membrane and in the phagolysosome membrane is

activated to convert molecular oxygen to superoxide anion (5). The superoxide anion undergoes a series of spontaneous reactions to form hydrogen peroxide, the hydroxyl radical, and perhaps singlet oxygen. All of these are highly reactive species that are damaging to living organisms (5). Superoxide anion directly reduces NBT to an insoluble purple formazan (158). The exact source of chemiluminescence is not known but it is associated with the generation of these reactive oxygen moieties (3). Therefore, the depression of NBT reduction and chemiluminescence indicates that an important component of the PMNs bactericidal mechanism is impaired by dexamethasone administration.

Dexamethasone administration caused a dramatic, prolonged depression of iodination by the bovine PMN preparations. The iodination reaction is a measure of the activity of the myeloperoxidase-hydrogen peroxide-halide antibacterial system in the PMN. Myeloperoxidase is released into the phagolysosome during degranulation. It catalyzes the reaction between hydrogen peroxide and iodide and results in the binding of iodide to tyrosine radicals of protein. Iodination is, therefore, dependent upon the presence of hydrogen peroxide which is generated by the burst of phagocytosis-associated oxidative metabolism and upon the release of myeloperoxidase into the phagolysosome. We have already stated that the oxidative metabolism of the PMN was depressed by dexamethasone administration; therefore, one would expect a lower amount of hydrogen peroxide to be available for iodination. Other workers have reported inhibition of lysosomal enzyme secretion by PMNs due to <u>in vitro</u>

treatment with corticosteroids (66). Therefore, one would expect a lowered amount of myeloperoxidase to be available to catalyze the iodination reaction. This inhibition of hydrogen peroxide formation and degranulation could both contribute to the depression of iodination which has been reported following the in vitro treatment of PMNs with corticosteroids (34). However, the prolonged suppression of iodination observed here suggests that another mechanism is at least partially responsible. The PMN preparations used in this study contained a mixed population of eosinophils and neutrophils. Eosinophils in the peripheral blood of normal cattle may make up from 2% to 20% of the total white blood cell population (125); this results in a fairly high percentage of eosinophils in the purified PMN preparations from normal animals (an average of 22.7% in this study). Corticosteroid administration causes eosinophils to sequester in lymphoid tissues (122) and thereby reduces the number of circulating eosinophils in the peripheral blood. This results in PMN preparations from dexamethasone-treated animals with a very low percentage of eosinophils (Figure 9). The depression of iodination (Figure 7) seems to parallel the depletion of eosinophils in the PMN preparation (Figure 9). We have shown that bovine eosinophils are four to five times more active than bovine neutrophils in the stimulated iodination reaction (115). Therefore, the depression of iodination is probably at least partially due to the lowered percentage of eosinophils in the PMN preparation. We believe

that the early depression of iodination was not totally due to a reduction in eosinophil numbers but was partially due to a direct effect of dexamethasone on the iodination ability of the neutrophil. In other experimentation, we have demonstrated that administration of adrenalcorticotropic hormone to cattle with very low numbers of eosinophils in their peripheral blood resulted in a depression of iodination by PMNs without a concomitant decrease in eosinophil numbers (118).

The inhibition of ingestion, oxidative metabolism, and ADCC by PMNs following dexamethasone administration is probably not due to the decreased percentage of eosinophils because: 1) Eosinophils were less efficient than neutrophils at ingestion of S. aureus and ADCC, and the two cell types produced equivalent NBT reduction (115) and 2) these parameters returned to normal within a few days, whereas iodination and the percentage of eosinophils remained depressed for the entire period of the study.

Bovine neutrophils have been demonstrated to be the most active bovine cell type tested in mediating ADCC against herpesvirus-infected target cells (120). The exact mechanism of this cytotoxicity is not known, but it is not dependent upon DNA, RNA, or protein synthesis within the PMN (29,153). The results observed here of depressed ADCC mediated by PMNs following the in vivo administration of dexamethasone, support the previous report of depressed ADCC by bovine PMNs treated in vitro with dexamethasone (153). This inhibition of the ability of

PMNs to mediate ADCC may play an important role in allowing bovine herpesvirus, which has been recrudesced by the administration of dexamethasone, to multiply (39,40,108).

In summary, a single pharmacologic dose of dexamethasone administered to cattle caused an enhancement of random migration by PMNs but an impairment of 1) the ingestion of bacteria by PMNs, 2) the oxidative metabolism of the PMN, 3) the myeloperoxidase-hydrogen peroxide-halide antibacterial system of the PMN, and 4) antibody-dependent PMN-mediated cytotoxicity. The impairment of PMN function may at least partially explain the detrimental effects associated with dexamethasone administration to cattle (27,40,131).

EFFECTS OF ADRENOCORTICOTROPIN ADMINISTRATION ON BOVINE POLYMORPHONUCLEAR LEUKOCYTE FUNCTION AND LYMPHOCYTE BLASTOGENESIS[1]

Summary

Yearling steers were treated with adrenocorticotropic hormone (ACTH) to determine the effect of elevated plasma cortisol concentration on bovine lymphocyte and polymorphonuclear leukocyte (PMN) function. The administration of ACTH caused a significant increase in serum cortisol concentration and depression of lymphocyte blastogenesis in response to phytohemagglutinin and concanavalin A. The response to pokeweed mitogen was also depressed but not significantly. Random migration by PMNs was significantly enhanced by ACTH treatment, but there was no effect on ingestion of Staphylococcus aureus, nitroblue tetrazolium reduction, or antibody-dependent cell-mediated cytotoxicity by PMNs. The iodination reaction, which evaluates the activity of the myeloperoxidase-hydrogen peroxide-halide antibacterial system of the PMN, was significantly impaired following ACTH treatment. These results indicate that specific parameters of lymphocyte and neutrophil function were impaired by elevated in vivo concentrations of plasma cortisol.

---

[1]Submitted for publication to the American Journal of Veterinary Research by J. A. Roth, M. L. Kaeberle, and W. H. Hsu.

Introduction

The glucocorticoids are generally considered to be anti-inflammatory and to suppress the immune system. The administration of dexamethasone to cattle can exacerbate infectious disease processes (22,27,79,85,130,131,143). Stress-induced elevation of plasma cortisol concentration is an important initiating factor in the pathogenesis of certain infectious diseases; one of the best examples is the "bovine respiratory disease complex" (BRD) (60). The bovine respiratory disease complex or "shipping fever" is the most costly of all diseases of feedlot cattle in the United States (63). It is an acute respiratory disease which occurs most commonly in beef calves following weaning and shipment to feedlots. A wide variety of etiologic agents including viruses, bacteria, and mycoplasma have been associated with BRD, but no single agent is consistently involved or capable of reproducing the syndrome (60). Stress has long been recognized to play an important role in the pathogenesis of BRD (62). A wide variety of factors may "stress" cattle at the time that they are weaned and shipped to the feedlot (60). A major response of the body to stress is the secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary gland and a resultant increase in blood cortisol concentration (139). Weaning of calves under good management conditions has been shown to increase blood cortisol concentration (35,56); shipment of newly weaned calves will potentiate this increase in blood cortisol (35,56,69,128). Handling

of calves (35) and surgical procedures (69) will also cause an increased blood cortisol concentration. Dexamethasone, a glucocorticoid which is approximately 30 times more potent than cortisol as an anti-inflammatory agent (43), has been shown to suppress lymphocyte blastogenesis (39,95) and polymorphonuclear leukocyte (PMN) function (113) when administered to cattle in pharmacologic doses. However, the effect of elevated plasma cortisol on bovine lymphocyte and PMN function has not been well-characterized. The purpose of the present study was to determine if high levels of plasma cortisol, induced by ACTH administration, would produce a measurable suppression of lymphocyte blastogenesis or PMN function in cattle.

Materials and Methods

Animals and experimental design

Ten head of apparently healthy 12- to 16-month-old Holstein-Friesian and Brown Swiss steers were used. They were serologically negative for infectious bovine rhinotracheitis and bovine viral diarrhea virus antibody. The animals were housed together; five of the animals were randomly assigned to a control group and five to an ACTH treatment group. The ACTH-treated group received 200 IU of ACTH[1] intramuscularly every 12 hours for 3 consecutive days. The timing of the ACTH injections,

---

[1]Adrenomone, kindly supplied by Burns-Biotec Laboratories, Omaha, NB.

blood sampling for evaluation of lymphocyte and neutrophil function, and blood sampling for cortisol radioimmunoassay is shown in Figure 10. Animals were bled prior to the initiation of ACTH treatment and then daily for three days while blood cortisol levels were elevated.

Leukocyte preparation

Mononuclear cells were isolated by a modification of the procedure of Bøyum (14). Twenty ml of blood was collected aseptically into 2 ml of acid-citrate-dextrose solution. This anticoagulated blood was diluted with 20 ml of sterile 0.015 M phosphate-buffered saline solution, pH 7.2 (PBSS) and layered over a column of 1.077 specific gravity Ficoll-Hypaque[1] in a 25 x 150 mm screw cap, siliconized test tube and centrifuged at 500 x g for 45 minutes. The lymphocytes were removed from the Ficoll-Hypaque plasma interface, washed in Hanks' balanced salt solution[2] and counted.

PMNs were isolated from the peripheral blood as previously described (114). Briefly, the anticoagulated blood was centrifuged, the plasma and buffy coat layer were discarded and the red blood cells were removed by hypotonic lysis with distilled water to yield a cell preparation of generally greater than 90% pure PMNs. The cells were adjusted to a concentration of 5.0×10^7 PMNs/ml in PBSS for use.

---

[1]Histopaque-1077, Sigma Chemical Co., St. Louis, MO.

[2]Grand Island Biological Co., Grand Island, NY.

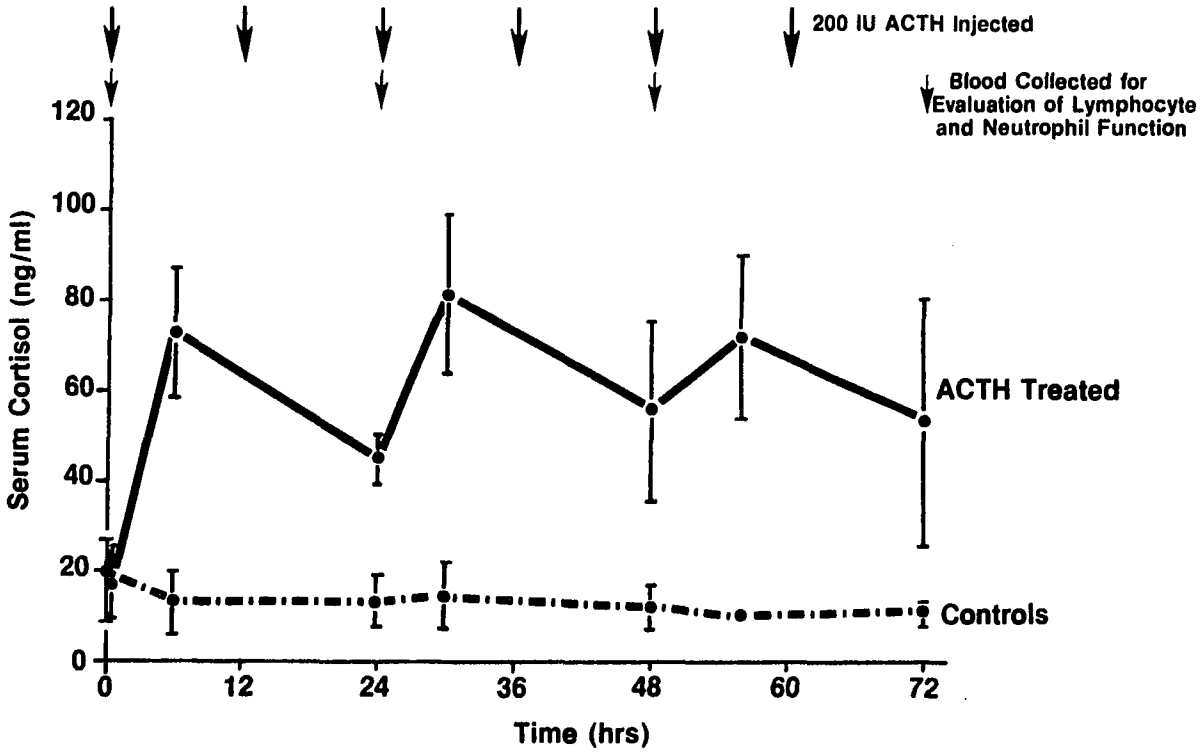


Figure 10. Serum cortisol concentration in the control and ACTH-treated animals during the course of the experimentation. The ACTH treatment group received 200 IU ACTH intramuscularly every 12 hours. Blood was collected prior to beginning the ACTH injections and at 24 hour intervals thereafter. Serum cortisol values are represented as the mean $\pm$ SD (n = 5)

Lymphocyte blastogenesis

Lymphocytes were cultured in microtiter plates[1] with 2.0 x 10^5 mononuclear cells in 0.15 ml of culture medium per well. The culture medium consisted of Medium 199 with Earle's salts[2] containing 15% heat-inactivated fetal calf serum and 1% Antibiotic-Antimycotic solution.[2] Control and mitogen-stimulated cultures were assayed in triplicate wells. Mitogen stimulated cultures received 25 µl of a dilution of mitogen [phytohemagglutinin-P(PHA),[3] concanavalin A (ConA),[4] or pokeweed mitogen (PWM)[2]] which had been predetermined to give optimal stimulation under identical culture conditions. The microtiter plates were incubated at 37 C in a humidified 5% CO_2 atmosphere. After 48 hours, 0.25 µCi of ^{3}[H] thymidine[5] was added to each well. Sixteen hours later the cultures were harvested onto glass fiber filters with an automated sample harvester,[6] the filters placed in 10 ml of toluene based cocktail[7] and counted in a liquid scintillation counter.

---

[1]Costar #3596, Cambridge, MA.

[2]Grand Island Biological Co., Grand Island, NY.

[3]Difco Laboratories, Inc., Detroit, MI.

[4]Miles Laboratories, Inc.

[5]Carrier Free in 0.1 M NaOH, New England Nuclear, Boston, MA.

[6]Flow Laboratories, Inc., Rockville, MD.

[7]Metric-Pak 2a70, Research Products International Corp., Elk Grove Village, IL.

PMN function tests

The procedures for evaluating random migration under agarose (114), ingestion of ^{125}I-labeled Staphylococcus aureus (114), nitroblue tetrazolium (NBT) reduction (114), iodination (114), and antibody-dependent cell-mediated cytotoxicity (ADCC) (113) by PMNs have been described in detail elsewhere. All PMN function tests were conducted in duplicate. Briefly, random migration under agarose was evaluated using plastic petri plates which contained a layer of 0.8% agarose with 10% fetal calf serum and 1% Antibiotic-Antimycotic in minimum essential medium.[1] Wells 2.0 mm in diameter were punched in the agar and filled with an aliquot of the PMN suspension. The migration plates were placed in an incubator with a humidified 5% CO_2 atmosphere at 37 C. After 18 hours, the plates were removed and the area of PMN migration was determined and expressed in mm^2. Heat-killed ^{125}I-iododeoxyuridine-labeled S. aureus was used to evaluate ingestion (114). Opsonized ^{125}I-S. aureus and PMNs were incubated together at 37 C with a bacteria to PMN ratio of 60:1. After 10 minutes, lysostaphin[2] was added and the incubation was continued for an additional 30 minutes. The PMNs were then washed twice in PBSS by centrifugation, and the amount of PMN-associated radioactivity was determined. The results are expressed as the

---

[1]Grand Island Biological Co., Grand Island, NY.

