Pathologic and immunobiologic changes in chemically-induced recrudescence of IBR infections in cattle

by

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INTRODUCTION

The respiratory form of infectious bovine rhinotracheitis (IBR) caused by Bovid herpesvirus-1 was first recognized as a distinct clinical entity in Colorado (Miller 1955) and California (Schroeder and Mays 1954) during the early 1950's. McKercher et al. (1955) and Jensen et al. (1955) initially described the clinical signs of hyperthermia, anorexia, coughing and mucopurulent discharge from the nostrils. Examination of the trachea, pharynx, tonsils and adjacent lymph nodes at necropsy often revealed extensive ecchymotic hemorrhages and focal necrotic ulceration. Histopathologic changes induced by the causative agent included acute to subacute necrotic rhinotracheitis especially prominent on the nasal septum, pharynx and anterior one-third of the trachea.

Madin et al. (1956) isolated the causative agent, a virus, from the nasal washings of experimentally infected calves. Their experiments clearly indicated that this virus, when inoculated intranasally into susceptible calves, caused disease with characteristic signs of the natural illness. The morphological and biophysical characteristics first defined by Tousimis (1958) and Armstrong et al. (1961) are typical of the herpes virus group. The virus is generally referred to as IBR virus (IBRV) even though the suggestions of viral taxonomists (Andrewes 1954; Roizman et al. 1973) prompted investigators to use such terms as herpes virus bovis (Nelson et al. 1972), bovine herpesvirus 1 (Michalski et al. 1976), and bovid herpesvirus 1 (Cilli and Castrucci 1976; Smith 1977).

A disease of cattle known as bläschenausschlag, coital exanthema or infectious pustular vulvovaginitis (IPV) was described in Europe in 1890 (Fenner 1890). Since that time it has been shown to be worldwide in its distribution. Gillespie et al. (1959) failed to show distinct differences between IBRV and IPV virus; therefore, references to IBR/IPV virus may often be found in the literature.

The diverse clinical manifestations of IBRV infection include bovine respiratory disease (Madin et al. 1956), conjunctivitis (Abinanti and Plummer 1961), vulvovaginitis, balanoposthitis (Grieg et al. 1958), abortions (Owen et al. 1964), and meningoencephalitis (French 1962; Barenfus and Schroeder 1963; Durham 1974). Though viral isolants made from these conditions seem to have a biological predilection for certain tissues, no unequivocal serologic, morphologic or biochemical differences in these strains have been demonstrated.

The persistent or chronic nature of IBRV infections has been demonstrated. Snowden (1965) was able to repeatedly isolate virus from experimentally infected cattle for as long as 578 days after initial exposure. Huck et al. (1973) reported evidence to support the theory of long term chronic IBR/IPV virus infection in maiden heifers. Male genital organs may also be chronically infected with the virus; therefore, the potential exists for venereal transmission through natural and artificial breeding (Böttcher and Mahler 1970). The failure of modified live virus vaccine to eliminate IBRV-induced respiratory disease from feedlot cattle (Kiesel et al. 1972; Curtis and Angulo 1974) even though many of the individuals may have been vaccinated 2 or 3 times (Jensen

et al. 1976) raises serious doubts about the effectiveness of these vaccines or the wisdom displayed by advocating their widespread use. Although vaccine induction of resistance to experimental challenge exposure may be demonstrated it is reasonable to expect that environmentally-induced stress-related immunosuppression or other viral infections (Mensik et al. 1976) may reactivate chronic IBRV infections. Chemically induced recrudescence of natural (Dennett et al. 1973; Dennett et al 1976) and experimental (Sheffy and Davies 1972; Sheffy and Rodman 1973) IBRV infections have been demonstrated. The modification of cell-mediated immune response by other viral infections could conceivably play a role in immunosuppression and IBRV reactivation (Mensik et al. 1976).

Hypersensitivity reactions (Darcel and Doward 1972; Girón et al. 1975) to herpesviral antigens in animals previously infected with herpesviruses suggests that the recovery from infection, recrudescence of chronic infections and resistance to disease may be intricately related to cell-mediated immune (CMI) mechanisms. The general knowledge of the pathogenicity and epidemiology of herpesvirus infections could be enhanced by using CMI techniques to evaluate the pathologic and immunobiologic changes that occur during acute, chronic, and chemically reactivated IBRV infections in cattle.

The objectives of these studies were to determine the effectiveness of immunosuppressive agents to cause recrudescence of chronic IBRV infections of cattle, to evaluate the histopathologic changes that occur in cattle due to chemically-induced reactivation of IBRV infections, to

compare changes in various immune mechanisms during immunosuppression, and to determine the effect of acute IBRV infections in immunosuppressed calves. Information provided by accomplishment of these objectives could provide valuable knowledge concerning the pathogenesis of IBRV infections in cattle and insights leading to improved methods of producing resistance to herpesvirus infections in all animals.

LITERATURE REVIEW

One of the most perplexing, yet intriguing virus-host relationships observed in nature is the interactions between herpesviruses and the multicellular organisms they infect. The research maze that surrounds this phenomenon is almost incomprehensible. Many theories are perpetrated to explain all or a portion of these well-known disease processes, and subsequent immunological relationships. Efforts to explain this mechanism have not abated since that so succinctly described by Du Castel in 1901 when he stated that "In our ignorance of the exact process of herpes, each medical generation creates a theory adapted to the ideas and discoveries of the moment" (quoted by Nahmias and Dowdle 1968, p. 111).

The Nature of Herpesvirus Infections

Much of the present knowledge of the pathogenesis of herpesvirus-cell interactions has been developed from the study of human herpesvirus 1 (Herpes simplex virus, HSV-1). A comprehensive review of the history of disease recognition and early research efforts with this virus is recorded by Nahmias and Dowdle (1968). They credit Herodotus, the Roman physician, as being the first to describe the disease with the statement, "the herpetic eruptions appeared about the mouth at the crisis of simple fever."

The perpetuation of viral diseases requires a mechanism for the causative virus to survive in nature. The obligate nature of viral replication in living cells mandate a host-to-host transfer, an equilibrium of compensatory virus cell interaction, or a state of latency. The terminology describing persistent viral infections of

host cells is not well-defined. Fenner (1968) used the term latency in a general sense, but Johnson (1970) used the term in a restricted sense to give the connotation of the covert nature of virus-cell relationships. Dulbecco's (1965) enunciation of the maintenance of equilibrium conditions of viral persistence in the cell depending upon the degree of cytocidal activity of the relationship brought some order to this controversy. His designation of a steady state of independent virus-cell interaction (IVCI), in which a noncytocidal infection of all cells occurs and dependent virus-cell interaction (DVCI) where a minority of cells are cytocidally infected and synchronous replication of the nucleic acid of both the cell and virus occurs with a minute amount of spontaneous release are useful designations. Jack (1974) reviews the use and meaning of these terms and uses 4 human herpesviruses to illustrate the type of host cell-virus relationships that exist. He places all viral infections into 2 major divisions, acute and nonacute infections. Acute infections refer to the classical type described by Fenner (1968) and Johnson (1970) with primary interaction of the virus and host cell and viral replication with or without cell death. This infection is eliminated or progresses on to the nonacute state. The nonacute stage is subdivided into chronic, latent or covert, and slow infections. The chronic infection is divided into continuously detectable and discontinuously detectable. The slow infections are subdivided into slow-degenerative and slow-neoplastic. A salient feature of this scheme then becomes apparent in that, in true latency or covert infections, the virus must be completely undetectable in the interval following recovery from acute infections until exacerbation of the illness due to recrudescence

of the virus, with recurrent illness that would progress through stages exhibiting features typical of the acute infection. The outline form developed below aids in understanding Jack's (1974) conclusions:

I Acute Infections - detectable virus with or without cell death

II Nonacute Infections

a. Chronic

1. Continuously detectable

2. Discontinuously detectable

b. Latent or covert - either integrated cell virus nucleic acids or undetectable

c. Slow

1. Slow degenerative

2. Slow neoplastic

Jack (1974) suggests that human cytomegalovirus infections are predominantly chronic infections that may show both continuous and discontinuous excretion from the host, with the degree of detectability dependent upon the age of the host at the time of primary infection, or subsequent state of health, such as hormonal influences and intense chemotherapy with cytotoxic substances. He describes the host virus relationship of Varicella-Zoster Virus (VZV) as an example of the latent or covert state. The appearance of zoster (shingles) in adult life following childhood Varicella (chicken pox) virus infection usually represents a single attack. The zoster syndrome may also occur in children if provoked by disease or chemotherapy. Each person so affected gives a reliable history of varicella infection or chicken pox in childhood. The facts that zoster patients can transmit Varicella virus (and not

vice versa), that no biologic or serologic differences can be detected in VZV strains, and that there are long intervening periods of lack of virus detection indicating a true covert or latent infection. The recurrence of active lesions at unusual anatomical sites led to the speculation that the initiator of the second attack must be the sensory ganglion supplying the cutaneous area involved. This theory is further enhanced by the evidence of acute inflammatory process in the regional ganglion of infected areas in fatal zoster cases. Jack states that "it is tempting to view the mitotically inactive neurone as the site for the long-term persistence of VZV," and if so, this would not necessitate a hypothesis of viral integration with host DNA. However, he further admits that if the virus is present in the satellite or glial cells that are capable of mitoses then an integrated state may be necessary to maintain it for long periods of time. Cytotoxic and steroid therapy, neoplasia and other infections may activate VZV infections but unlike CMV there is no ensuing chronicity or generalized spread, which indicates that the pathogenic or immune mechanisms are quite different.