[2]Sigma Chemical Co., St. Louis, MO.

percent of the ^{125}I-S. aureus which was ingested. The quantitative NBT reduction assay was performed by adding 5.0 x 10^6 granulocytes to a suspension of opsonized zymosan in 1.0 ml of Earle's balanced salt solution containing 0.4 mg of NBT.[1] After a 5.0 minute incubation in a 37 C water bath, 5.0 ml of cold N-ethylmaleimide[1] was added to stop the reaction. The purple formazan formed by the reduction of NBT was extracted with pyridine and the optical density at 580 nm was determined. The results are expressed as O.D./5.0 x 10^6 PMNs/5 min in 5.0 ml of pyridine. The standard reaction mixture for the determination of stimulated iodination contained 2.5 x 10^6 PMNs, 0.05 μCi[^{125}I],[2] 40 nmole NaI, and 0.5 mg of opsonized zymosan in 0.5 ml of Earle's balanced salt solution. Twenty minutes after the PMNs were added, the reaction was stopped by adding 2.0 ml of cold 10% trichloroacetic acid. The resulting precipitate was washed one additional time in 10% trichloroacetic acid and the amount of radioactivity in the precipitate was determined in a gamma counter. The results are expressed as nmole NaI/10^7 PMNs/hr. The antibody-dependent cell-mediated cytotoxicity (ADCC) assay was performed utilizing ^{51}Cr-labeled chicken red blood cells (CRBC) as target cells. The reaction mixture contained 2.5 x 10^5 ^{51}Cr-labeled CRBCs and 2.5 x 10^6 PMNs (effector to target cell ratio of 10:1) in 0.5 ml

---

[1]Sigma Chemical Co., St. Louis, MO.

[2]Carrier Free in 0.1 M NaOH, New England Nuclear, Boston, MA.

of Medium 199 containing 10% bovine anti-CRBC serum. Triton X controls, antibody controls, and PMN controls were included. After a three hour incubation at 37 C in a humidified 5% CO_2 atmosphere, the reaction tubes were centrifuged and an aliquot of supernatant solution was removed for gamma counting. The results were expressed as percent of specific ^{51}Cr release.

Determination of serum cortisol concentration

Blood for the cortisol assay was allowed to clot for 4 to 6 hours at 25 C. The serum was then removed and stored at -20 C until the cortisol assay was performed. A serum cortisol concentration was determined by a double antibody radioimmunoassay using a commercial kit.[1] The samples were assayed in duplicate and all of the samples were processed concurrently. The lower limit of detectability of the assay system was 10 ng/ml. All values below this limit were assigned a value of 10 ng/ml.

Hematologic studies

The total white blood cell (WBC) count in heparinized blood obtained by jugular venipuncture was determined by electronic counting.[2] Blood films for differential leukocyte counts were

---

[1]Diagnostic Products Corp., Los Angeles, CA.

[2]Coulter Electronics Co., Inc., Hialeah, FL.

prepared, stained with Wright's stain, and 100 cells were counted. Because lymphocytes and monocytes cannot be accurately differentiated in bovine blood by this method (58), they were counted together and termed mononuclear cells.

Statistical analysis

The control and ACTH treatment groups were both bled for the determination of lymphocyte and PMN function immediately prior to beginning the ACTH treatment. A mean and standard error of the mean (SEM) was determined for each parameter for each group and an analysis of variance procedure was performed to determine the level of significance of any differences between these two groups prior to ACTH administration. Both groups of 5 animals were bled 3 times during the ACTH treatment regimen. A mean and SEM was determined for each parameter for each group from these 3 bleedings. An analysis of variance procedure was performed to determine the level of significance of any differences between the two groups during the ACTH treatment regimen. Because there was significant day to day variability in test results, the data was blocked by day for the analysis of variance. Conservative degrees of freedom were used in the F test.

Results

Pre-treatment values

Prior to the administration of ACTH there was no significant difference between the two groups in any of the parameters studied.

Serum cortisol concentration

Serum cortisol concentration was significantly elevated by the intramuscular injection of 200 IU of ACTH every 12 hours. The two groups of control animals had mean serum cortisol concentrations of 12 and 13 ng/ml. At 6 hours post-ACTH injection, the mean serum cortisol concentration of the ACTH-treated animals was 75.5 ng/ml (Figure 10). However, by 12 hours post-ACTH administration, when blood was obtained for PMN and lymphocyte function evaluation, the serum cortisol level had dropped to 51.4 ng/ml. ACTH was administered every 12 hours for 6 injections; serum cortisol levels remained elevated for the entire 3 day period of the study.

White blood cell counts

The results of the WBC counts are shown in Table 5. The control group had a mean of 8,230 WBCs/mm^3 over the three day period of the ACTH treatment. The average WBC count for the ACTH-treated group was twice as high with 16,430 WBCs/mm^3. This increase in the total number of WBCs was due to an increase in the number of neutrophils.

Table 5. Average white blood cell counts over the three day treatment period in the control and ACTH treated groups (cells/mm^3)

	Control	ACTH-Treated
Total WBCs	8,230 ± 430[a]	16,430 ± 670**
Mononuclear cells	5,400 ± 310	4,870 ± 290
Mature neutrophils	2,530 ± 220	11,250 ± 580**
Band neutrophils	100 ± 20	280 ± 60*
Eosinophils	210 ± 40	30 ± 30**

[a]Mean ± SEM; n = 15.

*P < 0.05; **P < 0.01; When compared to controls.

The control group had a mean of 2,530 neutrophils/mm^3 while the ACTH-treated animals had a mean of 11,250 neutrophils/mm^3. The number of band neutrophils was also significantly higher in the ACTH-treated animals. This difference was very small, however, with the controls having an average of 100 band neutrophils/mm^3 and the ACTH-treated animals having an average of 280 band neutrophils/mm^3. The actual percentage of neutrophils which were band forms was lower in the ACTH-treated group (2.5%) than in the control group (4.0%). The mean number of mononuclear cells was lower in the ACTH-treated group (4,870/mm^3) than in the control group (5,400/mm^3) but this difference was not statistically significant. Treatment with ACTH caused the number of eosinophils in the peripheral circulation to drop to almost zero. The control animals also had a low number of circulating eosinophils (210 eosinophils/mm^3 or 2.5% of the total WBCs).

PMN function tests

PMNs from ACTH-treated animals had significantly enhanced random migration (86.3 mm^2) as compared to PMNs from the control animals (62.6 mm^2) (Table 6). Staphylococcus aureus ingestion, NBT reduction and ADCC by PMNs did not differ between the control and ACTH-treated groups (Table 6). Iodination by PMNs was significantly lower for the ACTH-treated group (30.8 nmole NaI/10^7 PMNs/hr) than for the control group (41.1 nmole NaI/10^7 PMNs/hr) (Table 6).

Table 6. PMN function parameters in the control and ACTH-treated groups during the three day treatment period

	Control	ACTH-Treated
Random migration (mm^2)	62.6 ± 5.2[a]	86.3 ± 5.1**
S. aureus ingestion (%)	28.1 ± 4.1	32.1 ± 4.8
NBT reduction (O.D.)	0.353 ± .028	0.368 ± .031
Iodination (nmole NaI/10^7 PMNs/hr	41.1 ± 2.5	30.8 ± 2.2*
Antibody-dependent cell-mediated cytotoxicity (%)	86.7 ± 1.6	88.3 ± 1.4

[a]Mean ± SEM (n = 15).

*P < 0.05; **P < 0.01; When compared to controls.

Lymphocyte blastogenesis

Lymphocytes from ACTH-treated animals had a significantly lower blastogenic response to the mitogens PHA and Con A when compared either as counts per minute or as stimulation indices (Table 7). The blastogenic response to PWM for lymphocytes from ACTH-treated animals was also lower than for the control animals but this was not statistically significant (Table 7).

Discussion

The treatment regimen of 200 IU of ACTH injected IM every 12 hours maintained elevated blood cortisol concentrations for the entire 3 days of the experiment. The blood cortisol concentration obtained was higher than physiologic concentrations reported for endotoxin-stressed dairy cows (105) or for weaned, transportation-stressed calves (35,56) but were lower than the peak levels found in newborn calves (70,80).

The effect of the ACTH treatment regimen on the total and differential WBC count was similar to that previously reported following the administration of 200 IU of ACTH in cows (56,106). There was a leukocytosis due to a neutrophilia with no left shift, no significant change in the absolute number of mononuclear cells and an eosinopenia. The ACTH-treated animals had significantly more immature "band" neutrophils than the control animals (Table 5); however, the actual number of bands was low and does not qualify as a left shift. In fact, the percentage of neutrophils which were bands was lower in the ACTH-treated group. Carlson and Kaneko (23) have shown that the increased

Table 7. Lymphocyte blastogenesis in response to mitogens in the control and ACTH-treated groups during the three day treatment period

Mitogen	Counts Per Minute		Stimulation Index[a]	
	Controls	ACTH-Treated	Controls	ACTH-Treated
None	607 ± 140[b]	866 ± 268	----	----
Phytohemagglutinin	50,357 ± 6,723	25,419 ± 5,431*	113.9 ± 16.4	59.3 ± 14.2*
Concanavalin A	61,909 ± 3,932	33,362 ± 5,037**	151.2 ± 19.8	77.3 ± 13.6**
Pokeweed Mitogen	32,237 ± 5,396	25,043 ± 6,399	73.0 ± 15.1	55.3 ± 12.4

[a]Stimulation index = (Mitogen stimulated cpm/Background cpm).

[b]Mean ± SEM (n = 15).

*P < 0.05; **P < 0.01; When compared to controls.

number of neutrophils in the circulation following physiologic stress or the administration of prednisolone was due to the input of mature neutrophils from the bone marrow storage pool, a decreased egress of neutrophils into the tissues, and to reduced margination of neutrophils. The decreased margination is probably due to the recognized ability of corticosteroids to reduce the stickiness of neutrophils and thereby to reduce their ability to adhere to vascular endothelium (24,30,88). The administration of 200 IU of ACTH did not significantly decrease the number of circulating mononuclear cells (Table 5) (106). The administration of the potent glucocorticoid 9 α-fluoroprednisolone has been reported to induce a lymphopenia and monocytosis in cattle (124). Dexamethasone administration to cattle has been reported to cause a lymphopenia but no consistent change in the number of monocytes (39). It has been suggested that a failure to detect a lymphopenia following the administration of ACTH to cattle may be due to the counting of lymphocytes and monocytes together as one cell type because a corticosteroid-induced monocytosis may compensate for the lymphopenia (124). We did not try to differentiate lymphocytes and monocytes because it has been shown that bovine lymphocytes and monocytes cannot be reliably differentiated by light microscopic observation of Wright's stained blood smears (58). Thus, the effect of glucocorticoids on monocytes in the peripheral blood of cattle is open to question. Corticosteroids in man have been shown to cause a monocytopenia (111). The eosinopenia induced by ACTH treatment was probably due to the

recognized effect of cortisol to induce migration of eosinophils to lymphoid organs (122).

Elevated serum cortisol concentration had no measurable effect on S. aureus ingestion, NBT reduction or ADCC by bovine PMNs (Table 6). We have previously reported that a single IM injection of dexamethasone (40 mg/adult animal) in cattle will depress all three of these activities by PMNs (113). The S. aureus ingestion assay as performed here evaluates ingestion by PMNs. It does not detect organisms which are simply adhered to the PMN surface, and it is not dependent on the bactericidal activity of the PMN (114). The NBT reduction assay evaluates the oxidative metabolism of the PMN which is a very important aspect of the PMN's bactericidal activity (5). The ability of bovine PMNs to mediate ADCC may play an important role in controlling bovine herpes virus infections (154). A single IM injection of dexamethasone (40 mg/adult animal) therefore inhibited ingestion, oxidative metabolism and ADCC by bovine PMNs whereas elevated plasma cortisol concentration did not have any of these effects. This difference between the effects of cortisol and dexamethasone on bovine PMN function is probably related to the observation that dexamethasone is much more potent than cortisol as an anti-inflammatory agent (43). It may be that cortisol has the same effects on PMN function as dexamethasone but the differences induced by cortisol are so subtle in comparison that the test procedures employed were not sensitive enough to allow detection.

Increased plasma cortisol did cause a significant decrease in iodination by bovine PMNs. The iodination reaction evaluates the activity of the myeloperoxidase-hydrogen peroxide-halide antibacterial system of the PMN. This myeloperoxidase catalyzed reaction is one of the most potent bactericidal mechanisms of the PMN (76). Dexamethasone administration also caused a decrease in iodination by bovine PMNs (113). However, those data were clouded by a dramatic difference in the number of eosinophils in the control and treated groups. We have reported that bovine eosinophils are approximately 4 to 5 times as active as bovine neutrophils at iodination, apparently because they contain 4 to 5 times as much peroxidase (115). A PMN preparation that had markedly fewer eosinophils would be expected to have lower iodination than a PMN preparation containing a relatively large percentage of eosinophils. In the present experiment, the control group of cattle had a low percentage of circulating eosinophils (2.5%; the normal range for cattle is 2% to 20%) (125), so that even though the ACTH-treated cattle had fewer eosinophils than the control cattle, we believe that the difference is not sufficient to account for the lower iodination by PMNs from the ACTH-treated group.

ACTH treatment caused an increase in random migration by PMNs. Dexamethasone has also been associated with increased random migration by bovine PMNs (113). The cortisol-induced increase in random migration and decrease in iodination by PMNs may be related. Agents which inhibit microtubule function will cause an enhancement of random

migration (142) and an inhibition of degranulation (49) in PMNs. The iodination reaction is dependent upon degranulation to release myeloperoxidase to catalyze the reaction. An inhibition of degranulation would result in a lower iodination value. Therefore, the observed increase in random migration and decrease in iodination may both be due to an inhibition of microtubule function. Stevenson and co-workers (140,142) have reported that the increase in random migration by PMNs associated with corticosteroid administration in man is indirect and is due to corticosteroids causing mononuclear phagocytes to release a polymorph migration stimulating factor. This factor may act by inhibiting microtubule formation. It is not known if the increased random migration and decreased iodination is due to a direct effect of cortisol on bovine PMNs or to an effect of cortisol on mononuclear phagocytes causing them to release a factor which inhibits microtubular assembly in PMNs.

Elevated plasma cortisol caused a significant depression of lymphocyte blastogenesis in response to PHA and Con A (Table 7). The blastogenic response to PWM was also lower in the ACTH-treated group, but the difference between the two groups was not statistically significant. Dexamethasone administration to cattle has also been shown to depress lymphocyte blastogenesis to PHA (39,95). The mitogens PHA and Con A are considered to be T lymphocyte specific, while PWM stimulates both B and T lymphocytes in man (103). If this is also the case in the bovine, then the suppression of lymphocyte blastogenesis

in response to PHA and Con A without a significant suppression of lymphocyte blastogenesis in response to PWM indicates that elevated serum cortisol affects bovine T lymphocytes to a greater extent than B lymphocytes. This is in agreement with reports of the effects of glucocorticoids on T and B lymphocyte function in man (45). The mechanism of the glucocorticoid-induced suppression of lymphocyte responsiveness to T cell mitogens is not known. It may be due to a direct effect of the steroid on lymphocytes to inhibit mitosis, to a corticosteroid-induced inhibition of T cell growth factor production by mononuclear cells (which has been reported to be important for blastogenesis) (135), or to a selective depletion of T lymphocytes from the peripheral blood. Glucocorticoids in man have been shown to cause the extravascular redistribution of circulating T lymphocytes into other compartments of the total body lymphocyte pool (45). This selective depletion of T lymphocytes from the peripheral blood may be responsible for the decreased responsiveness of peripheral blood lymphocytes in man to T cell mitogens; however, this is apparently not the case in the bovine. Wilkie et al. (157) have shown that the administration of dexamethasone to cattle causes a lymphopenia, but does not result in an altered proportion of erythrocyte rosette (T cells) or antibody-complement rosette forming lymphocytes (B cells).

In summary, the data presented here demonstrate that elevated serum cortisol levels in cattle will cause depressed lymphocyte blastogenesis and enhanced random migration but decreased iodination

by PMNs. The decreased iodination indicates that one of the most potent bactericidal mechanisms of the PMN is inhibited. This impairment of lymphocyte and neutrophil function may be partially responsible for the increased susceptibility of stressed cattle to the bovine respiratory disease complex.

EFFECT OF ESTRADIOL AND PROGESTERONE ON LYMPHOCYTE AND NEUTROPHIL FUNCTION IN STEERS[1]

Summary

Polymorphonuclear leukocyte (PMN) function and lymphocyte blastogenesis in response to mitogens were evaluated in castrated male cattle following the repeated administration of estradiol or progesterone. PMN function was evaluated using the following five parameters: (1) random migration under agarose, (2) ingestion of ^{125}I-labeled Staphylococcus aureus, (3) nitroblue tetrazolium (NBT) reduction, (4) iodination, and (5) antibody-dependent cell-mediated cytotoxicity (ADCC). The administration of estradiol produced no measurable effect on the total or differential white blood cell count, neutrophil function, lymphocyte blastogenesis, or blood cortisol levels. The administration of progesterone caused a significant enhancement of random migration by neutrophils and a depression of the activity of the myeloperoxidase-H_2O_2-halide antibacterial system (iodination) of the neutrophil. Progesterone administration did not cause a measurable effect on the ability of PMNs to ingest S. aureus, reduce NBT, or mediate ADCC, or upon the lymphocyte blastogenic response to mitogens. Progesterone did not cause a change in blood cortisol concentrations; therefore, the observed effects on PMN function were not due to

---

[1]Submitted for publication to Infection and Immunity by J. A. Roth, M. L. Kaeberle, and W. H. Hsu.

alterations in blood cortisol concentrations. Impairment of the iodination reaction indicates that high dosages of progesterone interfere with an important bactericidal mechanism of the neutrophil.

Introduction

The effect of estrogens and progesterone on the immune system is not well understood. High concentrations of estradiol *in vitro* have been reported to modify the activity of the myeloperoxidase-mediated microbicidal system of the neutrophil (75). High concentrations of progesterone *in vitro* have been reported to labilize neutrophil lysosomal membranes (110), and both estrogens and progesterone *in vitro* have been reported to inhibit the oxidative metabolism of the neutrophil (13), and to inhibit lymphocyte reactivity in the mixed lymphocyte reaction and to non-specific mitogens (31,126). This previous work was performed by treating isolated leukocytes *in vitro* with high concentrations of the steroid hormones. The effect of physiologic or pharmacologic levels of these hormones *in vivo* on neutrophil and lymphocyte function is not clear.

The purpose of the present experimentation was to determine if alterations in neutrophil function or lymphocyte responsiveness to mitogens could be detected following the administration of high dosages of estradiol or progesterone to cattle. Castrated male cattle were used in order to avoid interference from physiologic levels of

estradiol, progesterone, and testosterone found in intact female or male animals.

Materials and Methods

Animals and hormonal treatment

Ten head of apparently healthy 12- to 16-month-old Holstein-Friesian and Brown Swiss steers were used. They were serologically negative for infectious bovine rhinotracheitis and bovine viral diarrhea virus antibody. The estradiol and progesterone experiments were conducted separately using the same animals. The animals were rested for 4 days between experiments. For each experiment the ten animals were housed together; five of the animals were randomly assigned to a control group and five to a hormone treatment group. Estradiol-treated cattle were injected intramuscularly with 30 mg of estradiol cypionate[1] at 0700 hours and 20 mg of estradiol cypionate at 1600 hours daily for four days. Progesterone-treated cattle were injected intramuscularly with 10 cc of a solution containing 750 mg progesterone[2] in ethanol-propylene glycol (50:50) at 0700 hours and 1600 hours daily for four days. The control animals in the progesterone experiment received similar injections containing vehicle only. Blood for evaluation

---

[1]ECP, The Upjohn Co., Kalamazoo, MI.

[2]Sigma Chemical Co., St. Louis, MO.

of lymphocyte and neutrophil function was collected at 0900 hours daily for 3 days beginning 26 hours after the first injection of hormone.

Leukocyte preparation

Mononuclear cells were isolated by a modification of the procedure of Bøyum (14). Twenty ml of blood was collected aseptically into 2 ml of acid-citrate dextrose solution. This anticoagulated blood was diluted with 20 ml of sterile 0.015 M phosphate buffered saline solution, pH 7.2 (PBSS) and layered over a column of 1.077 specific gravity Ficoll-Hypaque[1] in a 25 x 150 mm screw cap, siliconized test tube and centrifuged at 500 x g for 45 minutes. The lymphocytes were removed from the Ficoll-Hypaque plasma interface, washed in Hanks' balanced salt solution[2] and counted.

PMNs were isolated from the peripheral blood as previously described (114). Briefly, the anticoagulated blood was centrifuged, the plasma and buffy coat layer were discarded and the red blood cells were removed by hypotonic lysis with distilled water to yield a cell preparation of generally greater than 90% pure PMNs. The cells were adjusted to a concentration of 5.0×10^7 PMNs/ml in PBSS for use.

---

[1]Histopaque-1077, Sigma Chemical Co., St. Louis, MO.

[2]Grand Island Biological Co., Grand Island, NY.

Lymphocyte blastogenesis

Lymphocytes were cultured in microtiter plates[1] with 2.0 x 10^5 mononuclear cells in 0.15 ml of culture medium per well. The culture medium consisted of Medium 199 with Earle's salts[2] containing 15% heat-inactivated fetal calf serum and 1% Antibiotic-Antimycotic solution.[2] Control and mitogen-stimulated cultures were assayed in triplicate wells. Mitogen stimulated cultures received 25 µl of a dilution of mitogen [phytohemagglutinin-P(PHA),[3] concanavalin A (ConA),[4] or pokeweed mitogen (PWM)[2]] which had been predetermined to give optimal stimulation under identical culture conditions. The microtiter plates were incubated at 37 C in a humidified 5% CO_2 atmosphere. After 48 hours, 0.25 µCi of ^{3}[H] thymidine[5] was added to each well. Sixteen hours later the cultures were harvested onto glass fiber filters with an automated sample harvester,[6] the filter pads were placed in scintillation vials containing 10 ml of toluene-based cocktail,[7] and the counts per minute

---

[1]Costar #3596, Cambridge, MA.

[2]Grand Island Biological Co., Grand Island, NY.

[3]Difco Laboratories, Inc., Detroit, MI.

[4]Miles Laboratories, Inc., Elkhart, IN.

[5]New England Nuclear, Boston, MA.

[6]Flow Laboratories, Inc., Rockville, MD.

[7]Metric-Pak 2a 70, Research Products International, Corp., Elk Grove Village, IL.

(cpm) of radioactivity was determined in a liquid scintillation counter.[1]

PMN function tests

The procedures for evaluating random migration under agarose (114), ingestion of ^{125}I-labeled Staphylococcus aureus (114), nitroblue tetrazolium (NBT) reduction (114), iodination (114), and antibody-dependent cell-mediated cytotoxicity (115) (ADCC) by PMNs have been described in detail elsewhere. All PMN function tests were conducted in duplicate. Briefly, random migration under agarose was evaluated using plastic petri plates which contained a layer of 0.8% agarose with 10% fetal calf serum and 1% Antibiotic-Antimycotic[2] in minimum essential medium.[2] Wells 2.0 mm in diameter were punched in the agar and filled with an aliquot of the PMN suspension. The migration plates were placed in an incubator with a humidified 5% CO_2 atmosphere at 37 C. After 18 hours the plates were removed and the area of PMN migration was determined and expressed in mm^2. Heat-killed ^{125}I-iododeoxyuridine-labeled S. aureus was used to evaluate ingestion (114). Opsonized ^{125}I-S. aureus and PMNs were incubated together at 37 C with a bacteria to PMN ratio of 60:1. After 10 minutes, lysostaphin[3] was added and the

---

[1]Model 2425, Packard Instrument Co. Inc., Downers Grove, IL.

[2]Grand Island Biological Co., Grand Island, NY.

[3]Sigma Chemical Co., St. Louis, MO.

incubation was continued for an additional 30 minutes. The PMNs were then washed twice in PBSS by centrifugation and the amount of PMN-associated radioactivity was determined. The results are expressed as the percent of the ^{125}I-S. aureus which was ingested. The quantitative NBT reduction assay was performed by adding 5.0 x 10^6 granulocytes to a suspension of opsonized zymosan in 1.0 ml of Earle's balanced salt solution containing 0.4 mg of NBT.[1] After a 5.0 minute incubation in a 37 C water bath, the reaction was stopped by the addition of 5.0 ml of cold N-ethylmaleimide.[1] The purple formazan formed by the reduction of NBT was extracted with pyridine and the optical density at 480 nm was determined. The results are expressed as O.D./5.0 x 10^6 PMNs/5 min in 5.0 ml of pyridine. The standard reaction mixture for the determination of stimulated iodination contained 2.5 x 10^6 PMNs, 0.05 μCi[^{125}I],[2] 40 nmole NaI, and 0.5 mg of opsonized zymosan in 0.5 ml of Earle's balanced salt solution. Twenty minutes after the PMNs were added, the reaction was stopped by adding 2.0 ml of cold 10% trichloroacetic acid. The resulting precipitate was washed once in 10% trichloroacetic acid and the amount of radioactivity in the precipitate was determined in a gamma counter. The results are expressed as nmole NaI/10^7 PMNs/hr. The antibody-dependent cell-mediated

---

[1]Sigma Chemical Co., St. Louis, MO.

[2]New England Nuclear, Boston, MA.

cytotoxicity (ADCC) assay was performed utilizing ^{51}Cr-labeled chicken red blood cells (CRBC) as target cells. The reaction mixture contained 2.5×10^5 ^{51}Cr-labeled CRBCs and 2.5×10^6 PMNs (effector to target cell ratio of 10:1) in 0.5 ml of Medium 199 containing 10% bovine anti-CRBC serum. Triton X controls, antibody controls, and PMN controls were included. After a three hour incubation at 37 C in a humidified 5% CO_2 atmosphere, the reaction tubes were centrifuged and an aliquot of supernatant solution was removed for gamma counting. The results were expressed as percent of specific ^{51}Cr release.

Determination of serum cortisol concentration

Blood for the cortisol assay was allowed to clot for 4 to 6 hours at 25 C. The serum was then removed and stored at -20 C until the cortisol assay was performed. Serum cortisol concentration was determined by a double antibody radioimmunoassay using a commercial kit.[1] The samples were assayed in duplicate and all of the samples were processed concurrently. The lower limit of detectability of the assay system was 5.0 ng/ml. For statistical evaluation, all values below this limit were assigned a value of 5.0 ng/ml.

---

[1]Diagnostic Products Corp., Los Angeles, CA.

Hematologic studies

The total white blood cell (WBC) count in heparinized blood obtained by jugular venipuncture was determined by electronic counting.[1] Blood films for differential leukocyte counts were prepared, stained with Wright's stain and 100 cells were counted. Because lymphocytes and monocytes cannot be accurately differentiated in bovine blood by this method (58), they were counted together and termed mononuclear cells.

Statistical analysis

The estradiol and progesterone experiments were each conducted using a group of five control and five hormone-treated animals. All ten animals were bled each day for the three day course of the experiment. The leukocytes from a single day's bleeding were all processed in parallel. A mean and standard error of the mean were determined for each parameter for each group from these three bleedings. An analysis of variance procedure was performed to determine the level of significance of any differences between the two groups within an experiment. Because there was significant day-to-day variability in test results the data was blocked by day for the analysis of variance. Conservative degrees of freedom were used in the F test.

---

[1]Coulter Electronics Co., Inc., Hialeah, FL.

Results

Effect of estradiol administration

The repeated administration of high dosages of estradiol cypionate to steers produced behavioral signs which are typical for estrus in female cattle. Estradiol administration had no significant effect on the total or differential white blood cell count (Table 8), lymphocyte blastogenesis (Table 9), serum cortisol levels (Table 8), or any of the parameters of neutrophil function which were evaluated (Table 10).

Effect of progesterone administration

The repeated administration of progesterone had no effect on total or differential white blood cell numbers (Table 8), lymphocyte blastogenesis (Table 9), or serum cortisol levels (Table 8). Progesterone administration did have an effect on certain neutrophil function parameters. Random migration under agarose by neutrophils was significantly enhanced in the progesterone-treated cattle ($P < .05$). Neutrophils from the control animals had a mean area of migration of 68.2 mm^2, whereas neutrophils from the progesterone-treated cattle had a mean area of migration of 80.0 mm^2 (Table 10). Neutrophils from the progesterone-treated animals had significantly depressed iodination (37.5 nmole NaI/10^7 PMNs/hr) as compared to the control animals (45.1 nmole NaI/10^7 PMNs/hr) ($P < .05$) (Table 10). There was no significant difference in the ability of neutrophils from control and progesterone-treated animals to ingest *S. aureus*, reduce NBT, or mediate ADCC (Table 10).

Table 8. Effect of estradiol or progesterone administration on the total and differential white blood cell count and serum cortisol levels in steers

Parameter	Estradiol experiment		Progesterone experiment	
	Controls (n=10)	Estradiol treated (n=10)	Controls (n=15)	Progesterone treated (n=15)
Total WBC's/mm^3	9,200 ± 600[a]	8,000 ± 600	9,600 ± 700	9,200 ± 300
Mononuclear cells/mm^3	5,800 ± 400	4,900 ± 300	5,500 ± 400	5,300 ± 200
Neutrophils/mm^3	3,200 ± 200	2,900 ± 500	3,900 ± 460	3,700 ± 200
Eosinophils/mm^3	190 ± 50	200 ± 40	200 ± 40	200 ± 40
Serum cortisol (ng/ml)	6.9 ± 1.3	13.1 ± 2.9	14.8 ± 1.2	13.7 ± 1.1

[a]Mean ± SEM: There were no statistically significant differences between the treated and control groups within an experiment.

Table 9. Effect of estradiol or progesterone administration on lymphocyte blastogenesis

Mitogen	Estradiol experiment		Progesterone experiment	
	Control (n=10)	Estradiol treated (n=10)	Control (n=15)	Progesterone treated (n=15)
Phytohemagglutinin				
Δ cpm[a]	32,800 ± 8,000[b]	27,500 ± 7,400	32,500 ± 6,600	36,100 ± 5,900
SI[c]	116.3 ± 34.6	84.6 ± 13.0	88.4 ± 20.1	95.5 ± 15.3
Concanavalin A				
Δ cpm	41,000 ± 8,000	44,000 ± 7,700	37,600 ± 6,200	40,300 ± 5,600
SI	137.2 ± 28.1	138.9 ± 15.0	111.8 ± 21.6	117.0 ± 20.1
Pokeweed mitogen				
Δ cpm	23,200 ± 3,500	25,500 ± 2,500	13,100 ± 3,000	12,200 ± 2,100
SI	79.2 ± 12.5	88.6 ± 9.8	31.4 ± 6.0	33.0 ± 5.6

[a]Δ cpm = difference in counts per minute = (cpm stimulated - cpm unstimulated).

[b]Mean ± SEM: There were no statistically significant differences between the treated and control groups within an experiment.

[c]SI = Stimulation index = (cpm stimulated/cpm unstimulated).

Table 10. Effect of estradiol or progesterone administration on neutrophil function in steers

Neutrophil function parameter	Estradiol experiment		Progesterone experiment	
	Controls (n=15)	Estradiol treated (n=15)	Controls (n=15)	Progesterone treated (n=14)
Random migration (mm^2)	55.5 ± 2.4[a]	53.7 ± 3.1	68.2 ± 3.3	80.0 ± 2.3*
S. aureus ingestion (%)	40.9 ± 3.2	44.8 ± 3.1	58.5 ± 4.9	55.0 ± 4.5
NBT reduction (O.D.)	0.32 ± 0.04	0.32 ± 0.04	0.34 ± 0.03	0.32 ± 0.02
Iodination (nmole NaI/10^7 PMNs/hr)	31.8 ± 2.1	34.0 ± 2.6	45.1 ± 2.6	37.5 ± 1.4*
Antibody-dependent cell-mediated cytotoxicity (%)	90.9 ± 1.6	94.6 ± 1.4	88.5 ± 3.3	85.6 ± 3.7

[a]Mean ± SEM.