The theory of Epstein-Barr Virus (EBV) is truly a covert or latent infection gains support from the fact that partial integration of the genome in blastoid lymphocytes has been demonstrated. Further evidence to support this position is found in the fact that conditions that induce lymphoid hyperplasia such as leprosy, sarcoidosis, lupus erythematosus and Hodgkins disease also cause increased activity of endogenous EBV. The establishment of lymphoblastoid cell lines only from lymphocytes

infected with EBV and its association with Burkitt's lymphoma and nasopharyngeal carcinoma indicates that this latent cell-virus relationship may also be carcinogenic.

The exact nature of the cell-virus relationships in nonacute HSV infections in humans is not known. Jack suggests that covert relationships analogous to the latency of VZV may exist in some individuals, whereas chronic infections with discontinuous excretion occurs in others. In a review of literature relating to the role of nervous tissue in maintenance of the chronic or latent nature of herpesvirus infections in man and experimental animals, Baringer (1975) concludes that the most significant HSV spread from the initial site of infection is through neuronal axon flow mechanisms, even though there is some evidence to indicate that contiguous cell-to-cell spread may occur along the supporting Schwann satellite cells of the nerve fibers and ganglions. A predominant number of reports by competent investigators seem to support the view that the neuron of the sensory ganglion innervating the site of epithelial lesion recurrence provides the essential milieu for residence of the virus for prolonged periods. Baringer (1975) concedes that at present the scientific evidence is insufficient to determine the exact mechanism involved in the neuron-virus relationship and the pathophysiological alterations within the host that trigger renewed virus replication within the ganglion and subsequent epithelial lesions in areas that it innervates. Two basic theories to explain this immunologic phenomenon have gained a degree of prominence.

First, the theory proposed by Roizman (1965) is palatable because it agrees with the supposition that mammalian viral latency is comparable to the prophage state of lysogenic bacteria. This theory contends that viral replication is restricted in a static state within virogenic cells, but the potential for that replication is preserved by the incorporation of the viral genome within chromosomes of the neuron. The work of Yamamoto et al. (1977), showing a HSV directed peak of thymidine kinase activity 46 days longer than infectious virus could be detected, tends to support this theory.

The second theory, described in some detail by Ennis (1973), proposes that HSV exists in a dynamic state within low metabolic neurons of the ganglion, in which the productive viral replication and related cytopathologic changes are effectively restricted by humoral and cellular host defense mechanisms. Recent investigations by Walz et al. (1976) indicate that the number of chronically infected ganglia and subsequent recurrence may be directly related to the effectiveness of viral replication at the initial site of infection, and the ability of the host to respond immunologically to the infection.

Recrudescence of Herpesvirus Infections in Man

The precipitation of recurrent herpesvirus eruptions in man is known to be associated with a number of quite variant biophysical conditions. Such conditions as elevated temperature, exposure to ultraviolet light, strong winds, menstruation, emotional stress or physical trauma may evoke recurrent eruptions. The stress of pregnancy may potentiate primary HSV infections into generalized disseminated disease (Young et al. 1976).

The common physiologic impact, of any, of these conditions is not known. Several methods of experimental induction of recurrent lesions have been successful. Good and Campbell (1948) reported that anaphylactic shock would induce recurrence of HSV in chronically infected mice. Other workers have shown that treatment with corticosteroids (Thygeson et al. 1953), epinephrine (Schmidt and Rasmussen 1960; Laibson and Kibrick 1966), and the induction of Arthus reaction may also cause the exacerbation of chronic HSV-1 infections in experimental animals (Anderson et al. 1961).

The exact mechanism involved in the variation of the host response to acute infections, the recovery from acute infections, the resistance to re-exposure to exogenous virus, and the development of lesions due to indogenous chronic or latent virus is not completely understood. Since recurrent lesions appear in patients with high serum antibody titers, most investigators believe that some other immune mechanism plays a predominant role. Recent investigations (Zisman et al. 1969; Mori et al. 1967) have shown that macrophages and thymus derived (T) lymphocytes are key factors in controlling chronic HSV infections in mice. An elegant series of experiments by Rager-Zisman and Allison (1976) using cyclophosphamide-treated HSV-infected mice has indicated that protection against this virus infection is predominantly T cell dependent. Their results also indicate that the macrophage plays an important role in the host's recovery process. Their studies seem to imply that humoral antibodies alone play no major role in the recovery of the host. However, they suggest that the data tends to support the theory that antibody-dependent cell-mediated cytotoxicity may well be

operative in this specific incidence. Blank and Haines (1976) demonstrated that patients with recurrent HSV infections and high antibody titers could easily be super-infected in epithelial tissues at remote sites from recurrent lesions with both indigenous and exogenous virus. They seem to believe that humoral antibody plays little or no role in preventing recurrent or exogenous HSV infections.

Latent Herpesvirus Infections in Other Animals

The mouse cytomegalovirus is another example of a chronic herpesvirus infection with discontinuous detectable excretion (Medearis 1964) but it appears that a latent infection may exist in lymphoid tissue. Olding et al. (1975) demonstrated more specifically that the latent infection resided within the B lymphocyte population and that the virus could be recovered readily from lipopolysaccharide-induced blastogenesis of B lymphocytes from chronically infected mice.

The infection of guinea pigs with a guinea pig herpesvirus leads to a chronic infection from which virus can be isolated from the blood and somatic tissue for periods up to 24 months following infection. Pregnant, chronically infected animals can transmit chronic infections to fetuses (Lam and Hsiung 1971).

The infection of rabbits by Herpesvirus cuniculi is considered to be both discontinuously detectable and latent since virus could be detected in some animals as long as 100 days after infection and primary kidney cell cultures failed to yield infectious virus but contained viral antigen that could be detected by immunofluorescence.

Recurrent IBR Infections in Cattle

The nature of the immune mechanisms responsible for the recovery of cattle from acute IBRV infections, resistance to re-infection and the recrudescence of chronic or latent infection has not until recently been the subject of critical, refined scientific evaluations. The role of environmentally-induced physiological stress in IBRV infections has been investigated (Bowes et al. 1970; Baczynski et al. 1975; Mihaljovíc et al. 1973), but careful cause-related scientific analysis is difficult. The role of shipping stress, hormonal imbalances during calving (Snowden 1965), and nutritional deficiencies (Crane 1965) have also been suspect. One of the most intriguing theories is based upon the fact that certain groups of viruses may suppress the cell-mediated immune (CMI) response of acutely infected animals or depress CMI in chronically infected animals thereby allowing recurrent disease to develop. Myxoviruses have been shown to affect the CMI response in other animals. The recent report (Mensik et al. 1976) of reactivation of IBRV infection in young calves by experimental PI-3 virus infection tends to support this hypothesis.

The report by Sheffy and Davies (1972) of the corticosteroid (CS) reactivation of IBRV from both naturally and experimentally infected cattle sparked a flurry of scientific activity. They reported that 5 of 6 bulls that had humoral anti-IBRV antibodies for a period of 4 years without obvious clinical disease shed IBRV in nasal secretions within 3-5 days post treatment with dexamethasone. Nineteen pregnant Holstein heifers 11 of which had been vaccinated with commercial vaccine, were exposed to virulent IBRV and treated with dexamethasone 3-9 months later

for a period of 6 or 7 days and slaughtered 24 hours after the last treatment. IBRV was recovered readily from nasal secretions and respiratory tract of treated animals, but much less frequently from somatic tissues such as nervous tissue, adrenals and reproductive tract. Virus was also recovered from the upper respiratory tract and ovarian tissues of 1 of 9 untreated animals. Sheffy and Rodman (1973) reported corticosteroid-induced recrudescence of commercial modified live IBRV vaccine given 2½ months previously by the intravenous, intrapreputial and intranasal routes. They indicated that even though commercial inactivated IBRV vaccine injected intramuscularly evoked humoral antibody development, it failed to protect against acute infection or the establishment of chronic infections that could be exacerbated with corticosteroids. Davies and Duncan (1974) reported the use of dexamethasone and ACTH to induce recrudescence of IBRV from cattle experimentally infected by intranasal and intravaginal inoculation. In these experiments virus could be isolated easily from respiratory and genital tissue 2-5 days post treatment. Darcel and Doward (1975) demonstrated that IBRV vaccine strain could be recovered from leukocytes and vaginal secretions of CS treated cattle six weeks after intramuscular injection of the modified live virus vaccine. CS injections have been used to determine that a chronic IBRV infection existed in naturally exposed animals (Böttcher and Mahler 1970; Huck et al. 1973; Bitsch 1973; Gibbs et al. 1975). In each instance the synthetic hormone treatment was used as a tool for determining the presence of virus in animals that were suspected to be chronically infected because they had antibody in their serums.