*$P < .05$: Level of significance of the difference between the designated value and the corresponding control value.

Discussion

The repeated administration of high dosages of estradiol to steers failed to produce any measurable effect on lymphocyte blastogenesis or neutrophil function. The actual blood level of estradiol in the estradiol-treated animals is not known. The recommended therapeutic dosage of estradiol cypionate in cattle is 3-10 mg per adult animal with a repeat injection in one week if necessary (151). The steers in this study received a total of 50 mg of estradiol cypionate per day for 4 days. The signs of behavioral estrus which were observed in the steers indicate that the hormone was being absorbed into the blood stream. There are reports that concentrations of estradiol which are greater than 10,000 times the physiologic concentration will inhibit the oxidative metabolism (13) and the myeloperoxidase-H_2O_2-halide antibacterial system (75) of the human neutrophil *in vitro*, and that estradiol concentrations of greater than 500 times the physiologic concentration will inhibit the lymphocyte blastogenic response to mitogens *in vitro* (126). These authors therefore concluded that estradiol has the potential to affect neutrophil function and lymphocyte blastogenesis. However, hormone levels this high are probably never attained *in vivo*; therefore, the *in vivo* affect of estradiol is not known. The NBT reduction assay evaluates an aspect of the oxidative metabolism of the neutrophil and the iodination reaction evaluates the activity of the myeloperoxidase-H_2O_2-halide antibacterial system (114). Our results indicate that even

suprapharmacologic levels of estradiol *in vivo* failed to affect neutrophil function or lymphocyte blastogenesis.

Progesterone was used at a very high dosage in this experimentation in order to maximize any potential effects. The recommended therapeutic dosage of progesterone in cattle is 500 to 1000 mg per adult animal repeated in 10 days if necessary (151). The progesterone-treated animals in this experiment received 1500 mg per day for four days. Unlike estradiol, progesterone did have a significant effect on certain aspects of neutrophil function (Table 10). There was a significant enhancement of random migration and inhibition of iodination but no significant effect on the ability of neutrophils to ingest *S. aureus*, reduce NBT, or mediate ADCC. The administration of adrenocorticotropic hormone (ACTH) to steers produced a nearly identical effect on neutrophil function (118). This suggests that the two steroid hormones, progesterone and cortisol, may influence neutrophil function through a similar mechanism. Synthetic progestins have been reported to have glucocorticoid-like activity (55). Stevenson and co-workers (140,142) have reported that the increase in random migration by neutrophils which is associated with corticosteroid administration in man is indirect and is due to corticosteroids causing mononuclear phagocytes to release a polymorph migration stimulating factor. This factor may act by inhibiting microtubule formation within the neutrophil. Agents which inhibit microtubule function will cause an enhancement of random migration (142) and an inhibition of degranulation (49) in neutrophils. The iodination

reaction is dependent upon degranulation to release myeloperoxidase from the lysosomes. An inhibition of degranulation would result in a lower iodination value. Therefore the observed increase in random migration and decrease in iodination may both be due to an inhibition of microtubule formation within the neutrophil. Progesterone and cortisol may have similar mechanisms for inhibiting microtubule formation. The effects on neutrophil function observed after the administration of progesterone were not due to elevated plasma cortisol levels in the progesterone-treated cattle. The radioimmunoassay data in Table 8 indicate that there was no significant difference in serum cortisol levels between the two groups of cattle. In addition, progesterone had no significant effect on the number of neutrophils or eosinophils in the peripheral blood (Table 8). Increased blood cortisol levels will characteristically cause a neutrophilia and eosinopenia in cattle (125).

The administration of progesterone to steers had no significant effect on the lymphocyte blastogenic response to mitogens. In this respect, the effect of progesterone administration differed from the effect of ACTH administration to cattle. Adrenocorticotropic hormone administration caused an inhibition of lymphocyte blastogenesis in response to phytohemagglutinin and concanavalin A (118). High concentrations of progesterone in vitro have been reported to inhibit human lymphocyte blastogenesis in the mixed lymphocyte reaction and in response to mitogens; physiologic levels of progesterone, however, did not have any effect (31,126). It has been postulated that the ability of progesterone

in high concentrations to inhibit lymphocyte reactivity is very important to the maintenance of pregnancy (31). In most species, the trophoblast cells of the placenta produce progesterone, resulting in a high local concentration of progesterone at the maternal-fetal interface. It is postulated that the progesterone concentration in this area is high enough to inhibit lymphocyte reactivity to foreign antigens, but the substantially lower progesterone concentration in the peripheral blood will not suppress lymphocyte reactivity. We do not know the serum concentration of progesterone achieved in our study, or how this relates to the local concentration of progesterone at the maternal-fetal interface, but our results indicate that suprapharmacologic doses of progesterone do not inhibit lymphocyte reactivity to mitogens in steers.

One cannot assume that estradiol and progesterone will have the same effect or lack of effect on neutrophil and lymphocyte function in cows as was observed in steers. In this experimentation, we used steers in order to determine if any effect could be observed when high dosages of hormone were used in animals that did not have significant background levels of estradiol, progesterone, or testosterone. The concentration and binding affinity of estradiol and progesterone receptors in the leukocytes of steers may be quite different from those in cows. In addition, estradiol and progesterone are present concurrently in cow blood in varying concentrations (137). The ratio of the two hormones may be an important determinant of the effect on the immune system. The results reported here indicate that high dosages of progesterone

impair an important bactericidal mechanism of the neutrophil in a manner which is similar to the action of cortisol. The effect of physiologic levels of progesterone on neutrophil function were not determined.

ASSOCIATION OF ELEVATED ESTRADIOL AND PROGESTERONE BLOOD LEVELS WITH ALTERED BOVINE POLYMORPHONUCLEAR LEUKOCYTE FUNCTION[1]

Summary

Polymorphonuclear leukocyte (PMN) function and serum concentrations of estradiol, progesterone, and cortisol were monitored concurrently in normal cows during the estrous cycle. Five parameters were used to evaluate PMN function: 1) random migration under agarose 2) ingestion of ^{125}I-labeled Staphylococcus aureus 3) nitroblue tetrazolium (NBT) reduction 4) iodination and 5) antibody-dependent cell-mediated cytotoxicity (ADCC). Elevated serum estradiol concentrations were associated with enhanced random migration but had no apparent effect on NBT reduction, iodination, or ingestion of S. aureus by bovine PMNs. Elevated serum estradiol was also associated with elevated serum cortisol. Elevated serum progesterone levels were associated with a depression of NBT reduction and iodination by PMNs but with enhanced random migration and ADCC. These results indicate that physiologic changes in steroid hormone levels during the normal estrous cycle of the cow are associated with alterations in PMN function.

---

[1]Submitted for publication to the American Journal of Veterinary Research by J. A. Roth, M. L. Kaeberle, and L. H. Appell.

Introduction

Observations reported several years ago indicated that the bovine uterus is more susceptible to infection during the luteal phase of the estrous cycle than during estrus (10,121). In 1953, Rowson et al. (121) reported that during estrus (when estradiol is the predominant hormone) the bovine uterus was remarkably resistant to infection introduced either as infected semen or as a culture of Corynebacterium pyogenes. However, during the luteal phase of the estrous cycle (when progesterone predominates) the bovine uterus was quite susceptible to infection and pyometritis frequently resulted. Furthermore, in experiments on ovariectomized cows, they demonstrated that exogenous progesterone promoted conditions suitable to the growth of certain bacteria, while exogenous estrogen rendered the bovine uterus relatively resistant to infection. The authors speculated that the action of estrogen in promoting the development of the terminal blood supply to the uterine mucosa and stimulating uterine contraction may be responsible for the enhanced uterine resistance to bacterial infection. They also speculated that estrogen and progesterone may affect, by unknown mechanisms, general body resistance to bacterial infection.

There have since been reports which indicate that estrogen and progesterone may have general effects on resistance through their actions on the immune system. Estrogen and progesterone have been reported to have various effects on lymphocyte function (31,44,81,

126,132,147) and on polymorphonuclear leukocyte (PMN) function (13,67, 75,110) in *in vitro* test systems. The administration of pharmacologic dosages of estrogens or progesterone has been reported to alter the phagocytic activity of the reticuloendothelial system in mice (98,99,100). In cattle, the repeated administration of high dosages of progesterone to steers caused an enhancement of random migration and an inhibition of iodination by neutrophils. There was no significant effect on lymphocyte blastogenesis. The repeated administration of high dosages of estradiol to steers failed to produce any significant effect on either neutrophil or lymphocyte function (117). All of the above research involved either the treatment of leukocytes *in vitro* with high concentrations of hormone or the *in vivo* administration of pharmacologic dosages of hormone. The effect of physiologic levels of estradiol and progesterone on neutrophil and lymphocyte function is not clear.

Since metritis is an economically important disease of cattle and because PMNs play an important role in bacterial defense in the uterus (73), the present experimentation was undertaken to determine if physiologic levels of estradiol and progesterone present during the estrous cycle produced a detectable alteration of PMN function in cows.

Materials and Methods

Animals and experimental design

Six apparently healthy adult (bovine) cows of various breeds were used in this experimentation. The animals were determined to have normal estrous cycles by periodic palpation of the ovaries per rectum and were given no pharmacologic agents immediately prior to or during the course of the experimentation. Blood was obtained from each of five animals three times per week for four weeks for experimental evaluation of PMN function and determination of hormone levels. After resting the animals for two weeks and substituting one cow, the bleeding schedule and experimentation were repeated. A four-week bleeding schedule was used because a normally cycling cow should experience estrus at least once during that period. The cows were examined by palpation per rectum to determine ovarian activity each day that they were bled. In order not to bias results, personnel performing the ovarian palpations, PMN function tests, and hormone assays did not share their data until after the completion of the experimentation.

PMN isolation

PMNs were isolated as previously described (114). Briefly, peripheral blood was collected into acid-citrate-dextrose solution, centrifuged, and the plasma and buffy coat layer were discarded. The packed red blood cell layer was treated with two volumes of cold,

phosphate-buffered distilled water for 50 seconds; isotonicity was restored by adding one volume of cold, phosphate-buffered 2.7% NaCl. The remaining cells were washed with 0.015 M phosphate-buffered saline solution, pH 7.2 (PBSS) by centrifugation and were suspended in PBSS to a concentration of 5.0×10^7 PMNs (neutrophils plus eosinophils) per ml. The cells were held at room temperature and were used in all five PMN function tests within three hours of the time they were standardized. The cell isolation procedure generally yielded a PMN preparation of greater than 90% purity.

Migration under agarose

The ability of the PMNs to migrate under agarose was evaluated as previously described (114). The test was performed in 60 x 15 mm tissue culture petri plates containing 5.0 ml of agar gel consisting of 0.8% agarose,[1] 10% fetal calf serum, and 1% Antibiotic-Antimycotic in minimum essential medium. Wells 2.0 mm in diameter were punched in the agarose and were filled with the PMN suspension. The plates were incubated at 37 C in a 5% CO_2 enriched atmosphere overnight. The cells were then fixed with formaldehyde and stained with modified Wrights stain. The area of migration was determined by calculating the total area of migration and subtracting the area of the inner well. The test was conducted in duplicate and the results are expressed as the square mm of migration.

---

[1] #57035 Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY.

Ingestion of Staphylococcus aureus

The ingestion of ^{125}I-labeled S. aureus by PMNs was evaluated as previously described (114). The S. aureus were labeled by growth in the presence of [^{125}I] iododeoxyuridine[1] and 5-fluorodeoxyuridine.[2] The ^{125}I-labeled S. aureus was washed by centrifugation in PBSS, and killed by heating to 60 C for one hour, washed again in PBSS and resuspended to a concentration such that a 1:10 dilution in PBSS had an optical density of 0.4 at λ = 600 nm. The ingestion assay mixture contained 100 µl of ^{125}I-labeled S. aureus, 50 µl of PMN suspension (2.5 x 10^6 PMNs, bacteria to PMN ratio $\approx$ 60:1), 50 µl of a 1:10 dilution of bovine anti-S. aureus serum, and 0.3 ml of Earle's balanced salt solution (EBSS).[3] The reactants were combined and incubated at 37 C. After ten minutes, 0.5 IU of lysostaphin[2] was added, and the incubation was continued for an additional 30 minutes to destroy the uningested S. aureus. The PMNs were then washed twice in PBSS by centrifugation and the amount of radioactivity associated with the PMNs was determined in a gamma counter. The test was conducted in duplicate, and the results are expressed as the percent of the total S. aureus ingested.

---

[1]New England Nuclear, Boston, MA.

[2]Sigma Chemical Co., St. Louis, MO.

[3]GIBCO Laboratories, Grand Island, NY.

Nitroblue tetrazolium reduction

The quantitative nitroblue tetrazolium (NBT) reduction test was performed as previously described (114). The reaction mixture contained 0.4 mg of NBT,[1] 1.0 mg of preopsonized zymosan, and 5.0 x 10^6 PMNs in 1.0 ml of EBSS. The reactants were combined and incubated at 37 C. After 5.0 minutes, 5.0 ml of cold 1 mM N-Ethylmaleimide[1] in PBS was added to stop the reaction. The purple formazan formed by the reduction of NBT was pelleted by centrifugation, resuspended in 5.0 ml of pyridine by sonication and placed in a boiling water bath for ten minutes. The pyridine was then clarified by centrifugation and the optical density at λ = 580 nm was determined. The test was conducted in triplicate, and the results are reported as the optical density per 5.0 x 10^6 PMNs per 5.0 minutes in 5.0 ml of pyridine.

Iodination

The iodination procedure was performed as previously described (114). The standard reaction mixture contained 40 nmole NaI, 0.05 µCi [^{125}I],[2] 2.5 x 10^6 PMNs, and 0.5 mg of preopsonized zymosan in a total volume of 0.5 ml EBSS. The reactants were combined in 12 x 75 mm

---

[1]Sigma Chemical Co., St. Louis, MO.

[2]Carrier free in 0.1 M NaOH, New England Nuclear, Boston, MA.

polystyrene snap cap test tubes[1] and incubated for twenty minutes at 37 C with end-over-end tumbling. The reaction was terminated by the addition of 2.0 ml of cold 10% trichloroacetic acid. The resulting precipitate was washed one additional time in 10% trichloroacetic acid, and the amount of radioactivity in the precipitate was determined in a gamma counter. The test was conducted in duplicate, and the results are expressed as nmole NaI/10^7 PMNs/hr.

Antibody-dependent cell-mediated cytotoxicity (ADCC)

The ADCC reaction was conducted as previously described (115). The standard reaction mixture contained 2.5 x 10^6 PMNs, 2.5 x 10^5 ^{51}Cr-labeled chicken red blood cells and 50 µl of bovine anti-chicken red blood cell antiserum in a total volume 0.5 ml of Medium 199.[2] The test was conducted in duplicate and Triton-X controls, antibody controls, and granulocyte controls were also included. The reactants were combined and incubated at 37 C for 3.0 hours, the tubes were centrifuged and an aliquot of supernatant solution was removed for gamma counting. The results are expressed as the percent of ^{51}Cr specifically released.

---

[1] #2058 Falcon, Oxnard, CA.

[2] GIBCO Laboratories, Grand Island, NY.

Radioimmunoassay of steroid hormones

Blood for hormone assays was collected at the time that the animals were bled to obtain PMNs (between 8:00 and 9:30 A.M.). The blood was allowed to clot for 4 to 6 hours; the serum was then removed and stored at -20 C. For the estradiol and progesterone assays, the serum was extracted with hexane. Radioimmunoassays were conducted using commercial reagents;[1] the bound and free steroid were separated by absorption with dextran coated charcoal. Serum cortisol was also assayed by radioimmunoassay using commercial reagents.[2]

Statistical analysis

PMNs from each bleeding were assigned to one of four hormone level groups on the basis of the serum concentration of estradiol and progesterone. Estradiol concentrations of > 19 pg/ml were designated as high estradiol levels. Progesterone concentrations of > 1.7 ng/ml were designated as high progesterone levels. The PMNs, therefore, were classified into one of four hormone level groups (Figure 11): the high estradiol-high progesterone group, high estradiol-low progesterone group, low estradiol-high progesterone group, or the low estradiol-low progesterone group. There were only three blood samples which were classified in the high estradiol-high progesterone group (Figure 11).

---

[1]Diagnostics Biochem International, Inc., Tampa, FL.

[2]Clinical Assays, Inc., Cambridge, MA.

	High progesterone > 1.7 ng/ml	Low progesterone ≤ 1.7 ng/ml
High estradiol > 19 pg/ml	3	18
Low estradiol ≤ 19 pg/ml	15	78

Figure 11. Classification of the PMNs into hormone-level group on the basis of the serum concentration of estradiol and progesterone. The number of PMN preparations classified into each hormone level group is indicated

This was an insufficient number for any further analysis of this group. A mean and standard error of the mean were obtained for each PMN function parameter and hormone level in each of the three remaining hormone-level groups.

An analysis of variance procedure was performed to determine the probability that the various parameters differed between the high estradiol-low progesterone group and the low estradiol-low progesterone group and between the low estradiol-high progesterone group and the low estradiol-low progesterone group. Because the experiment was designed so that 5 animals were bled concurrently and 2 groups of 5 animals were used, the data were blocked by a group of 5 animals for the analysis of variance procedure.

Results

Estradiol, progesterone, and cortisol serum concentrations during the estrous cycle

The mean serum concentrations of progesterone, estradiol, and cortisol during the estrous cycle are shown in Figures 12-14. The stage of the estrous cycle was determined by palpation of the ovaries per rectum. Each of the cows underwent one estrus period during each 4 week bleeding period. There was a relatively large variability in hormone levels between cows during their estrous cycles. In general, progesterone concentration was low during estrus and higher between estrus periods

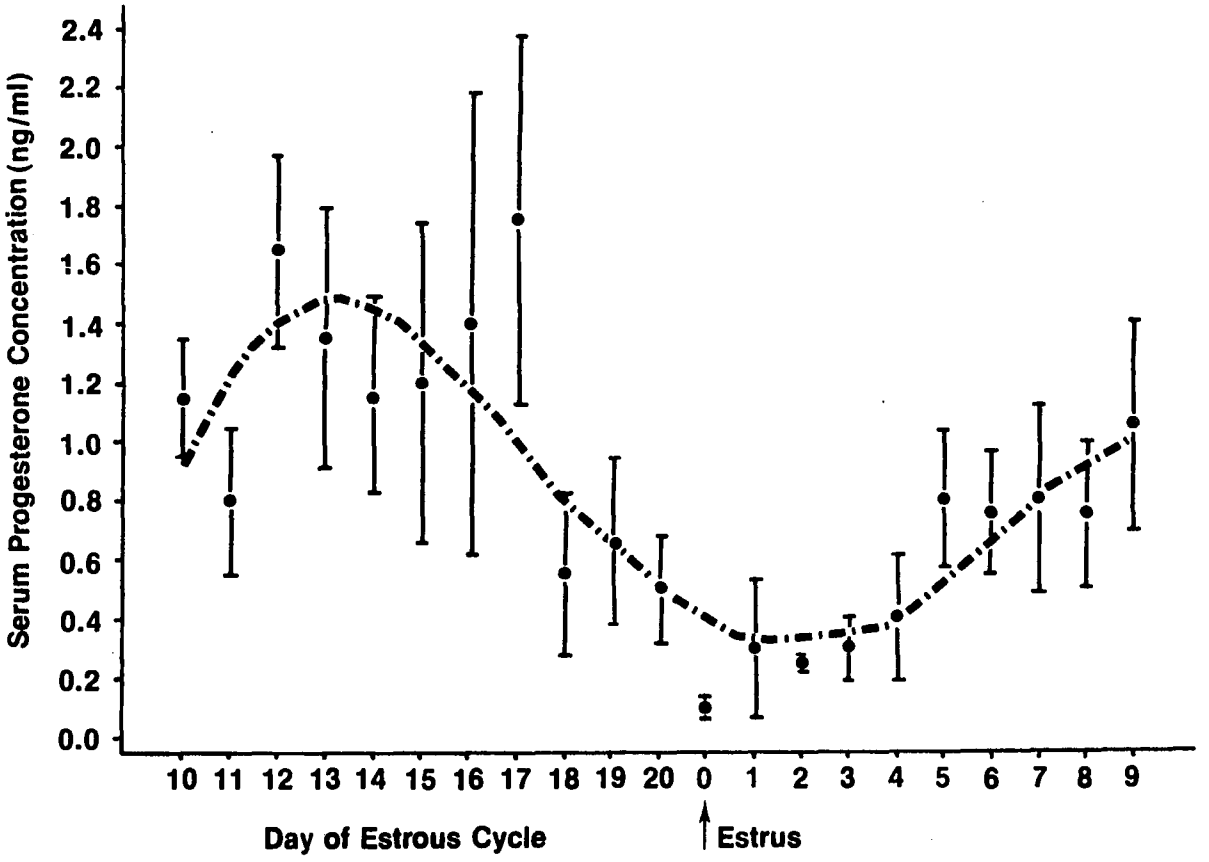


Figure 12. Mean serum progesterone concentration during the estrous cycle. Each point represents the mean ± SEM for 2 to 9 animals

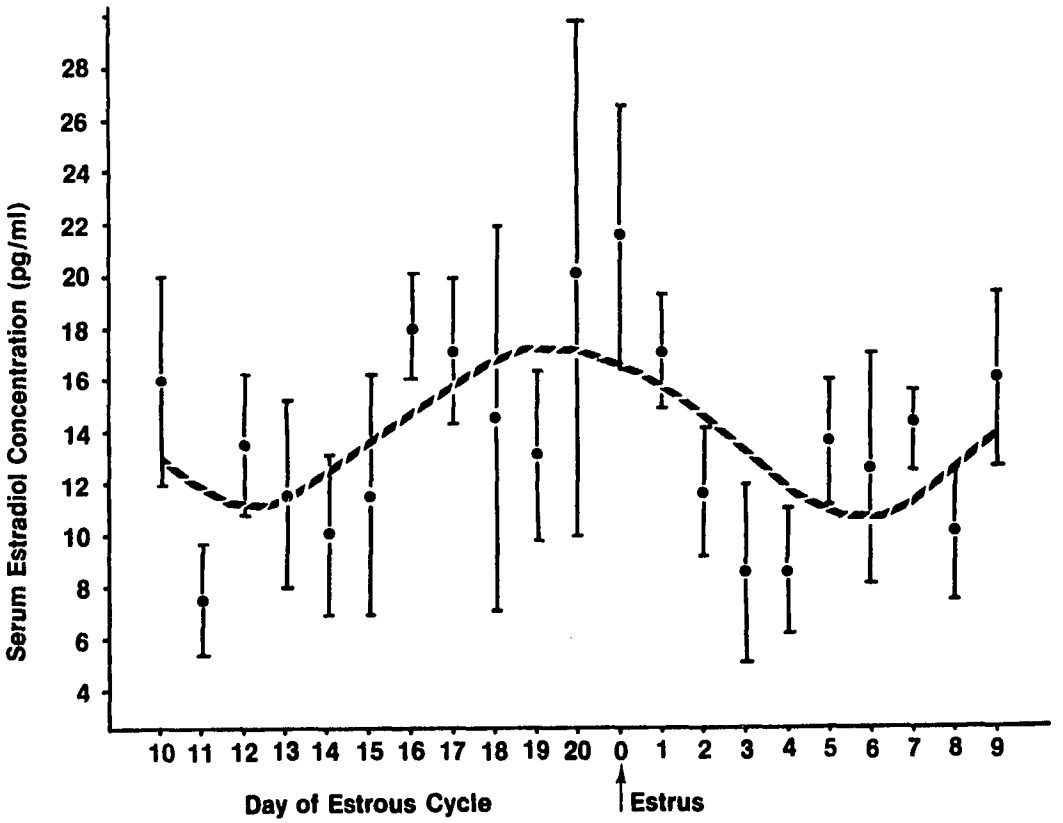


Figure 13. Mean serum estradiol concentration during the estrous cycle. Each point represents the mean ± SEM for 2 to 9 animals

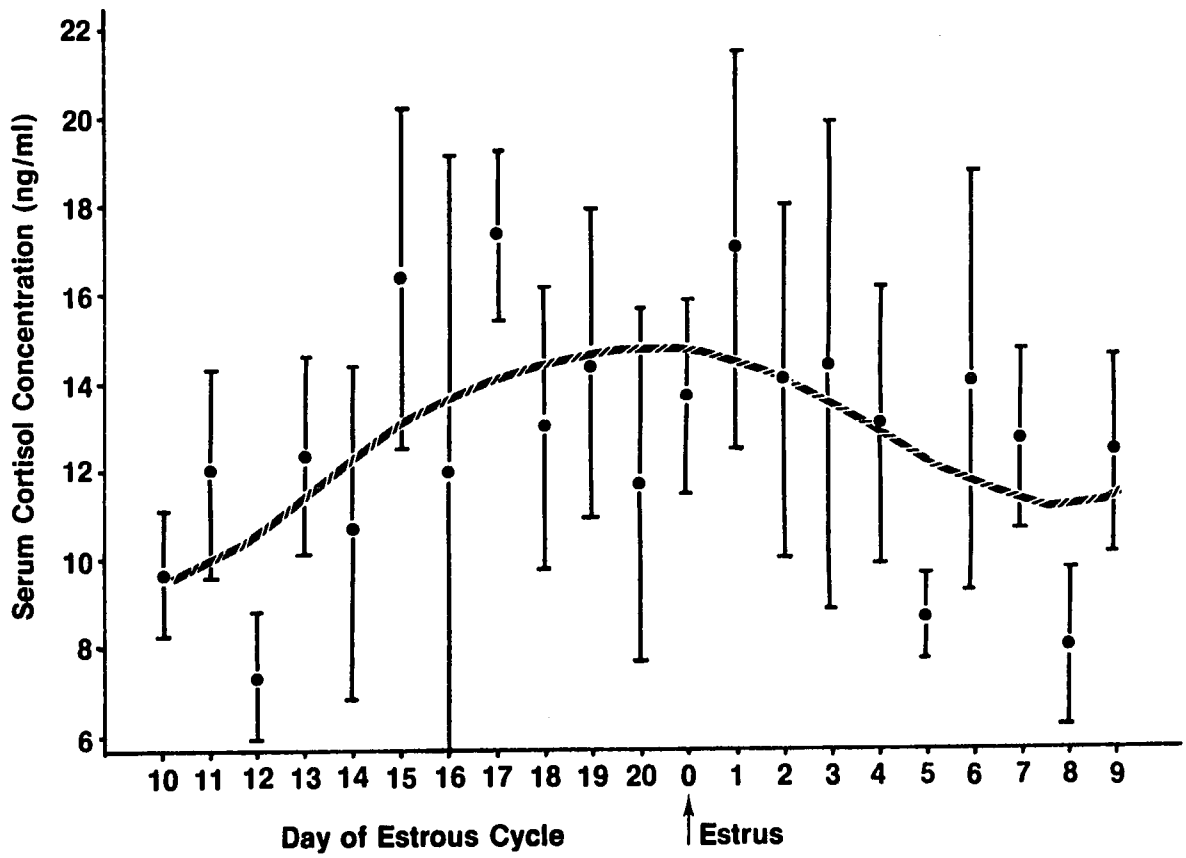


Figure 14. Mean serum cortisol concentration during the estrous cycle. Each point represents the mean ± SEM for 2 to 9 animals

(Figure 12). Estradiol and cortisol concentrations were generally high during estrus and lower between estrus periods (Figures 13,14). The distribution of PMN preparations in each hormone-level group is shown in Figure 11.

Effect of elevated serum progesterone concentration

The effect of elevated serum progesterone was determined by comparing PMN function in the low estradiol-high progesterone group to PMN function in the low estradiol-low progesterone group. PMNs obtained from blood containing a relatively high level of progesterone displayed significantly enhanced random migration and ADCC but depressed NBT reduction and iodination (Table 11). There was no significant effect on S. aureus ingestion. High progesterone levels in the serum were not associated with any change in estradiol or cortisol concentrations in the blood (Table 11).

Effect of elevated serum estradiol concentration

The effect of elevated serum estradiol was determined by comparing PMN function in the high estradiol-low progesterone group to PMN function in the low estradiol-low progesterone group. PMNs obtained from blood with a relatively high estradiol concentration had significantly enhanced random migration when compared to PMNs from blood with lower concentrations of estradiol (Table 11). There was no significant difference in S. aureus ingestion, NBT reduction, iodination, or ADCC by PMNs from the two different groups. A significantly higher cortisol concentration was present in the serum samples that had a high estradiol level (Table 11).

Table 11. PMN function and serum hormone concentration in cattle grouped by serum estradiol and progesterone level

Parameter	Low Estradiol Low Progesterone Group	High Estradiol Low Progesterone Group	Low Estradiol High Progesterone Group
Random migration (mm^2)	55.7 ± 2.5 (64)[a]	72.9 ± 4.4 (15)**	71.6 ± 7.2 (9)*
S. aureus ingestion (%)	48.4 ± 1.7 (78)	45.4 ± 3.7 (18)	42.8 ± 4.1 (15)
NBT reduction (O.D.)	0.32 ± 0.01 (78)	0.34 ± 0.02 (18)	0.24 ± 0.01 (15)**
Iodination *nmole NaI/10^7 PMNs/hr	36.2 ± 2.0 (78)	40.9 ± 3.8 (18)	24.6 ± 1.9 (15)**
Antibody-dependent cell-mediated cytotoxicity (%)	55.7 ± 3.1 (49)	45.1 ± 5.2 (11)	73.0 ± 2.9 (12)**
Serum progesterone (ng/ml)	0.58 ± 0.06 (78)	0.52 ± 0.10 (18)	2.4 ± 0.2 (15)**
Serum estradiol (pg/ml)	10.3 ± 0.7 (78)	27.1 ± 1.7 (18)**	11.0 ± 1.0 (15)
Serum cortisol (ng/ml)	11.5 ± 0.7 (78)	17.4 ± 2.1 (18)**	10.4 ± 1.2 (15)

[a]Mean ± SEM (n).

*$P < 0.05$.

**$P < 0.01$; Level of significance of the difference between the designated group and the low estradiol-low progesterone group.

Discussion

Several previous reports have indicated that the uterus is relatively susceptible to infection when blood progesterone levels are high and is relatively resistant to infection when blood estrogen levels are high (10,59,73,121). The mechanism of this altered susceptibility to infection is not known, but several possibilities have been suggested, including: 1) increased uterine blood flow induced by estrogens (121), 2) enhancement of uterine contractility by estrogens (121), 3) depression of PMN function by progesterone (59,73), and 4) enhancement of PMN function by estrogens (59,73). Our results indicate that a relatively high physiologic level of progesterone occurring during a normal estrous cycle does impair certain PMN functions which are important for normal bactericidal activity. This inhibition of PMN function by progesterone may be responsible for the increased susceptibility of the uterus to bacterial infection when blood progesterone levels are high. High physiologic levels of estradiol did not cause alterations of those PMN functions which are directly related to bactericidal activity. The increased resistance of the uterus to bacterial infection when estradiol levels are high may be related to the fact that progesterone blood levels are relatively low at this time. Therefore, there is a lack of inhibition of PMN function by progesterone. In addition, the high estradiol levels will result in enhanced uterine blood flow and contractility.

Elevated serum levels of progesterone or estradiol were associated with enhancement of random migration by bovine PMNs. Similar results were obtained in steers that were administered dexamethasone (113) or ACTH (118). Blood which contained a relatively high level of estradiol also contained a significantly higher concentration of cortisol (Table 11). Since the administration of estradiol to steers does not result in an increase in random migration by PMNs (117), but ACTH administration to steers does cause an increase in random migration (118) it is tempting to speculate that the increase in PMN random migration observed in the high estradiol group was due to a concurrent increase in serum cortisol concentration.