Davies and Carmichael (1973) studied the effect of dexamethasone and ACTH upon the cell-mediated immune response during primary and recurrent infections. They suggested that suppression of cell-mediated immunity, as measured by lymphocyte transformation in whole blood cultures, occurred during CS induced recrudescence but that adrenocorticotropic hormone (ACTH) and trigenimal neurectomy induced recrudescence without a concomitant immunosuppression. Rouse and co-workers have recently published a series of papers (Rouse and Babiuk 1974a, 1974b, 17975a, 1975b; Rouse et al. 1976; Babiuk and Rouse 1975; Babiuk et al. 1975) concerning their investigations into the immune mechanisms of acute and chronic IBR infections. Their conclusion is that almost all well-known immune mechanisms play a role in the recovery from, the maintenance of, and the recurrence of IBRV infections.

Ontogeny of Immunocytes and Phagocytes

The varied expressions of immune responsiveness and resistance to disease are a complex of synergistic and complementary antagonistic interrelationships of cells and their metabolic products. The current knowledge of the ontogeny of these cellular and humoral interactions and the in vitro methodology used to study them have been reviewed by Globerson (1976). The review deals primarily with the embryogenesis, mechanisms of developing immunocompetence, and the interrelationships of immune responsiveness of B and T lymphocytes and macrophages. The synergistic interactions of these three cell populations have been studied more carefully than others but the role of granulocytic leukocytes

in resistance to disease, and their potential role in regulating the immune response cannot be ignored.

Ontogeny of the B lymphocyte

The discovery by Glick et al. (1956) of the existence of a distinct population of immunoglobulin producing cells in chickens that were dependent upon their development in the bursa of Fabricius led to the current terminology of B-cell or B-lymphocyte. Though there has been no agreement on the bursa-equivalent compartment in mammals Globerson's evaluation of the research surrounding the controversy seems to indicate that: (1) the embryogenesis of the B-lymphocyte in mammals begins in the yolk sac with the possibility of some stem cell development in the liver, (2) the migration of the stem cell from the yolk sac to the embryonic liver where these and indigenous stem cells develop the capability of producing immunoglobulin-M, (3) further migration of these precursor B-lymphocytes to the bone marrow for the final stage of maturation. Thus, in mammals, a concert relationship of the fetal liver and the bone marrow seems to perform an analogous function as the avian bursa of Fabricius. The existence of humoral factors necessary for final stage maturation in the bone marrow microenvironment is suspected. The ability of B-lymphocytes to produce immunoglobulins is dependent both upon soluble factors produced by T-lymphocytes and the physical presence of monocytes.

Ontogeny of the macrophage

The macrophage plays an important role in resistance to disease. Although Globerson (1976) maintains that the preponderance of scientific

literature suggests the progenitor cells of the promonocyte of the bone marrow may have its origin in the embryonic liver, he admits that the macrophage developing in the bone marrow may originate from other distinct precursors. This idea is preferred by Gordon and Cohn (1973) in their review of the macrophage. Though this controversy has not been settled, all agree that the continuing development of immune competence takes place in the bone marrow from which the monocyte is released upon maturation. The review by Gordon and Cohn (1973) of the present knowledge of the role of the monocyte details many specific functions of the macrophage in the immune response and describes many of their properties. They enter the blood stream from the bone marrow and comprise, as monocytes, from 3-5% of the circulating leukocytes. Their half-life in the blood stream is approximately 22 hours, due to random migration between endothelial cells of capillaries. The tissue monocyte matures into a more active cell, the tissue macrophage, which divides rarely and has a 60 day life span. These cells are particularly prominent as Kupffer cells of the liver alveolar macrophages of the lung, and histiocytes of the spleen and bone marrow. Macrophages are often found in large accumulations, granulomata, in certain local inflammatory conditions. These "epitheloid" cells, though less endocytic, may have many more mitochondria in their cytoplasm than other macrophages. They may fuse to form multinucleated giant cells. Macrophages are highly phagocytic in the fetus and neonate but their digestive processes are poorly developed at this stage. This enzymatic process develops rapidly after birth and may be related to "sensitization" to a multitude of

conventional antigens. The role of the macrophage in the immune process has many ramifications including phagocytosis, digestion, antigen processing and interaction with other immunocytes through contact and soluble factors produced by the macrophage.

Ontogeny of the T lymphocyte

The ontogeny of T cells (thymus processed lymphocyte) reviewed by Globerson (1976) can be summarized as follows: The exact origin of the primordial cell that differentiates into T lymphocytes is unknown. The progenitor cells for T lymphocytes originate in the fetal yolk sac and migrate to the thymus; nevertheless the critical marker (θ antigen) for T cell identification in the mouse develops within the microenvironment of the thymus. Certain thymic humoral factors (THF) are necessary for the maturation of immature T cells into mature cells bearing the θ antigen and having the capability of inducing the graft-versus-host (GHV) response. It has also been demonstrated that whereas only the THF is required for θ Ag development, direct contact with viable thymus is required for other T cell functions such as T helper activity and the GHV response. The exact nature of this essential thymic microenvironment is unknown. It appears that the T cell function in neonatal life lags behind the potential to produce B cell responses.

Function and Relationship of Immunocytes and Phagocytes

The intricate developmental and functional relationships between B and T lymphocytes have been reviewed by Cooper and Lawton (1974). They

contend that both cells arise from a common pluripotential stem cell in the yolk sac and follow separate developmental pathways through the fetal liver, bone marrow, and thymus to develop varied but interdependent functional immune expressions.

B lymphocyte function

The classic expression of humoral immunity mediated through the production of immunoglobulins by the plasmacyte (mature B lymphocyte) is well-recognized. The principal impact of the B lymphocyte upon resistance to disease is through the production of at least 5 well-defined immunoglobulin (Ig) classes. These Ig molecules (antibodies: IgM, IgG, IgA, IgD, and IgE) are synthesized and secreted as a result of antigen exposure and are capable of combining with the specific stimulating antigenic moiety upon contact. Claman (1973) points out that the Clonal Selection Theory of "one cell -- one antibody" of Talmadge and Burnet is no longer adequate to explain antibody production, since we now know that macrophages and T lymphocytes play important roles in immunoglobulin production. Their response is mediated through macrophage antigen processing and T "helper" cell stimulation of lymphokine production that functions to turn on antigen-specific immunoglobulin production by a given B lymphocyte. However, he also points out that some antigens can turn on B lymphocytes to immunoglobulin production without macrophage-T-cell processing. It is interesting that all these antigens are polymers (polysaccharides, dextrans, and lipopolysaccharides) and all induce antibodies of the IgM class. It is generally recognized (Merigan 1974)

that immunoglobulin-mediated disease resistance predominates in such viral infections as enteroviruses, flaviviruses and certain myxoviruses.

T lymphocyte function

It has been suggested that cell-mediated immune responses are the primary resistance factors in such viral infections as herpesviruses, measles virus and visna virus (Allison 1974). T lymphocytes are responsible for the reactions of cell-mediated immunity (CMI) expressed as delayed hypersensitivity, allograft and tumor immunity and graft versus host reactions and their in vitro correlates. Expressions of CMI are mediated through the production of several different lymphokines in response to specific antigen - T cell interactions. Some of the more important factors include: (a) chemotactic factors for monocytes and neutrophils; (b) blastogenic factors; (c) macrophage migration inhibition factors; (d) factors that enhance the capacity of macrophages to destroy intracellular parasites; (e) interferon; and (f) factors capable of lysing target cells (lymphotoxin) (Pierce and Benacerraf 1976). This list of T lymphocyte origin factors could be expanded based upon the fact that these substances have usually been recognized and described solely upon their biologic activities. Therefore, a logical question of the possibility of a single or a few mediating substances with different biological manifestations being related to the assay system has not been adequately answered. David (1973), however, has reviewed the biologic nature and physiochemical properties of many of these lymphokines. He concluded that even though several of the factors, recognized and named according

to biologic reactivity in a specific assay system, may be similar at least the six listed above appear to represent different biochemical substances. In addition to the secretion of these chemical substances by antigen-activated T cells, several other biologic properties of such cells are also believed to be important in CMI. Under some circumstances activated T lymphocytes appear to be able to act as aggressor or "killer" cells when faced with certain specific antigen-induced cell membrane alterations (Cerottini et al. 1970a, 1970b). Woodruff and Woodruff (1975) suggested that this function may play an important role in recovery from and resistance to viral diseases.

The current knowledge of the functional specificity of T lymphocytes has been reviewed by Paul and Benacerraf (1977). They summarize the T cell regulatory function upon B lymphocytes in six different categories: (1) the stimulation of IgG production; (2) the selection of precursors of cells that secrete a given class of Ig and allotypic variants of the Ig subclass; (3) the increase in affinity of the specific antibody produced; (4) the selective activation of a clonally restricted hapten-specific antibody; (5) the expression of common idiotypes on antibody molecules specific for distinct antigenic determinants presented on the same carrier molecule; and (6) the selection of antibody forming cells which produce hapten-specific antibody of opposite negative charge fo the hapten-carrier complex. In addition to the positive influence of helper effect of T cells upon immunoglobulin production by B lymphocytes, there is considerable scientific evidence to indicate that T cells may serve a regulatory function upon B cells to depress immunoglobulin over-production or even T cell response to specific antigens (Katz 1972; Gershon 1974).