The administration of prednisolone to human patients has also been reported to enhance in vitro PMN random migration (140). From that experimentation, the author reported that the enhancement of migration was dependent upon the presence of mononuclear leukocytes in the PMN preparation. He suggested that the steroid reacts in vivo with mononuclear leukocytes to produce a polymorph migration stimulator. It isn't known if a similar mechanism is responsible for the results observed here, or if the elevated steroid levels had a direct effect on PMN migration.

The association between elevated serum cortisol and estradiol suggests that they may be causally related. There is evidence that glucocorticoids will activate the biosynthetic pathway for estrogen in the placenta at term and that the resulting increase in estrogen

concentration may be important in the induction of parturition (138). Whether a similar phenomenon occurs in steroid synthesizing tissues of the non-pregnant female, is apparently not known. The relationship of serum glucocorticoid concentrations to the bovine estrous cycle is not clear. Serum glucocorticoid concentration in cows has previously been reported to not change during the estrous cycle (127), to be elevated at the time of estrus (129), or to be depressed at the time of estrus (1).

The data in Table 11 indicate that elevated serum progesterone levels were associated with depressed NBT reduction by PMNs. NBT is a soluble yellow compound that is directly reduced by superoxide anion to an insoluble purple formazan (158). During the burst of oxidative metabolism which normally occurs following phagocytosis, the PMN produces superoxide anion, the hydroxyl radical, hydrogen peroxide (H_2O_2), and perhaps singlet oxygen (5). This burst of oxidative metabolism is important to the PMNs bactericidal ability (5). NBT reduction by PMNs is therefore a close correlate of the burst of oxidative metabolism and evaluates an important component of the PMNs bactericidal activity. Bodel et al. (13) previously reported that incubation of human leukocytes with either estradiol or progesterone in vitro inhibited the PMN's oxidative metabolism. However, they used concentrations of estradiol that were more than 10^4 times the level usually found in plasma. Our results indicate that in the cow, physiologic concentrations of progesterone in vivo depress the oxidative metabolism of PMNs.

High serum progesterone concentration was associated with depressed iodination (Table 11). The iodination reaction evaluates the activity of the myeloperoxidase-H_2O_2-halide antibacterial system in the PMN. It is dependent upon the generation of H_2O_2 during the burst of oxidative metabolism and upon the release of myeloperoxidase from the primary granules. The myeloperoxidase-H_2O_2-halide reaction is one of the most potent bactericidal mechanisms of the PMN (76). A hormonally induced change in the iodination activity of PMNs could be due to a change in the PMNs oxidative metabolism, a change in the degranulation rate, or a direct effect of the hormone on the myeloperoxidase catalyzed reaction. The results of the NBT reduction assay shown in Table 11 indicate that PMN oxidative metabolism was also depressed in the high progesterone group. This suggests that the decrease in iodination may have been due to a decreased availability of H_2O_2 for the myeloperoxidase catalyzed reaction. We have previously demonstrated that progesterone administration to steers results in an inhibition of iodination without a concurrent inhibition of NBT reduction. Thus, progesterone inhibits the iodination reaction by a mechanism which is independent of the oxidative metabolism. The inhibition of iodination may be due to an inhibition of degranulation which would result in a reduced availability of myeloperoxidase to catalyze the iodination reaction. Agents which inhibit microtubule function will cause an inhibition of degranulation (49) and an enhancement of random migration (142). Therefore, one potential explanation for the concurrent decrease in iodination and

enhancement of random migration by PMNs is that progesterone directly or indirectly inhibits microtuble function in the PMN. We have previously shown that variations in the percentage of eosinophils present can markedly influence the iodination activity of a PMN preparation (115). The altered iodination rate was apparently not due to changes in the ratio of eosinophils to neutrophils in the PMN preparations, because there was no significant association between hormone level and the percentage of eosinophils in the PMN preparations.

Antibody-dependent cell-mediated cytotoxicity by PMNs was enhanced when serum progesterone levels were high (Table 11). Elevated serum estradiol was associated with depressed ADCC (Table 11), although this was not statistically significant ($0.05 < P < 0.10$). It seems paradoxical that elevated serum progesterone levels were associated with depressed oxidative matabolism, and iodination but enhanced ADCC by PMNs. The method by which PMNs mediate ADCC is not known; more than one mechanism may be involved. There is evidence that the burst of oxidative metabolism is important (29) and that the superoxide anion and H_2O_2 which are released extracellularly can be cytotoxic (97,133). Grewal et al. (52) have reported evidence indicating that a second mechanism is involved. They used bovine PMNs and herpesvirus-infected target cells and demonstrated that the PMN plasma membrane makes direct contact with the target cell membrane. They suggested that this results in the production of transmembrane channels and target cell destruction. Our evidence that increased serum progesterone is associated with depressed oxidative

metabolism but enhanced ADCC by PMNs indicates that the burst of oxidative metabolism by the PMN is not important in mediating ADCC under the experimental conditions that we used. A possible mechanism for progesterone enhancement of ADCC, which would be compatible with the observed progesterone associated depression of oxidative metabolism and iodination by PMNs, is that progesterone has a direct effect on the PMN membrane which enables it to more efficiently contact and damage the target cell membrane. Other workers have previously suggested that progesterone may exert some of its influence on lymphocytes through a direct action on the lymphocyte membrane (31).

The concentration of progesterone observed during the estrous cycle was lower than has been previously reported (1,136,137). This is probably due to the fact that we assayed progesterone levels in the serum after allowing the blood to clot at room temperature for 4 to 6 hours. Vahdat et al. (150) have demonstrated that allowing whole bovine blood to stand before separating the serum and the cells will result in a decrease in the progesterone concentration. This decrease in progesterone concentration was apparently proportional to the amount of progesterone originally present because the relative level of progesterone varied as expected during the estrous cycle (Figure 12) (136,137). Therefore, it is valid to group the blood samples which had relatively higher progesterone concentrations into a high progesterone group. However, the level of progesterone which is designated as being high in this assay and which was associated with altered PMN function should not be

extrapolated to levels of progesterone reported by other workers.

As indicated above, the serum progesterone level correlated well with the stage of the estrous cycle as determined by palpation of the ovaries per rectum. However, the stage of the estrous cycle could not be correlated with a significant change in PMN function. Failure of correlation could be related to the physiologic level of progesterone necessary to alter PMN function. Apparently, an exceptionally high physiologic level of progesterone was required to alter PMN function sufficiently so that it could be detected within the limits of sensitivity of the assay procedures which we used. The individual cows had differing maximum serum concentrations of hormone, and the peak concentrations occurred at somewhat different times in the cycle for different cows. Therefore, progesterone levels were not maintained in each cow long enough so that altered neutrophil function could be associated with particular days of the estrous cycle.

The results reported here indicate that elevated levels of progesterone or estradiol during the normal bovine estrous cycle are associated with a more profound effect on PMN function than was the repeated administration of high dosages of estradiol or progesterone to steers (117). This is surprising because the steers would presumably have a higher concentration of the hormone in the blood. The reason for this observed difference is not known, but there are some factors which should be considered. The intracellular concentration and affinity of receptors for estradiol and progesterone in the steers

are not known. One might expect that steers would have fewer receptors for these hormones than cows, or receptors of lower affinity. A difference in receptor characteristics could explain the decreased sensitivity of PMNs from steers to the actions of estradiol and progesterone. It may be that the ratio between estradiol and progesterone is important for the production of maximal effects on PMNs. The cows had physiologic levels of both hormones present. The steers would have had one hormone present in high concentration and the other hormone only at very low levels if at all. In the cow, the blood concentration of follicle stimulating hormone, leuteinizing hormone and prostaglandins (71) also change during the estrous cycle. The cyclic changes in these hormones may be responsible for some of the observed changes in PMN function. Another factor which must be considered is that the data obtained from cycling cows is observational data on untreated cows. Therefore, the experiment could not be designed so that there were equal numbers of animals in each of the hormone level groups on each day that the PMN assays were performed. This could allow some of the daily variability which was inherent in the PMN assay procedures to bias the results.

We can state with a high degree of confidence that progesterone inhibits iodination and enhances random migration by PMNs, because these effects were observed both in the normally cycling cows and in steers which received progesterone injections (117). The effects which were observed only in cycling cows (enhancement of random migration associated

with elevated estradiol, and enhancement of ADCC and inhibition of NBT reduction by PMNs associated with elevated progesterone) may be due to factors which are present in cycling cows but absent in steers as discussed previously. Our results do indicate that physiologic changes in steroid hormone levels that occur during the normal estrous cycle of the cow are associated with alterations in PMN function. The altered PMN function may partially explain the increased susceptibility of the uterus to bacterial infection under the influence of progesterone.

GENERAL SUMMARY

The experimentation reported in this dissertation covers three general areas: 1) characterization and comparison of neutrophil and eosinophil function in cattle, 2) the effects of glucocorticoids on bovine lymphocyte and neutrophil function, and 3) the effects of estradiol and progesterone on bovine lymphocyte and neutrophil function.

A method was developed for separating eosinophils and neutrophils in a purified form from the peripheral blood of normal cattle. The two cell types had differing activities in the various functional assays. Neutrophils were significantly more active than eosinophils in the ingestion of S. aureus and in antibody-dependent cell-mediated cytotoxicity. Eosinophils were much more active than neutrophils in the resting and stimulated iodination test and they contained higher levels of peroxidase. There was no difference between the two cell types in their ability to reduce nitroblue tetrazolium or migrate under agarose. These results were discussed in relation to results reported for human eosinophils and neutrophils. The relevance of these results to the in vivo function of neutrophils and eosinophils was also discussed.

The administration of the synthetic glucocorticoid, dexamethasone, to cattle had a marked effect on all aspects of neutrophil function which were evaluated. It caused enhanced random migration under agarose but impaired ingestion of S. aureus, nitroblue tetrazolium reduction, chemiluminescence, iodination, and antibody-dependent cell-mediated cytotoxicity by neutrophils. The administration of adrenocorticotropic

hormone (ACTH) to cattle produced increased serum cortisol levels which were similar to physiologic levels found in stressed cattle. The effect of increased serum cortisol on neutrophil function was not as dramatic as the effect of dexamethasone. Increased serum cortisol caused enhanced random migration and impaired iodination, as did dexamethasone, but increased cortisol had no effect on S. aureus ingestion, NBT reduction, or antibody-dependent cell-mediated cytotoxicity. Increased serum cortisol also caused an impairment of lymphocyte blastogenesis in response to non-specific mitogens. These results were discussed in relation to other clinical and experimental evidence for immunosuppression by glucocorticoids in the bovine.

The effect of the repeated administration of high dosages of estradiol and progesterone to steers was evaluated to determine if pharmacologic levels of these hormones had the potential to influence bovine lymphocyte or neutrophil function. Estradiol at this high dosage produced no significant effect on lymphocyte blastogenesis or neutrophil function. Progesterone had no significant effect on lymphocyte blastogenesis but it did produce significant alterations in certain aspects of neutrophil function. Neutrophils from the progesterone treated steers displayed enhanced random migration and impaired iodination. There was no significant effect on their ability to ingest S. aureus, reduce NBT, or mediate antibody-dependent cell-mediated cytotoxicity. The effects of progesterone administration on neutrophil function were very similar to the effects of ACTH

administration; however, serum cortisol levels were not elevated in the progesterone-treated steers. This experimentation demonstrated that pharmacologic levels of estradiol had no measurable effect on neutrophil function in steers but pharmacologic levels of progesterone were capable of altering bovine neutrophil function.

The effect of physiologic concentrations of estradiol and progesterone on bovine neutrophil function was investigated by evaluating neutrophil function during the estrous cycle of cows; function was then correlated with the serum level of estradiol and progesterone. Neutrophils obtained from blood samples with elevated estradiol displayed enhanced random migration; there was no effect on any of the other neutrophil function parameters evaluated. This enhancement of random migration may have been due to a concurrent elevation of serum cortisol concentration. Neutrophils obtained from blood samples with elevated serum progesterone levels displayed enhanced random migration under agarose and ability to mediate ADCC, impairment of NBT reduction and iodination, and normal S. aureus ingestion. This differed from the effects of progesterone administration to steers in that progesterone in steers did not alter NBT reduction or ADCC by neutrophils. Potential reasons for the differing effects of progesterone on neutrophils from steers and cows were discussed.

The results of the experimentation reported here indicated that specific parameters of bovine lymphocyte and neutrophil function are impaired by elevated blood levels of dexamethasone, cortisol, and

progesterone. The observed alterations in neutrophil and lymphocyte function are undoubtedly part of the reason for the increased susceptibility to infectious disease that has been associated with these hormones. The challenge of the future will be to further characterize the immunosuppression induced by these hormones and to attempt to find pharmacologic agents which are capable of restoring the host defense mechanisms to normal in the presence of these hormones.

LITERATURE CITED

1. Abilay, T. A., H. D. Johnson, and M. Madan. 1974. Influence of environmental heat on peripheral plasma progesterone and cortisol during the bovine estrous cycle. J. Dairy Sci. 58:1836-1840.

2. Arave, C. W., J. L. Walters, and R. C. Lamb. 1978. Effect of exercise on glucocorticoids and other cellular components of blood. J. Dairy Sci. 61:1567-1572.

3. Babior, B. M. 1978. Oxygen-dependent microbial killing by phagocytes. N. Engl. J. Med. 298:659-668.

4. Babiuk, L. A., and B. T. Rouse. 1979. Immune control of herpesvirus latency. Can. J. Microbiol. 25:267-274.

5. Badwey, J. A., and M. L. Karnovsky. 1980. Active oxygen species and functions of phagocytic leukocytes. Ann. Rev. Biochem. 49:695-726.

6. Baehner, R. L., and R. B. Johnston. 1971. Metabolic and bactericidal properties of human eosinophils. Br. J. Haemat. 20:277-285.

7. Beeson, P. B., and D. A. Bass. 1977. The Eosinophil. W. B. Saunders Company, Philadelphia, Pa. 269 pp.

8. Belding, M. E., and S. J. Klebanoff. 1970. Peroxidase-mediated virucidal systems. Science 167:195-196.

9. Black, W. D. 1974. Therapeutics of corticosteroids in the bovine animal and problems surrounding their use. Proc. Ann. Conv. Am. Assoc. Bov. Pract. 7:63-66.

10. Black, W. G., L. C. Ulberg, H. E. Kidder, J. Simon, S. H. McNutt, and L. E. Casida. 1953. Inflammatory response of the bovine endometrium. Am. J. Vet. Res. 14:179-183.

11. Bloom, J. C., S. J. Kenyon, and T. G. Gabuzda. 1979. Glucocorticoid effects on peripheral blood lymphocytes in cows infected with bovine leukemia virus. Blood 53:899-912.

12. Bloom, J. C., V. K. Ganjam, and T. G. Gabuzda. 1980. Glucocorticoid receptors in peripheral blood lymphocytes from bovine leukemia virus-infected cows with persistent lymphocytosis. Cancer Res. 40:2240-2244.

13. Bodel, P., G. M. Dillard, S. S. Kaplan, and S. E. Malawista. 1972. Anti-inflammatory effects of estradiol on human blood leukocytes. J. Lab. Clin. Med. 80:373-384.

14. Bøyum, A. 1966. Ficoll Hypaque method for separating mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. Suppl. 1966:77.

15. Brandon, M. R., A. J. Husband, and A. K. Lascelles. 1975. The effect of glucocorticoid on immunoglobulin secretion into colostrum in cows. Aust. J. Exp. Biol. Med. Sci. 53:43-48.

16. Bujak, J. S., and R. K. Root. 1974. The role of peroxidase in the bactericidal activity of human blood eosinophils. Blood 43:727-736.

17. Burnet, F. M. 1959. The clonal selection theory of acquired immunity. Vanderbilt University Press, Nashville, Tenn. 208 pp.