Monocyte/Macrophage function

With the expansion of our scientific knowledge concerning the mammalian immune system, it becomes more apparent that the mononuclear phagocyte or the macrophage plays a central role in resistance to disease. The macrophage-lymphocyte interaction is the main line of body defense. Macrophages serve as primary effector cells in amplifying the immune response. Its influence is exerted through its interrelationships with lymphocytes upon immunoglobulin production by antigen processing and as an effector cell in delayed hypersensitivity. The general knowledge of macrophage origin and function in resistance to disease has been reviewed by Gordon and Cohn (1973) and Unanue (1976). In addition to the classic phagocytosis and digestion of invading pathogens and lysosomal enzyme secretion, they emphasize the role of the macrophage in modulating the activity of surrounding cells and tissues through chemical secretory products. These reviews point out that activated macrophages secrete several substances that have a regulating effect upon other cell activities: (1) a mitogenic principle that is highly stimulatory for lymphocyte proliferation; (2) a substance that promotes the rapid differentiation or maturation of B-lymphocytes to the mature plasmacyte-antibody secreting cell; (3) an enhancement of T cell function; (4) a colony stimulating factor that promotes the proliferation of bone marrow stem cells that differentiate into granulocytic and monocytic phagocytes; (5) inhibitory or lytic molecules that seem to depress DNA synthesis and cause certain cells to lyse; (6) complement factors, C_4, C_2 and possibly C_3 and C_5; (7) pyrogens; and (8) interferon. The reviewers hasten to add that since

these biologic factors have not been identified chemically more than one of these activities may be due to a single chemical entity. Allison (1974) summarized the current knowledge of the role of macrophages in the mammalian immune response to virus infection. He emphasizes the well-known fact that one primary function of the macrophage is to phagocytize and digest viruses invading the lung and circulating in the blood stream to prevent further invasion and multiplication in parenchymal tissues. The macrophage-virus relationship then becomes one of extreme importance. The ability of certain viruses to replicate within the macrophage and thereby spread to nearby tissues is well-known.

The resistance of certain strains of mice to mouse hepatitis virus and yellow fever virus was found to be genetically related to the ability of their macrophages to destroy these viruses, whereas the macrophages of nonresistant strains supported viral replication. Allison (1974) reviews the data that indicate that peritoneal macrophages can be used to block lethal herpes simplex virus infections in young mice and that macrophages from adult donors do so more effectively than those from immature donor mice. The human counterpart is the severe generalized herpes simplex infection in newborn children. Allison (1974) is quick to point out, however, that scientific investigations support the idea that the maturation of macrophages in their virucidal capacity may be linked to cell-mediated immunity and stimulation with bacterial or other antigens. The recruitment of inflammatory mononuclear phagocytes into areas of virus multiplication is a long-recognized phenomenon, and as stated previously, there are many indications that immunologically specific T lymphocytes are responsible for this chemotaxis in viral infections.

Chemical Immunosuppression

The mode of action of cyclophosphamide

Cyclophosphamide is one of the most commonly used alkylating agents for immunosuppressive therapy. The main site of action of the alkylating agents is their interference of nucleic acid synthesis and subsequent cell mitosis. These compounds also cause a miscodification of messenger, transfer, and ribosomal RNA and thereby interfere with protein synthesis (Miescher et al. 1976). Turk and Poulter (1972) studied the effect of cyclophosphamide treatment upon lymphoid tissue of mice and guinea pigs. They found that cyclophosphamide produces a selective depletion upon lymphoid cells from the corticomedullary junction and lymph follicles of mouse and guinea pig, cervical, axillary and mesenteric lymph nodes following 1 and 3 daily injections of cyclophosphamide at the rate of 300 to 400 mg/kg body weight. The lymphoid cells around the post capillary venules and paracortical areas were spared. Lymphocytes were markedly depleted in the lymphoid follicles of the spleen except around the central arteriole of the white pulp. In short, their work showed that cyclophosphamide treatment caused a selective depletion of lymphoid cells in areas classically populated with B cells but had little or no effect upon cells in the areas routinely populated with T cells. Stockman et al. (1973) reported that the effect of cyclophosphamide treatment of mice exerted a more severe and longer-lasting depression upon the B lymphocyte than upon the T lymphocyte. Turk et al. (1972) reported an increased intensity and prolongation of contact hypersensitivity reactions in guinea pigs treated with 300 mg/kg body weight of cyclophosphamide. They concluded

that the treatment did not alter T lymphocyte function but markedly reduced B lymphocyte function. Hunninghake and Fauci (1976) studied the effect of cyclophosphamide treatment upon peripheral blood leukocytes of guinea pigs. They found a marked 5-day sequential reduction in total numbers of neutrophils and lymphocytes but no significant reduction in monocytes. Askenase et al. (1975) reported that cyclophosphamide treatment amplified rather than depressed the development of delayed-type hypersensitivity of mice to sheep red blood cells. A comparative study of the effects of immunosuppressive drugs upon cell-mediated cytolysis was conducted by Borel (1976) who reported that corticosteroids caused a marked inhibition of this T lymphocyte mediated response but that cyclophosphamide treatment showed no effect upon this activity in mice. Pekárek et al. (1976) showed that both cyclophosphamide and hydrocortisone interfered with the action of the T cell produced lymphokine (MIF) action upon macrophage migration inhibition tests.

Finally, several studies concerning the effect of cyclophosphamide treatment upon the recovery and resistance to viral infections have been conducted. Rager-Zisman and Allison (1973) studied the effect of cyclophosphamide therapy upon coxsackie B-3 virus infection in mice. They found that young adult, cyclophosphamide-treated mice injected intraperitoneally with coxsackie B-3 virus developed fatal infections whereas nontreated controls developed mild transient infections. They found a marked decrease in antibody production, high titers of virus and severe lesions in target organs in cyclophosphamide-treated mice but stated that the macrophage reactions in virus infected tissues were close to

normal. Two reports (Worthington et al. 1972; Worthington and Baron 1973) of the effect of cyclophosphamide treatment of vaccinia-virus-infected mice give solid evidence that drug treatment results in increased mortality and decreased antibody production to the virus but has no effect upon interferon production and the cell-mediated immune response to the virus. McFarland et al. (1972) studied the effect of cyclophosphamide treatment of mice upon the characteristic mononuclear inflammatory response due to Sindbis virus encephalitis. They found that the treatment of mice with a single injection of cyclophosphamide as long as 24 hours after intracerebral inoculation with Sindbis virus caused ablation of the perivascular mononuclear response characteristic of most viral infections.

Chin and Hudson (1974) studied the ultrastructural changes in mouse peritoneal macrophages that occurred following a single intravenous injection of a sublethal dose of cyclophosphamide. They found that rapid destruction of lymphocytes, neutrophils, eosinophils, and mast cells occurred as early as 6 hours following cyclophosphamide treatment. The morphology and phagocytizing ability of peritoneal macrophages were unchanged by the treatment. Investigations by Turk and Parker (1973) indicate that cyclophosphamide treatment enhances and amplifies the Jones-Mote type hypersensitivity in guinea pigs but they indicate that the potentiating effect of the drug on the reaction is modulated by a depression of B lymphocyte function which serves as a moderator of the Jones-Mote response.

Mode of action of corticosteroids

Corticosteroids (CS) are used extensively for their anti-inflammatory and immunosuppressive effect. However, the exact mechanism through which they modify immunologic phenomena is not known. Claman (1975) has reviewed the literature concerned with the mode of action of corticosteroids, especially as it relates to their effect upon the cell-mediated immune response. He concludes that even though much of the early literature seemed to support the idea that CS acted through their lytic effect on T lymphocytes the preponderance of more recent investigations indicates that the primary effect is rather upon macrophage function. CS administration seems to decrease blood levels of monocytes, inhibit the response to T cell produced migration inhibition factor and profoundly inhibits phagocytic and digestive mechanisms of macrophages. However, the effect of CS upon lymphokine production by T lymphocytes is controversial. Whereas there are reports (Weston et al. 1973; Barlow and Rosenthal 1973) that indicate no interference in MIF production by CS, other investigations (Barlow et al. 1975) provide evidence to indicate that a single injection of CS produces no measurable effect on MIF production but that continued daily administration caused marked decrease in antigen-induced MIF production with depression of cell-mediated lymphocyte function. Wahl et al. (1975) demonstrated that pharmacological levels of CS inhibited both macrophage chemotactic factor (MCF) and macrophage inhibition factor (MIF) production by T lymphocytes. They also showed that the drug blocked the action of MIF on macrophages but did not interfere with the action of MCF. Fauci (1975) has shown that CS have a