18. Butler, W. T., and R. D. Rossen. 1973. Effects of corticosteroids on immunity in man. I. Decreased serum IgG concentration caused by 3 or 5 days of high doses of methylprednisolone. J. Clin. Invest. 52:2629-2640.

19. Butterworth, A. E. 1977. The eosinophil and its role in immunity to helminth infection. Curr. Topics in Microbiol. Immunol. 77:127-168.

20. Cabello, G. 1980. Plasma cortisol and aldosterone levels in healthy and diarrhoeic calves. Br. Vet. J. 136:160-167.

21. Cabello, G., and D. Levieux. 1980. Comparative absorption of colostral IgG1 and IgM in the newborn calf: Effects of thyroxine, cortisol and environmental factors. Ann. Rech. Vet. 11:1-7.

22. Callow, L. L., and R. J. Parker. 1969. Cortisone-induced relapses in Babesia argentina infections of cattle. Aust. Vet. J. 45:103-104.

23. Carlson, G. P., and J. J. Kaneko. 1976. Influence of prednisolone on intravascular granulocyte kinetics of calves under nonsteady state conditions. Am. J. Vet. Res. 37:149-151.

24. Chiang, J. L., R. Patterson, J. J. McGillen, J. P. Phair, M. Roberts, K. E. Harris, and K. S. Riesing. 1980. Long-term corticosteroid effect on lymphocyte and polymorphonuclear cell function in asthmatics. J. Allergy Clin. Immunol. 65:263-268.

25. Chretien, J. H., and V. F. Garagusi. 1972. Corticosteroid effect on phagocytosis and NBT reduction by human polymorphonuclear neutrophils. J. Reticuloendothel. Soc. 11:358-367.

26. Chretien, J. H., and V. F. Garagusi. 1973. Correction of corticosteroid-induced defects of polymorphonuclear neutrophil function by ascorbic acid. J. Reticuloendothel. Soc. 14:280-286.

27. Christie, B. M., R. E. Pierson, P. M. Braddy, D. E. Flack, D. P. Horton, R. Jensen, E. A. Lee, E. E. Remmenga, and K. G. Rutt. 1977. Efficacy of corticosteroids as supportive therapy for bronchial pneumonia in yearling feedlot cattle. Bov. Pract. 12:115-117.

28. Claman, H. N. 1972. Corticosteroids and lymphoid cells. N. Engl. J. Med. 287:388-397.

29. Clark, R. A., and S. J. Klebanoff. 1977. Studies on the mechanism of antibody-dependent polymorphonuclear leukocyte-mediated cytotoxicity. J. Immunol. 119:1413-1418.

30. Clark, R. A. F., J. I. Gallin, and A. S. Fauci. 1979. Effects of *in vivo* prednisone on *in vitro* eosinophil and neutrophil adherence and chemotaxis. Blood 53:633-641.

31. Clemens, L. E., P. K. Siiteri, and D. P. Stites. 1979. Mechanism of immunosuppression of progesterone on maternal lymphocyte activation during pregnancy. J. Immunol. 122:1978-1985.

32. Cline, M. J., J. Hanifin, and R. I. Lehrer. 1968. Phagocytosis by human eosinophils. Blood 32:922-934.

33. Comline, R. S., L. W. Hall, R. B. Lavelle, P. W. Nathanielsz, and M. Silver. 1974. Parturition in the cow: Endocrine changes in animals with chronically implanted catheters in the foetal and maternal circulations. J. Endocrinol. 63:451-472.

34. Cooper, M. R., L. R. DeChatelet, and C. E. McCall. 1972. The in vitro effect of steroids on polymorphonuclear leukocyte metabolism. Proc. Soc. Exp. Biol. Med. 141:986-990.

35. Crookshank, H. R., M. H. Elissalde, R. G. White, D. C. Clanton, and H. E. Smalley. 1979. Effect of transportation and handling of calves upon blood serum composition. J. Anim. Sci. 48:430-435.

36. Cummins, J. M., and B. D. Rosenquist. 1977. Effect of hydrocortisone on the interferon response of calves infected with infectious bovine rhinotracheitis virus. Am. J. Vet. Res. 38:1163-1166.

37. Cummins, J. M., and B. D. Rosenquist. 1979. Leukocyte changes and interferon production in calves injected with hydrocortisone and infected with infectious bovine rhinotracheitis virus. Am. J. Vet. Res. 40:238-240.

38. Dale, D. C., and R. G. Petersdorf. 1975. Corticosteroids and Infectious Disease. Pages 209-222. In D. L. Azarnoff, ed., Steroid Therapy. Saunders, Philadelphia.

39. Davies, D. H., and L. E. Carmichael. 1973. Role of cell-mediated immunity in the recovery of cattle from primary and recurrent infections with infectious bovine rhinotracheitis virus. Infect. Immun. 8:510-518.

40. Davies, D. H., and J. R. Duncan. 1974. The pathogenesis of recurrent infections with infectious bovine rhinotracheitis virus induced in calves by treatment with corticosteroids. Cornell Vet. 64:340-366.

41. DeChatelet, L. R., R. A. Migler, P. S. Shirley, D. A. Bass, and C. E. McCall. 1978. Enzymes of oxidative metabolism in the human eosinophil. Proc. Soc. Exp. Biol. Med. 158: 537-541.

42. DeChatelet, L. R., R. A. Migler, P. S. Shirley, H. B. Muss, P. Szejda, and D. A. Bass. 1978. Comparison of intracellular bactericidal activities of human neutrophils and eosinophils. Blood 52:609-617.

43. Dluhy, R. G., S. R. Newmark, D. P. Lauler, and G. W. Thorn. 1975. Pharmacology and chemistry of adrenal glucocorticoids. Pages 1-14 in D. L. Azarnoff, ed. Steroid therapy. Saunders, Philadelphia, Penn.

44. Fabris, N., L. Piantanelli, and M. Muzzioli. 1977. Differential effect of pregnancy or gestagens on humoral and cell-mediated immunity. Clin. Exp. Immunol. 28:306-314.

45. Fauci, A. S. 1979. Immunosuppressive and anti-inflammatory effects of glucocorticoids. Pages 449-465 in J. D. Baxter, and G. G. Rousseau, eds. Glucocorticoid hormone action. Springer-Verlag, Berlin.

46. Fuenfer, M. M., E. A. Carr, and H. C. Polk. 1979. The effect of hydrocortisone on superoxide production by leukocytes. J. Surg. Res. 27:29-35.

47. Fulton, R. W., and B. D. Rosenquist. 1976. In vitro interferon production by bovine tissues: Effects of hydrocortisone. Am. J. Vet. Res. 37:1493-1495.

48. Glasser, L., D. W. Heustis, and J. F. Jones. 1977. Functional capabilities of steroid-recruited neutrophils harvested for clinical transfusion. N. Engl. J. Med. 297:1033-1036.

49. Goldstein, I., S. Hoffstein, J. Gallin, and G. Weissmann. 1973. Mechanisms of lysosomal enzyme release from human leukocytes: microtubule assembly and membrane fusion induced by a component of complement. Proc. Nat. Acad. Sci. 70: 2916-2920.

50. Grewal, A. S., and L. A. Babiuk. 1979. Induction, isolation and surface marker studies on bovine eosinophils. J. Immunol. Methods 25:65-75.

51. Grewal, A. S., B. T. Rouse, and L. A. Babiuk. 1977. Mechanisms of resistance to herpesviruses: Comparison of the effectiveness of different cell types in mediating antibody-dependent cell-mediated cytotoxicity. Infect. Immun. 15:698-703.

52. Grewal, A. S., M. Carpio, and L. A. Babiuk. 1980. Polymorphonuclear neutrophil-mediated antibody-dependent cell cytotoxicity of herpesvirus-infected cells: Ultrastructural studies. Can. J. Microbiol. 26:427-435.

53. Grover, W. H., H. H. Winkler, and D. E. Normansell. 1978. Phagocytic properties of isolated human eosinophils. J. Immunol. 121:718-725.

54. Guidry, A. J., M. J. Paape, and R. E. Pearson. 1976. Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids, and neutrophil phagocytosis in the cow. Am. J. Vet. Res. 37:1195-1200.

55. Guthrie, G. P., and W. J. John. 1980. The in vivo glucocorticoid and anti-glucocorticoid actions of medroxyprogesterone acetate. Endocrinol. 107:1393-1396.

56. Gwazdauskas, F. C., W. B. Gross, T. L. Bibb, and M. L. McGilliard. 1978. Antibody titers and plasma glucocorticoid concentrations near weaning in steer and heifer calves. Can. Vet. J. 19:150-154.

57. Gwazdauskas, F. C., M. J. Paape, D. A. Peery, and M. L. McGilliard. 1980. Plasma glucocorticoid and circulating blood leukocyte responses in cattle after sequential intramuscular injections of ACTH. Am. J. Vet. Res. 41: 1052-1056.

58. Hammer, R. F., and A. F. Weber. 1974. Ultrastructure of agranular leukocytes in peripheral blood of normal cows. Am. J. Vet. Res. 35:527-536.

59. Hawk, H. W., G. D. Turner, and J. F. Sykes. 1960. The effect of ovarian hormones on the uterine defense mechanism during the early stages of induced infection. Am. J. Vet. Res. 21:644-648.

60. Hoerlein, A. B. 1980. Shipping Fever. Pages 99-106 in H. E. Amstutz, ed. Bovine medicine and surgery. American Veterinary Publications, Inc., Santa Barbara, CA.

61. Hoerlein, A. B., and D. L. Jones. 1977. Bovine immunoglobulins following induced parturition. J. Am. Vet. Med. Assoc. 170:325-326.

62. Hoerlein, A. B., and G. L. Marsh. 1957. Studies on the epizootiology of shipping fever in calves. J. Am. Vet. Med. Assoc. 131:123-127.

63. Horton, D., and P. M. Braddy. 1980. Disease prevention in feedlot cattle. Pages 91-98 in H. E. Amstutz, ed. Bovine medicine and surgery. American Veterinary Publications, Inc., Santa Barbara, Calif.

64. Hudson, S., M. Mullford, W. G. Whittlestone, and E. Payne. 1975. Bovine plasma corticoids during parturition. J. Dairy Sci. 59:744-746.

65. Husband, A. J., M. R. Brandon, and A. K. Lascelles. 1973. The effect of corticosteroid on absorption and endogenous production of immunoglobulins in calves. Aust. J. Exp. Biol. Med. Sci. 51:707-710.

66. Ignarro, L. J., and S. Y. Cech. 1975. Lysosomal enzyme secretion from human neutrophils mediated by cyclic GMP: Inhibition of cyclic GMP accumulation and neutrophil function by glucocorticosteroids. J. Cyclic Nuc. Res. 1:283-292.

67. Jaccard, F., and G. Cimasoni. 1979. Female sex hormones and lysosomal stability in gingival polymorphonuclear leukocytes. Experientia. 35:1291-1292.

68. Jensen, R., R. E. Pierson, P. M. Braddy, D. A. Saari, L. H. Lauerman, J. J. England, H. Keyvanfer, J. R. Collier, D. P. Horton, A. E. McChesney, A. Benitez, and R. M. Christie. 1976. Shipping fever pneumonia in yearling feedlot cattle. J. Am. Vet. Med. Assoc. 169:500-506.

69. Johnston, J. D., and R. B. Buckland. 1976. Response of male holstein calves from seven sires to four management stresses as measured by plasma corticoid levels. Can. J. Anim. Sci. 56:727-732.

70. Johnston, N. E., and W. D. Oxender. 1979. Effect of altered serum glucocorticoid concentrations on the ability of the newborn calf to absorb colostral immunoglobulin. Am. J. Vet. Res. 40:32-34.

71. Kaltenbach, C. C., and T. G. Dunn. 1980. Endocrinology of reproduction. Pages 85-113 in E. S. E. Hafez, ed. Reproduction in farm animals. Lea and Febiger, Philadelphia, Penn.

72. Kerr, W. R., J. L. McGirr, and M. Robertson. 1949. Specific and non-specific desensitisation of the skin in trichomonas sensitive bovines. J. Comp. Path. 59:133-154.

73. Killingbeck, J., and G. E. Lamming. 1963. Influence of uterine secretions on phagocytosis. Nature 198:111-112.

74. Kimball, H. R., G. H. Ford, and S. M. Wolff. 1975. Lysosomal enzymes in normal and Chediak-Higashi blood leukocytes. J. Lab. Clin. Med. 86:616-630.

75. Klebanoff, S. J. 1979. Effect of estrogens on the myeloperoxidase-mediated antimicrobial system. Infect. Immun. 25:153-156.

76. Klebanoff, S. J. 1979. Oxygen-dependent antimicrobial systems of the neutrophil. Pages 87-91 in D. Schlessinger, ed. Microbiology - 1979. American Society for Microbiology, Washington, D.C.

77. Klebanoff, S. J., D. T. Durack, H. Rosen, and R. A. Clark. 1977. Functional studies on human peritoneal eosinophils. Infect. Immun. 17:167-173.

78. Kunesh, J. P. 1977. Clinical use of glucocorticoids in large animals. Vet. Med. Small Anim. Clin. 72:611-613.

79. Kuttler, K. L., and L. G. Adams. 1977. Influence of dexamethasone on the recrudescence of Anaplasma marginale in splenectomized calves. Am. J. Vet. Res. 38:1327-1330.

80. LaMotte, G. B., and R. J. Eberhart. 1976. Blood leukocytes, neutrophil phagocytosis, and plasma corticosteroids in colostrum-fed and colostrum-deprived calves. Am. J. Vet. Res. 37:1189-1193.

81. Lopatin, D. E., K. S. Kornman, and W. J. Loesche. 1980. Modulation of immunoreactivity to periodontal disease-associated microorganisms during pregnancy. Infect. Immun. 28:713-718.

82. MacGregor, R. R., P. J. Spagnuolo, and A. L. Lentnek. 1974. Inhibition of granulocyte adherence by ethanol, prednisone, and aspirin, measured with an assay system. N. Engl. J. Med. 291:642-646.

83. MacIntyre, D. E., J. D. Pearson, and J. L. Gordon. 1978. Localization and stimulation of prostacyclin production in vascular cells. Nature 271:549-551.

84. Mandell, G. L., W. Rubin, and E. W. Hook. 1970. The effect of an NADH oxidase inhibitor (hydrocortisone) on polymorphonuclear leukocyte bactericidal activity. J. Clin. Invest. 49: 1381-1388.

85. Martin, W. B., and F. M. M. Scott. 1979. Latent infection of cattle with bovid herpesvirus 2. Arch. Virol. 60:51-58.

86. Massip, A. 1979. Haematocrit, biochemical and plasma cortisol changes associated with diarrhoea in the calf. Br. Vet. J. 135:600-605.

87. May, I., I. Manoiu, C. Donta, M. Tetu, C. Vior, and St. Moldovan. 1979. Stess und Immunität beim Rind. Arch. Exp. Veterinaemed., Leipzig. 33:87-98.

88. McGillen, J., and J. Phair. 1979. Polymorphonuclear leukocyte adherence to nylon: Effect of oral corticosteroids. Infect. Immun. 26:542-546.

89. McGillen, J. R., Patterson, and J. P. Phair. 1980. Adherence of polymorphonuclear leukocytes to nylon: Modulation by Prostacyclin (PGI_2), corticosteroids, and complement activation. J. Infect. Dis. 141:382-388.

90. Melby, J. C., and M. St. Cyr. 1961. Comparative studies on absorption and metabolic disposal of water soluble corticosteroid esters. Metabolism 10:75-82.

91. Merrill, W. G., and V. R. Smith. 1954. A comparison of some cellular and chemical constituents of blood at time of parturition and after administration of adrenocorticotrophin. J. Dairy Sci. 37:546-551.

92. Mickenberg, I. D., R. K. Root, and S. M. Wolff. 1972. Bactericidal and metabolic properties of human eosinophils. Blood 39:67-80.

93. Muller, L. D., G. L. Beardsley, R. P. Ellis, D. E. Reed, and M. J. Owens. 1975. Calf response to the initiation of parturition in dairy cows with dexamethasone or dexamethasone with estradiol benzoate. J. Anim. Sci. 41:1711-1716.

94. Muscoplat, C. C., D. W. Johnson, K. A. Pomeroy, J. M. Olson, V. L. Larson, J. B. Stevens, and D. K. Sorensen. 1974. Lymphocyte surface immunoglobulin: Frequency in normal and lymphocytotic cattle. Am. J. Vet. Res. 35:593-595.

95. Muscoplat, C. C., R. E. Shope, A. W. Chen, and D. W. Johnson. 1975. Effects of corticosteroids on responses of bovine peripheral blood lymphocytes cultured with phyto-hemagglutinin. Am. J. Vet. Res. 36:1243-1244.

96. Narita, M., S. Inui, K. Namba, and Y. Shimizu. 1978. Neural changes in recurrent infection of infectious bovine rhinotracheitis virus in calves treated with dexamethasone. Am. J. Vet. Res. 39:1399-1403.

97. Nathan, C. F., S. C. Silverstein, L. H. Brukner, and Z. A. Cohn. 1979. Extracellular cytolysis by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. J. Exp. Med. 149:100-113.

98. Nicol, T., D. L. J. Bilbey, L. M. Charles, J. L. Cordingley, and B. Vernon-Roberts. 1964. Oestrogen: The natural stimulant of body defense. J. Endocrinol. 30:277-291.

99. Nicol, T., B. Vernon-Roberts, and D. C. Quantock. 1965. The influence of various hormones on the reticulo-endothelial system: Endocrine control of body defense. J. Endocrinol. 33:365-383.

100. Nicol, T., B. Vernon-Roberts, and D. C. Quantock. 1967. The effect of testosterone and progesterone on the response of the reticuloendothelial system and reproductive tract to oestrogen in the male mouse. J. Endocrinol. 37:17-21.

101. Niilo, L. 1970. The effect of dexamethasone on bovine coccidiosis. Can. J. Comp. Med. 34:325-328.

102. Nugent, C. A., K. Eik-nes, and F. H. Tyler. 1959. A comparative study of the metabolism of hydrocortisone and prenisolone. J. Clin. Endocrinol. 19:526-534.

103. Oppenheim, J. J., and B. Schecter. 1980. Lymphocyte transformation. Pages 233-245 in N. R. Rose, and H. Friedman, eds. Manual of clinical immunology. American Society for Microbiology, Washington, D. C.

104. Paape, M. J., D. W. Carroll, A. J. Kral, R. H. Miller, and C. Desjardins. 1974. Corticosteroids, circulating leukocytes, and erythrocytes in cattle: Diurnal changes and effects of bacteriologic status, stage of lactation, and milk yield on response to adrenocorticotropin. Am. J. Vet. Res. 35: 355-362.

105. Paape, M. J., W. D. Schultze, C. Desjardins, and R. H. Miller. 1974. Plasma corticosteroid, circulating leukocyte and milk somatic cell responses to Escherichia coli endotoxin-induced mastitis. Proc. Soc. Exp. Biol. Med. 145:553-559.

106. Paape, M. J., C. Desjardins, A. J. Guidry, R. H. Miller, and V. R. Smith. 1977. Response of plasma corticosteroids and circulating leukocytes in cattle following intravenous injection of different doses of adrenocorticotropin. Am. J. Vet. Res. 38:1345-1348.

107. Parrillo, J. E., and A. S. Fauci. 1978. Mechanisms of corticosteroid action on lymphocyte subpopulations. III. Differential effects of dexamethasone administration on subpopulations of effector cells mediating cellular cytotoxicity in man. Clin. Exp. Immunol. 31:116-125.

108. Parrillo, J. E., and A. S. Fauci. 1979. Mechanisms of glucocorticoid action on immune processes. Ann. Rev. Pharmacol. Toxicol. 19:179-201.

109. Pastoret, P. O., L. A. Babiuk, V. Misra, and P. Griebel. 1980. Reactivation of temperature-sensitive and non-temperature-sensitive infectious bovine rhinotracheitis vaccine virus with dexamethasone. Infect. Immun. 29:483-488.

110. Persellin, R. H., and L. C. Ku. 1974. Effects of steroid hormones on human polymorphonuclear leukocyte lysosomes. J. Clin. Invest. 54:919-925.

111. Rinehart, J. J., A. L. Sagone, S. P. Balcerzak, G. H. Ackerman, and A. F. LoBuglio. 1975. Effects of corticosteroid therapy on human monocyte function. N. Engl. J. Med. 292:236-241.

112. Rosenquist, B. D., and R. W. Loan. 1969. Interferon induction in the bovine species by infectious bovine rhinotracheitis virus. Am. J. Vet. Res. 30:1305-1312.

113. Roth, J. A., and M. L. Kaeberle. 1981. Effects of in vivo dexamethasone administration on in vitro bovine polymorphonuclear leukocyte function. Infect. Immun., Submitted for publication.

114. Roth, J. A., and M. L. Kaeberle. 1981. Evaluation of bovine polymorphonuclear leukocyte function. Vet. Immunol. Immunopathol., In press.

115. Roth, J. A., and M. L. Kaeberle. 1981. Isolation of neutrophils and eosinophils from the peripheral blood of cattle and comparison of their functional activities. J. Immunol. Methods, Accepted for publication.

116. Roth, J. A., L. H. Appell, and M. L. Kaeberle. 1981. Association of elevated estradiol and progesterone blood levels with altered bovine polymorphonuclear leukocyte function. Am. J. Vet. Res., Submitted for publication.

117. Roth, J. A., M. L. Kaeberle, and W. H. Hsu. 1981. Effect of estradiol and progesterone on lymphocyte and neutrophil function in steers. Infect. Immun., Submitted for publication.

118. Roth, J. A., M. L. Kaeberle, and W. H. Hsu. 1981. Effects of adrenocorticotropin administration on bovine polymorphonuclear leukocyte function and lymphocyte blastogenesis. Am. J. Vet. Res., Submitted for publication.

119. Rouse, B. T., R. C. Wardley, and L. A. Babiuk. 1976. Antibody-dependent cell-mediated cytotoxicity in cows: Comparison of effector cell activity against heterologous erythrocyte and herpesvirus-infected bovine target cells. Infect. Immun. 13:1433-1441.

120. Rouse, B. T., L. A. Babiuk, and P. M. Henson. 1978. Neutrophils as mediators of antiviral immunity. Experientia 34: 346-348.

121. Rowson, L. E. A., G. E. Lamming, and R. M. Fry. 1953. The relationship between ovarian hormones and uterine infection. Vet. Record 65:335-340.

122. Sabag, N., M. A. Castrillon, and A. Tchernitchin. 1978. Cortisol-induced migration of eosinophil leukocytes to lymphoid organs. Experientia 34:666-667.

123. Sanderson, C. J., and J. A. Thomas. 1978. A comparison of the cytotoxic activity of eosinophils and other cells by ^{51}chromium release and time lapse microcinematography. Immunology 34:771-780.

124. Schalm, O. W., J. Lasmanis, and E. J. Carroll. 1965. The use of a synthetic corticoid on experimental coliform (Aerobacter aerogenes) mastitis in cattle: The response of leukocytes and the effect of hormone-induced neutrophilia. Am. J. Vet. Res. 26:851-857.

125. Schalm, O. W., N. G. Jain, and E. J. Carroll. 1975. Veterinary hematology. Lea and Febiger, Philadelphia, Penn. 807 pp.

126. Schiff, R. I., D. Mercier, and R. H. Buckley. 1975. Inability of gestational hormones to account for the inhibitory effects of pregnancy plasmas on lymphocyte responses in vitro. Cellular Immunol. 20:69-80.

127. Schwalm, J. W., and H. A. Tucker. 1978. Glucocorticoids in mammary secretions and blood serum during reproduction and lactation and distributions of glucocorticoids, progesterone, and estrogens in fractions of milk. J. Dairy Sci. 61: 550-560.

128. Shaw, K. E., and R. E. Nichols. 1964. Plasma 17-hydroxycorticosteroids in calves - The effects of shipping. Am. J. Vet. Res. 25:252-253.

129. Shaw, K. E., S. Dutta, and R. E. Nichols. 1960. Quantities of 17-hydroxycorticosteroids in the plasma of healthy cattle during various physiologic states. Am. J. Vet. Res. 21: 52-53.

130. Sheffy, B. E., and D. H. Davies. 1972. Reactivation of a bovine herpesvirus after corticosteroid treatment. Proc. Soc. Exp. Biol. Med. 140:974-976.

131. Shope, R. E., C. C. Muscoplat, A. W. Chen, and D. W. Johnson. 1976. Mechanism of protection from primary bovine viral diarrhea virus infection. I. The effects of dexamethasone. Can. J. Comp. Med. 40:355-359.

132. Siiteri, P. K., F. Febres, L. E. Clemens, R. J. Chang, B. Gondos, and D. Stites. 1977. Progesterone and maintenance of pregnancy: Is progesterone nature's immunosuppressant? Ann. N. Y. Acad. Sci. 286:384-397.

133. Simchowitz, L., and I. Spilberg. 1979. Evidence for the role of superoxide radicals in neutrophil-mediated cytotoxicity. Immunology 37:301-309.

134. Simmons, S. R., and M. L. Karnovsky. 1973. Iodinating ability of various leukocytes and their bactericidal activity. J. Exp. Med. 138:44-63.

135. Sloman, J. C., and P. A. Bell. 1980. Cell cycle-specific effects of glucocorticoids on phytohaemagglutinin-stimulated lymphocytes. Clin. Exp. Immunol. 39:503-509.

136. Stabenfeldt, G. H. 1974. Physiologic, pathologic and therapeutic roles of progestins in domestic animals. J. Am. Vet. Med. Assoc. 164:311-317.

137. Stabenfeldt, G. H., L. E. Edqvist, H. Kindahl, B. Gustafson, and A. Bane. 1978. Practical implications of recent physiologic findings for reproductive efficiency in cows, mares, sows, and ewes. J. Am. Vet. Med. Assoc. 172:667-675.

138. Steele, P. A., A. P. F. Flint, and A. C. Turnbull. 1976. Activity of steroid C-17, 20 lyase in the ovine placenta: Effect of exposure to foetal glucocorticoid. J. Endocrinol. 69:239-246.

139. Stephens, D. B. 1980. Stress and its measurement in domestic animals: A review of behavioral and physiological studies under field and laboratory situations. Adv. Vet. Sci. Comp. Med. 24:179-210.

140. Stevenson, R. D. 1976. Effect of steroid therapy on in vitro polymorph migration. Clin. Exp. Immunol. 23:285-289.

141. Stevenson, R. D. 1977. Mechanism of anti-inflammatory action of glucocorticosteroids. Lancet 8005:225-226.

142. Stevenson, R. D., A. C. Gray, and N. P. Lucie. 1978. Stimulation of capillary tube polymorph migration: an indirect glucocorticoid effect on microtubular function. Clin. Exp. Immunol. 33:478-485.

143. Stockdale, P. H. G., and L. Niilo. 1976. Production of bovine coccidiosis with Eimeria zuernii. Can. Vet. J. 17:35-37.

144. Stossel, T. P. 1974. Phagocytosis. N. Engl. J. Med. 290: 717-723, 774-780, 833-839.

145. Stott, G. H. 1980. Immunoglobulin absorption in calf neonates with special considerations of stress. J. Dairy Sci. 63:681-688.

146. Stott, G. H., and E. J. Reinhard. 1978. Adrenal function and passive immunity in the dystocial calf. J. Dairy Sci. 61:1457-1461.

147. Thompson, J. S., M. K. Crawford, R. W. Reilly, and C. D. Severson. 1967. The effect of estrogenic hormones on immune responses in normal and irradiated mice. J. Immunol. 98:331-335.

148. Tuchinda, M., R. W. Newcomb, and B. L. De Vald. 1972. Effect of prednisone treatment on the human immune response to keyhole limpet hemocyanin. Int. Arch. Allergy 42:533-544.

149. Vadas, M. A., J. R. David, A. Butterworth, N. T. Pisani, and T. A. Siongok. 1979. A new method for the purification of human eosinophils and neutrophils, and a comparison of the ability of these cells to damage schistosomula of Schistosoma mansoni. J. Immunol. 122:1228-1236.

150. Vahdat, F., J. P. Hurtgen, H. L. Whitmore, S. D. Johnston, and C. L. Ketelsen. 1979. Effect of time and temperature on bovine serum and plasma progesterone concentration. Theriogenology 12:371-374.

151. Venino, D. H., and J. R. Hall. 1979. Veterinarians' product and therapeutic reference. 5th ed. Therapeutics Communications, Inc., Caldwell, N. J. 534 pp.

152. Vukotic, M., J. Vujosevic, D. Kuzmanov, M. Zeremski, M. Movsesijan, and B. Jovanovic. 1978. Serum immunoglobulin G levels in calves from dexamethasone induced parturition. Acta Veterinaria, Yugoslavia 28:113-118.

153. Wardley, R. C., L. A. Babiuk, and B. T. Rouse. 1976. Polymorph-mediated antibody-dependent cytotoxicity - modulation of activity by drugs and immune interferon. Can. J. Microbiol. 22:1222-1228.

154. Wardley, R. C., B. T. Rouse, and L. A. Babiuk. 1976. Antibody dependent cytotoxicity mediated by neutrophils: A possible mechanism of antiviral defense. J. Reticuloendothel. Soc. 19:323-332.

155. Weiland, F., and O. C. Straub. 1975. Frequency of surface immunoglobulin bearing blood lymphocytes in cattle affected with bovine leukosis. Res. Vet. Sci. 19:100-102.

156. Weissmann, G., J. E. Smolen, and H. Korchak. 1980. Prostaglandins and inflammation: Receptor/cyclase coupling as an explanation of why PGEs and PGI_2 inhibit functions of inflammatory cells. Adv. in Prostaglandin and Thromboxane Res. 8:1637-1653.

157. Wilkie, B. N., F. Caoili, and R. Jacobs. 1979. Bovine lymphocytes: Erythrocyte rosettes in normal, lymphomatous and corticosteroid-treated cattle. Can. J. Comp. Med. 43:22-28.

158. Yost, F. J., and I. Fridovich. 1974. Superoxide radicals and phagocytosis. Arch. Biochem. Biophys. 161:395-401.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to the many individuals who willingly gave assistance and advice in the conduct of this experimentation and in the preparation of this dissertation. Dr. M. L. Kaeberle played a major role in conceiving of and designing the experimentation, interpreting the results, and preparing the manuscripts. I am especially grateful to Dr. Kaeberle for his considerable efforts in procuring and maintaining a well-equipped research laboratory and suitable animal housing, without which this research would not have been possible. I also wish to acknowledge the valuable assistance provided by Messrs. E. C. Johnson, R. D. Hubbard, E. W. Field, G. J. Friedman, D. L. Stanley and T. M. Sullivan in the conduct of the experimentation.

Dr. W. H. Hsu and L. H. Appell played an important role in certain phases of this research. Dr. Hsu performed the cortisol radioimmunoassays on steer sera and gave valuable advice in the preparation of certain of the manuscripts. Dr. Appell was a co-investigator in the experimentation on steroid hormones in cows and assisted in the preparation that manuscript. Dr. R. F. Nachreiner at Michigan State University performed the radioimmunoassays for the experimentation on steroid hormones in cows.

I am also grateful to Dr. R. A. Packer, Dr. J. P. Kluge, Dr. D. C. Beitz, and Dr. L. E. Evans, the other members of my graduate committee, for their time and valuable advice.

I wish to thank Mrs. Marjorie Davis for the many hours which she spent typing numerous drafts of the manuscripts in this dissertation and for striving for excellence with each draft.

My greatest expression of appreciation goes to my wife, Jeanne, for her patience and understanding during the course of my graduate work and to our children, Claire and David, for helping me to view my graduate training and research in the proper perspective.

This experimentation was supported by a grant from the Cooperative State Research Service of the United States Department of Agriculture and by a formula fund grant provided by the United States Department of Agriculture.