two-fold effect upon circulating lymphocytes. First it produces a lymphocytopenia related to redistribution of lymphocytes to other body compartments, particularly the bone marrow, and secondly a direct interference by suppressing lymphocyte proliferation. Schlesinger and Israël (1975) investigated the effect of CS administration upon different subpopulations of thymic lymphocytes. They found that cortisol depleted mouse thymic spleen-seeking lymphocytes to a greater extent than the population of lymph-node-seeking thymic cells, but that the drug completely eliminated the small population of thymus antigen positive lymph-seeking cells (those primarily responsible for the cell-mediated immune response). Fauci and Dale (1974) also studied the effect of hydrocortisone treatment on subpopulations of human lymphocytes and determined that transient selective depletion of monocytes and subpopulations of human T lymphocytes followed single dose treatments of the drug. Several investigations concerned with the effect of CS treatment upon macrophage function have been reported (Rinehart et al. 1974; Hunninghake and Fauci 1975; Dimitriu 1976). The studies of Rinehart et al. (1974) indicated that blood monocytes have a unique sensitivity to corticosteroid therapy. They were able to demonstrate impaired random movement, specific chemotaxis and bactericidal activity of monocytes after only 60-90 min. exposure to low levels of hydrocortisone. Their observations suggested that defective monocyte function contributes to impaired cellular immune resistance in patients receiving corticosteroids. Investigations by Hunninghake and Fauci (1975) demonstrated that CS treatment caused a marked decrease in cytotoxic effector function of alveolar macrophages by interfering with

its capability to bind to the target cell. Dimitriu (1976) studied the effect of CS treatment to modify the capacity of sensitized T lymphocyte products to induce cytotoxic properties to mouse peritoneal macrophages. He found that the effector lymphokine {macrophage arming factor (MAF)} production was not impaired but that macrophages from treated animals failed to respond to the effects of MAF during CS treatment. Whether or not these impaired functions are related to the stabilization of the macrophage lysosomal membranes and impaired intracellular digestion (DeDuve et al. 1962; Weissman and Dingle 1961) and concomitant aggression of macrophages to susceptible target cells by exocytosis of lysosomal enzymes as suggested by Hibbs (1974) is merely theoretical.

Many investigators have studied the effect of CS treatment upon the production of interferon in virus infected tissues. Their reports (Kilbourne et al. 1961; DeMaeyer and DeMaeyer 1963; Smart and Kilbourne 1966a, 1966b; Mendelson and Glasgow 1966; Rytel and Baylay 1973) agree that the drug causes a marked reduction in interferon levels in virus infected host cells. Results reported by Smart and Kilbourne (1966a, 1966b) which suggested that cortisone inhibited the action of preformed interferon contradicted the investigations of DeMaeyer and DeMaeyer (1963). Whereas most of these reports emphasize the importance of interferon in recovery from and elimination of viral infections, the work of Rytel and Balay (1973) emphasizes that its inhibition by corticosteroid treatment of immunosuppressed patients may play an important role in reactivation in chronic virus infections.

Finally, Schreiber et al. (1975) have shown that in vitro CS treatment of human mononuclear phagocytic cells inhibited both IgG and

complement receptor activity of the phagocytic cell membrane. This important immune mechanism was depressed at drug dosage levels that had no inhibitory effect upon the ability of the monocyte to phagocytize latex and neutral red particles.

Delayed-Type Hypersensitivity to Specific Antigens

Bosman and Feldman (1970) reviewed the cellular events that occur in the pathogenesis of delayed-type hypersensitivity (DTH) reactions following the intradermal injection of antigen to which the host had been sensitized. In the same report they summarize their own investigations into the composition, morphology and source of cells in skin reactive tissue in sensitized rats. A reasonably rational brief description of the cellular response to DTH reactions can be formulated from this report as follows: First, at the site of injection of the sensitizing antigen a relatively small number of antigen specific T lymphocytes accumulate, react with the specific protein, and produce a number of inflammatory reagents. Three principal factors involved in the continued DTH response appear to be macrophage chemotactic factor (MCF), macrophage inhibitory factor (MIF), cytotoxins, and permeability factor. The combined action of MCF and MIF directs the subsequent site accumulation of nonspecific bone marrow-derived macrophages from the adjoining arterioles and capillaries. The 24-48 reaction is predominantly of the monocyte/macrophage moiety (approximately 80%).

DTH reactions to intradermal injections of viral antigens have been demonstrated following herpesvirus infections in man (Nagler 1944; Nagler 1946; Rose and Malloy 1947), chickens (Dawe et al. 1971), pigs

(Skoda et al. 1968; Yotov 1973; Smith and Mengeling 1977), and cattle (Darcel and Doward 1972; Girón et al. 1975). Both reports of the use of skin test in IBRV infected cattle indicate that not all animals with serum virus-neutralizing antibodies responded with a positive reaction and that one animal in an infected group of cattle repeatedly tested negative to SNV but was posibive to the DTH response (Girón et al. 1975).

MATERIALS AND METHODS

Cell Cultures

Embryonic bovine turbinate (EBT) cells

Primary cell cultures were prepared from the surface epithelial tissues of the turbinates of fetal calves following the basic procedures of McClurkin et al. (1974). Modifications included the use of only surface epithelial tissue and the use of Hank's balanced salt solution (HBSS) to replace PBS in the initial culture preparation. Metabolic depression, degenerative changes and support of viral replication seemed to be inhibited in 37th to 45th passage levels; therefore, new primary cells were prepared when these levels were reached. Each new primary cell culture and second and subsequent random passage levels were tested for indigenous bovine viral diarrhea (BVD) virus and mycoplasmas; only those found to be free were maintained. Cell stocks were maintained in 75 cm^2 plastic flasks[1] and transferred at a 1:2 ratio from 1 to 6 week intervals depending upon the need for cells. Routine weekly supplies were prepared by Central Services, National Animal Disease Center (NADC), Ames, Iowa.

Embryonic bovine kidney (EBK) cells

EBK cells were prepared in a manner similar to that described for EBT cells and were used only initially in passage of viral stocks and to prepare exposure viral suspensions for experiment 2.

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[1]Falcon Plastics, Inc., Oxnard, CA.

Colostrum-deprived isolation reared (CDIR) calves

Except for cattle in experiment 1, all experimental cattle were CDIR calves. They were obtained from commercial farms by the Animal Supply Section, NADC, and hand-reared in isolation by a modification of the technique reported by Edward et al. (1967). Modifications of the technique included keeping all calves deep-bedded on fir shavings under heat lamps for the first week of life and a feeding schedule of 4 times each day of dried milk replacer with antibiotics.[1]

The supplier's recommendations were followed on the daily amount of dried milk replacer but approximately one-half the recommended daily amount of water was used for reconstitution. All calves were fed 4 times each day at approximately 8 hour intervals for the first week and gradually changed to 3 then 2 feedings at 8:00 AM and 4:00 PM at 2 weeks of age. Reconstituted milk replacer was fed from hand-held plastic nippled bottles that were cleaned and immersed in 0.1% Roccal[2] solution between feedings. First-day-of-life blood samples were taken and serum was separated from clotted blood and tested for IBRV antibodies.

Conventional cattle

Eleven feeder steers approximately 11 months of age that had been raised in the conventional manner in commercial herds, were used in experiment 1. They had been purchased by the Animal Supply Section, NADC, from herds that were free of IBRV antibodies.

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[1]Land O' Lakes Creameries, Minneapolis, MN.

[2]Alkyl dimethyl ammonium chloride, National Laboratories, Montvale, NJ.

Virus Strains and Virological Techniques

Seed stock and IBRV working-stock pools were prepared in large batches from infected EBK and EBT cell monolayers that had been tested and found free of BVD and mycoplasmas. Pools were divided into small aliquots and stored frozen at -90 C until ready to be used. Aliquots of the pool were titrated immediately after preparation and during each major incident when the stock was used.

IBRV-Colo strain

This IBRV strain was obtained, as a reference seed stock virus, from Dr. T. W. Tamoglia, USDA, Veterinary Services Biologics Laboratory, Large Animal Viral Products Section, Ames, Iowa. He had received the strain from Dr. T. L. Chow, Colorado State University, Fort Collins, Colorado, who stated that it was an isolate of bovine respiratory disease origin that had been passed 5 times in embryonic bovine kidney (EBK) cells. Dr. Tamoglia passed the isolate once in EBK cells, exposed a nonimmune calf intranasally, and prepared the seed stock from primary EBK cells inoculated with nasal secretions collected 10 days post inoculation. The seed stock virus was passed 2 times in primary EBK cells in our laboratory for exposure pools of virus (Experiment 1) or continued 1-3 additional passages in EBT cells for all other experiments. All cells used for passage and virus pool production were free from BVDV and mycoplasmas.

IBRV-LA strain

The LA (Los Angeles) strain of IBRV was obtained from the American Type Culture Collection (ATCC No. VR 188) Rockville, Maryland. The isolate was passed 2 times in EBK cells for exposure pools of virus.

IBRV-K22 strain

The K22 strain (Potgieter and Maré 1974) IBRV was obtained from Dr. L. Potgieter, Department of Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa. It was a strain received from Dr. D. G. McKercher, School of Veterinary Medicine, Davis, California. The strain was isolated from a clinical case of infectious pustular vulvovaginitis and has been passed approximately 28 times in bovine cell cultures. It had been plaque purified 3 times and passed 9 times in Madin-Darby bovine kidney cells (MDBK:21) when we received the seed stock. The stock pool (exposure virus suspension, Experiment 4) was prepared from first seed stock passage EBT cells. The titer of this virus pool was $1.0 \times 10^{8.2}$ pfu/ml of suspension.

IBRV-Jensal strain

The Jensal strain (Potgieter and Maré 1974) was a modified live virus vaccine strain. The seed stock virus pool was prepared from a commercial vaccine[1] by Dr. Potgieter in the 3rd passage in MDBK:21 cells. The stock pool (exposure virus suspension, Experiment 4) was prepared from first seed stock passage in EBT cells. The titer of this virus pool was $1.2 \times 10^{8.2}$ pfu/ml of suspension.

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[1]Jensen-Salisbury Laboratories, Kansas City, MO.

Virus isolation techniques

Nasal swabs were taken from cattle only after cleaning of the anterior nares. Cotton-tipped applicators were inserted approximately 6 inches into the dorsal meatus of the nasal passage, removed and placed into 2.5 ml of pre-cooled MEM containing 4X antibiotics and mycostatin. The samples were agitated, stored frozen at -90 C until tested, or the nasal swabs expressed of absorbed fluids, the tubes were centrifuged at 600 Xg for 20 min and 0.1 ml of the supernatant fluid placed on each of 3 monolayer cultures of EBT cells and observed daily for 10 days for the development of viral-induced CPE. Cell cultures with CPE typical of IBR were harvested by freezing within 24 hours of the initial observance of CPE. Cultures without obvious CPE were passed onto new monolayer cultures after 10 days of incubation. Two such passages were made and observed for 10 days each before a final negative result was recorded. Selected positive samples (those with typical CPE) from each experiment were identified as IBRV by serological methods.

Tissue samples from experimental animals taken during postmortem examination were minced, ground in a Tenbroeck tissue grinder, and prepared to a 20% suspension in MEM containing antibiotics and mycostatin. One-tenth ml of the supernatant fluid was removed and placed into each of 3 monolayer EBT cultures. The samples were handled in the same manner as described above for nasal swab samples.

Immunological Methods

Serological tests to quantitate the amount of viral antibody in a serum sample and to identify viral isolates essentially as described by Lennette and Schmidt (1969) were used in these studies. Fluorescent antibody procedures (Brown et al. 1968) for the identification of IBRV in infected tissues were used occasionally.

Serum-virus neutralization (SVN)

Serum neutralization of viral plaque reduction was used to quantitate IBRV antibody in serum samples. Tests were performed in EBT cells. Confluent monolayer cultures of EBT cells were prepared in either 75 cm^2 plastic flasks or 60 mm plastic Petri dishes[1]. Cultures were incubated at 35 C and Petri dishes were kept in a 5% CO_2 humidified chamber. Overlay media was prepared by dissolving 7.0 gm Ion agar 2S[2] in 857 ml of boiling distilled water then cooled to 44 C in a water bath. The following reagents were then added to the 44 C agar: (1) 97 ml of 10X minimum essential medium (MEM), Eagle, with Earle's salts,[3] (2) 30 ml heat inactivated (56 C for 30 min) fetal calf serum, (3) 14 ml 7.5% $NaCHO_2$ in H_2O, (4) 5 mg Gentocin[4] (1 ml), and (5) 35 units Mycostatin[5]/ml. The mixture

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[1]Falcon Plastics, Inc., Oxnard, CA.

[2]Colab Laboratories, Glenwood, IL.

[3]Grand Island Biological Compound, Grand Island, NY.

[4]Gentamicin sulfate, Schering Corp., Kenilworth, NJ.

[5]Nystatin, E. R. Squibb and Sons, Inc., New York, NY.

was allowed to remain at 44 C until dispensed (within 1 hr) in 5 ml amounts as an agar overlay.

Virus-challenge stock pool aliquots were diluted in MEM containing 5% guinea pig serum (complement) to give approximately 100 plaque forming units/0.1 ml. Sera to be tested were heat inactivated at 56 C for 30 min. and 2-fold dilutions (1:2 through 1:256 routinely) were prepared in MEM. A test control negative and a test control positive serum of known antibody titer were included in each group of serum to be tested. Equal amounts of the challenge virus-complement suspension and the appropriate serum dilutions were mixed and allowed to react overnight at 4 C. The reaction was continued for 1 hr at room temperature prior to placing the mixture on cell monolayers. Medium was removed from cell culture monolayers and 0.2 ml of each serum-virus mixture was added to each of 3 monolayer cultures. The mixture was adsorbed to the cell monolayer for 90 min at 35 C in a humid, 5% CO_2 atmosphere. During the adsorption period the Petri plates were agitated every 15-20 min to redistribute the suspension. Following the adsorption period the monolayer was rinsed with 4 ml of MEM and 5 ml of the liquid (warmed) freshly prepared agar was added to each dish and allowed to cool to room temperature, inverted and incubated for 5-7 days before plaques were counted. Agar overlay was gently removed from monolayer cultures; they were stained for 2-3 minutes with a 1% crystal violet tincture of 20% ethyl alcohol. Plaques were counted and the antibody titer was recorded as the reciprocal of the serum dilution that had neutralized 50% or more of the plaques when compared to the negative serum control.

Whole blood cultures

The whole blood culture techniques of Park and Good (1972) as modified by Johnson and Muscoplat (1973) were further modified by increasing the volume of whole blood cultured to 0.25 ml amounts diluted with 0.25 ml of RPMI-1640[1] medium containing 15% fetal calf serum. Further modifications included the lengthening of the prelabeling incubation period to 48 hours, and the adaptation of a more convenient harvesting method with the apparatus described below.

Whole blood cultures were prepared as follows: 0.25 ml of venous heparinized blood was mixed with 0.25 ml of RPMI-1640 containing 15% fetal calf serum. Duplicate cultures were prepared for each mitogen dilution or specific immunostimulant dilution. Duplicate control cultures containing cell control antigen and RPMI-1640 only were also prepared from each blood sample. Preselected dilutions of phytohemagglutinin-M (PHA-M), specific viral mitogens (IBRV-Ag) and cell control antigens were added in 50 µl amounts to each culture at the time of set-up. The cultures were incubated in a humidified CO_2 incubator at 37 C for 48 hours and then pulsed with 1.0 µci of ^3HdT[2] and the incubation continued for another 14 hours. The initial efforts to conduct whole blood cultures following the methods of Good and Campbell (1948) and Johnson and Muscoplat (1973) gave erratic results because of the numerous cumbersome techniques

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[1]Roswell Park Memorial Institute Medium #1640, Grand Island Biological Co., Grand Island, NY.

[2]Thymidine (Methyl ^3H) 6.7 Ci/M mole, New England Nuclear Co., Boston, MA.

of the harvesting procedures; therefore, a method for harvesting was developed whereby whole blood could be harvested by lysing erythrocytes with distilled water and using glass fiber filters[1] to retain DNA of the stimulated cells in culture. Microsized filters and equipment used in the Otto-Heller cell precipitator/harvester could not be used because the debris in the large volume whole blood cultures rapidly clogged all filter pores. A manifold-type multiple macrosized filter apparatus was designed (Figs. 1 and 2). The fabrication was completed by the Plastic Shop of the Equipment and Preventive Maintenance Section, NADC. The body of the apparatus was constructed to two solid blocks of plastic (25 cm x 10 cm x 10 cm and 25 cm x 10 cm x 5 cm). Conical wells 2.5 cm in diameter at the base were drilled 2.0 cm apart and filled with a filter base support taken from a SX 2500 Milipore filter[2] (see Fig. 2). Effluent holes were drilled to connect from each filter and fitted to connect to a vacuum line. Matching holes were drilled in the top block and fitted with plastic syringe barrels. Grooves were constructed in the bottom part of the top block to seat a 2.50 cm diameter rubber "O" ring (Fig. 2). Metal clamps (Fig. 1) were affixed to secure the blocks together in an air tight fashion. During harvesting the effluent tube was fastened to a vacuum line and distilled water-lysed cultures poured into plastic syringe barrels and washed with 40-50 volumes of distilled water. Twelve cultures could be harvested before the manifold was disassembled, filters removed and replaced with new filters for the next

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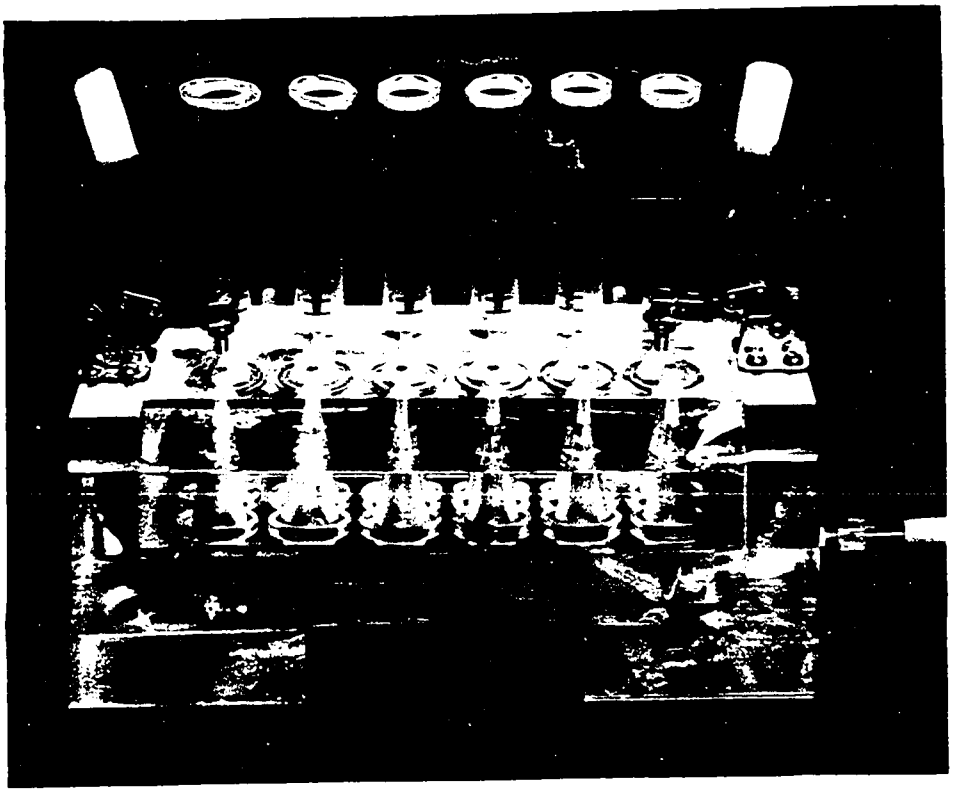
[1]Glass Fiber Filter, 934AH, Reeve Angel Co., Clifton, NJ.

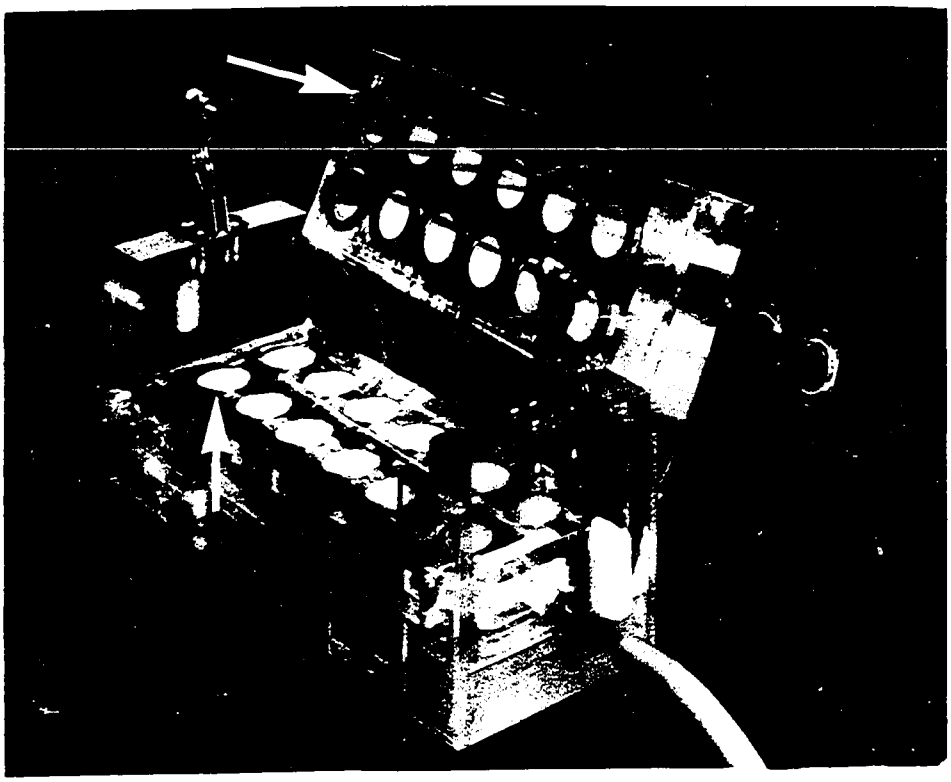
[2]Millipore Corp., Bedford, MA.

Figure 1. A manifold-type multiple macrosized filter apparatus designed to harvest whole blood cultures.

Notice locking clamps and 6 plastic syringe barrels in position for harvesting. Six other adapters are available.

Figure 2. Whole blood culture harvester opened to demonstrate rubber "O" ring seal and filter base (arrows).





culture. The filter discs were placed into scintillation counting vials and dried overnight. The following morning each scintillation counting vial containing the disc was filled with 10 ml of POPOP scintillation counting fluid and the amount of radioactivity determined by counting in a Model 3380 Packard scintillation counter[1]. The stimulation index was calculated by dividing the average counts per minute of 2 IBRV-Ag or PHA-M stimulated cultures by the average counts per minute in 2 control cultures that had been mock stimulated with a control antigen prepared from noninfected EBT cells by the same method used to prepare the IBRV-Ag.

Isolated lymphocyte microcultures

Blood was collected by venipuncture of the jugular vein and immediately placed into tubes containing 50 USP units of heparin per ml of blood. Heparinized blood was diluted 1:4 in physiological saline (1 part blood, 3 parts saline) mixed thoroughly and 10 ml was carefully layered over 4.0 ml of Ficoll-Hypaque solution. The suspension was centrifuged at 400 Xg for 30 min at room temperature. The banded layer of lymphocytes was removed, resuspended (washed) in 20 volumes of HBSS and centrifuged at 250 Xg for 30 min at room temperature. The supernatant was removed, the washing step repeated and the cells were suspended in a small amount of RPMI-1640 medium containing 15% homologous serum. The cell suspension was counted in a Model 75 Sanborn Frommer cell counter[2] and diluted to a final concentration of 2.0×10^6 cells per ml in RPMI-1640 containing 15%

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[1]Packard Instrument Co., Downers Grove, IL.

[2]Sanborn Co., Waltham, MA.

homologous serum and 100 units of penicillin in 100 mg streptomycin per ml of medium. The cultures were prepared in flat-bottomed, 96-well micro-filter-type tissue culture plates[1]. Appropriate numbers of microtiter plate wells were filled with 0.15 ml of the cell suspension from each animal. Desired amounts of each mitogenic stimulant were added to triplicate cultures. The plate was incubated at 37 C in a humidified 5% CO_2 atmosphere. Following 50 hours of incubation each culture was pulsed with 0.25 µci of ^{3}HdT in 0.05 ml RPMI-1640 and the incubation continued for 20 hours. Initially 1.0 µci of ^{3}HdT per culture was used; later it was found that 0.25 µci was just as effective. Cultures were harvested from the plates with an Otto Hiller cell precipitator/harvester, Model A[2], that lysed the cultures with distilled water and deposited the prcipitate on glass fiber filter paper that retains DNA molecules. The filter papers were dried overnight in scintillation counting vials filled with 10 ml of POPOP scintillation counting fluid and the radioactivity counted on a Packard scintillation counter. The stimulation index was calculated by dividing the average counts per minute of 3 stimulated cultures by the average counts per minute in 3 control cultures that had been mock stimulated with a control antigen prepared from noninfected EBT cells by the same method used to prepare the IBRV-Ag.

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[1]Falcon Plastics, Inc., Oxnard, CA.

[2]Otto Hiller Co., Madison, WI.

Determination of B cell - T cell percentages

Lymphocytes were harvested and separated by the same technique described for isolated lymphocyte microcultures. Suspensions were prepared containing 4.0×10^6 cells/ml in HBSS and 0.1 ml of this suspension was incubated with 0.2 ml of a 1:30 dilution of anti-bovine globulin rabbit serum conjugated with fluorescein isothiocyanate[1]. The mixture was incubated at room temperature for 30 min with occasional gentle mixing. The cells were then washed 2 times with 5 ml of HBSS containing 0.1% sodium azide. After the second centrifugation slides were prepared with 1 drop of cell suspension, coverslipped and ringed with glycerine. Two hundred cells were counted using reflected fluorescent microscopy and phase contrast microscopy simultaneously. Cells having evidence of specifically stained immunoglobulins on their surfaces were enumerated as B lymphocytes whereas all other mononuclear cells in the same size range were considered to be T lymphocytes.

Preparation of Ficoll-Hypaque solution

Ten gm of Ficoll 400[2] (high mol. wgt. dextran 400,000) was dissolved in 100 ml distilled water by low temperature heating and vigorous stirring

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[1]Fluorescein-isothiocyanate-conjugated antibovine globulin rabbit serum was provided by Dr. George M. Brown, APHIS-Virological Reagents Section, who had prepared the antiglobulin in rabbits by repeated injections of bovine globulin supplied to him by Dr. M. Kaeberle, Iowa State University, Ames, Iowa.

[2]Pharmacia Fine Chemicals, Piscataway, NJ.

(Solution A). Solution B was prepared by mixing 20 ml of 50% hypaque sodium[1] in 9.4 ml of distilled water; 29.4 ml of Solution B was then mixed with 53.33 ml of Solution A and the specific gravity carefully adjusted to 1.080. The mixture was then sterilized by filtration through an Amicon filter (Model 402, XM-50)[2].

Preparation of POPOP scintillation counting fluid

Five grams of PPO[3] and 0.3 grams of Ortho-1-methyl POPOP[3] were dissolved in 1,000 ml of toluene. The preparation was stored in colored bottles at 4 C until used.

IBRV antigen and cell control antigen preparation for skin testing, lymphocyte microculture and whole blood immunostimulation

IBRV antigen was prepared by inoculating monolayer EBT cell cultures with IBRV (1.0 - 2.0 ml of 1.0 x $10^{8.5}$ pfu/ml). Cells were harvested by two rapid freeze-thaw cycles when maximal viral CPE was observed, usually 48 hours post infection. Stock preparations with titers less than 1.0 x $10^{8.0}$ pfu/ml were discarded. The cell suspension was sonicated for 20 seconds with a Branson sonifier and centrifuged at 400 Xg to remove large particle cell debris. The supernatant suspension was then concentrated 50 to 100 X by filtration through an Amicon X-M 50 filter. The IBRV suspension was heat-inactivated for 2 hours at 56 C. Aliquots were

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[1]Winthrop Laboratories, New York, NY.

[2]Amicon Corp., Lexington, MA.

[3]Packard Instrument Co., Downers Grove, IL.

inoculated onto EBT cell monolayers and passed three times to assure that all IBRV had been inactivated. Control cell antigens were prepared from noninfected cells from the same lot of EBT cells each time and an antigen was prepared.

Exposure of cattle to IBRV

All cattle except those in experiment 2 were exposed to IBRV by the aerosol route. The method described by Sinclair and Tamoglia (1972) was used in all other initial and challenge exposures. All animal contact parts used to expose each animal had been sterilized prior to the inoculation procedure. Cattle in experiment 2 were exposed to IBRV by the aerosol route by nebulizing IBRV suspensions with a Collison generator through a Henderson tube apparatus (Beard and Easterday 1965).

Histopathology

Selected tissues, 3.0 x 5.0 cm or smaller, were taken from cattle during postmortem examination or by surgical biopsy, fixed overnight in 10% formalin buffered with calcium phosphate, processed by dehydration in graded ethanol and infiltrated and embedded in commercial paraffin using an autotechnicon[1]. Embedded tissues were sectioned at 6.0 μ and stained with hematoxylin-eosin (H&E) and Giemsa stains.

Experimental Design

Experiment 1

Eleven 600 to 800 pound Holstein crossbred feeder steers approximately 1 year of age were used in experiment 1. They were fed a growing ration

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[1]Technicon Corporation, Tarrytown, NY.

composed of ground corn mixed with soybean meal and free choice good quality alfalfa hay. They were maintained in an area isolated from other cattle. All had been used in a vaccine trial experiment and had been challenged by intranasal exposure 90 days previously with 4.0 ml of a virus suspension containing $10^{6.4}$ $TCID_{50}$ of IBRV (McKercher isolate) in its 8th cell culture passage. Following challenge all animals had developed hyperthermia, necrotic nasal plaques, and had shed virus in their nasal secretions for 8-12 consecutive days beginning 1-2 days after challenge. (The animals were made available following the vaccine trial conducted by Dr. T. W. Tamoglia.) The steers were divided into 3 groups based upon their previous history of vaccination, IBRV challenge and serological response. Group I consisted of 4 steers treated with 30 mg of dexamethasone[1] intravenously for 5 consecutive days. Group II contained 4 steers injected subcutaneously with 10 ml of a 1:1000 solution of epinephrine[2] for 5 consecutive days. Group III was composed of 3 animals used as controls.

Each steer was examined for clinical signs of respiratory disease. Rectal temperatures were recorded, nasal swabs were taken for viral isolation and blood samples were drawn for total leukocyte determinations each day for 10 days from the beginning of the drug treatments. One animal (#3031) was killed and necropsied on the 8th day post treatment. This experiment was begun in November of 1972 and was designed to determine

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

[2]Haver-Lockhart Laboratories, Shawnee, KA.

if corticosteroids (CS) could be used to cause recrudescence of IBRV infections in previously exposed feedlot steers.

Experiment 2

This experiment was conducted to determine the effect of dexamethasone treatment on acute IBRV infections. Four 8-week-old CDIR calves were used in the experiment. Two calves (#7222 and #7255) were treated for 5 days with 15 mg of dexamethasone administered intravenously. They were subsequently inoculated intranasally by atomizing 2.5 ml of an IBRV suspension containing $1.0 \times 10^{8.2}$ pfu/ml. Each animal was also given 5.0 ml of the same IBRV suspension by intratracheal (IT) injection by inserting a 20 gage 1½ inch needle between tracheal rings in the mid-tracheal area. Prior to IT inoculation the skin of the injection site was clipped free of hair and disinfected with ethyl alcohol. Following IBRV exposure, dexamethasone dosage was increased to 20 mg daily. Two calves (#7228 and #7256) not treated with dexamethasone served as acute IBRV infected controls. These 2 animals were exposed to the same amount of virus by the same method. Nasal swabs for viral isolation, heparinized blood samples for clinical hematology, and rectal temperatures were taken daily. Postmortem examinations were conducted on calves that died as a result of the disease or that were killed 17 days after initial IBRV exposure. Tissues and organs were exposed and separate sets of sterile instruments used to take tissue samples for viral isolation and histological examination.

Experiment 3

Ten CDIR Holstein and Holstein-cross cattle approximately 6 months old were used in this experiment. The experiment was designed to determine the nature of corticosteroid-induced recrudescence in cattle experimentally exposed to low doses of IBRV and to compare the effect of 2 virus strains (IBRV-Colo and IBRV-LA) in recurrent infections. Cattle in group I (4 animals) were exposed to aerosols of IBRV-Colo (titer $10^{6.5}$ pfu/ml) generated in a modified Henderson tube by a Collison nebulizer that produced droplets averaging 2 μ in diameter. Cattle in group II were exposed to approximately the same amount of IBRV-LA by the same technique. Group III (2 animals) served as controls on initial exposure. All animals including controls were challenged with IBRV-Colo strain at a higher dosage rate (titer $10^{8.3}$ pfu/ml) by the same exposure method 2½ months after initial exposure. All cattle were skin tested for DTH response to inactivated IBRV antigen 4 months after challenge exposure. Areas were clipped free of hair on the neck and 0.1 ml amounts of the antigen were injected intradermally on the neck and caudal fold. Cell control antigen was injected on the opposite side. Sites were evaluated at 24 and 48 hours post injection.

Six months after challenge exposure all animals were skin tested again and injected intravenously with dexamethasone for 10 consecutive days. Blood samples were drawn at 1, 2, 4, 8, 12, 24, 48, 72, 96 hours and 7, 4, and 28 days after the initial injection to determine the leukocyte response to corticosteroids and also to evaluate the biochemical changes in plasma in response to corticosteroid injection. Nasal swabs

were taken for viral isolation attempts daily for 15 days and serum samples for anti-IBRV antibody evaluations were taken on a weekly basis. At the end of the 10-day treatment period, the skin test response was evaluated following corticosteroid treatment. One year after initial IBRV exposure, the microculture isolated lymphocyte technique was adapted for use in cell-mediated immunity studies and a series of studies was begun on these cattle. Base line determinations for blastogenic responses of lymphocyte cultures of these cattle to PHA-M and inactivated IBRV and control antigens were conducted. Dexamethasone treatment by the intravenous route (30 mg/animal) was instituted and its effect on the lymphocyte blastogenic response was evaluated. It became necessary during dexamethasone treatment to process (by the Ficoll-Hypaque technique) approximately 8-10 times the amount of blood used previously in order to harvest the required amount of lymphocyte to complete the tests. Total leukocyte counts, virus shedding, B lymphocyte - T lymphocyte ratios and IBRV antibody responses were determined following dexamethasone treatment.

Finally, 2 of these cattle were used to evaluate the cellular inflammatory infiltrate in response to the intradermal skin test and to evaluate the changes induced due to dexamethasone treatment. Surgical biopsies were taken of the skin test site 12, 24, 48 and 72 hours after intradermal injection. Dexamethasone treatment (20 mg I.V.) was again instituted for 10 days and the intradermal skin test was again administered in 6 different sites on the neck. Surgical biopsies were taken at 12, 24, 48, and 72 hours after intradermal injection of the inactivated IBRV antigen.

Experiment 4

The objective of experiment 4 was to determine the potential of using specific antigen-induced blastogenesis of isolated lymphocyte microcultures and DTH skin testing to differentiate strains of IBRV and to compare the pathogenicity of a vaccine strain (Jensal), IPV (K-22), and IBRV-Colo for cattle exposed intranasally to similar doses of these agents. An additional objective was to evaluate the effectiveness of dexamethasone to cause IBRV recrudescence in cattle exposed to modified live virus vaccine strains of IBRV.

Six, 6-month-old calves were divided into 3 groups of 2 calves each and held in separate quarters during the entire experiment. Two calves each were exposed intranasally to similar doses of Jensal (modified live virus vaccine), K-22 (IPV), and Colo (field strain) of IBRV.

During the acute infection isolated lymphocyte microcultures were exposed to homologous and heterologous inactivated IBRV antigens and their blastogenic response evaluated. Six weeks after initial exposure all animals were skin-tested with homologous and heterologous strain antigens and the DTH response evaluated. Finally, 6 months post exposure all 6 calves were treated for 10 days with dexamethasone and during this time nasal swabs for viral isolation and blood samples for lymphocyte microculture were collected, prepared and evaluated.

Experiment 5

This experiment was designed to compare the effectiveness of whole blood cultures and isolated lymphocyte cultures to detect

the development of T lymphocyte sensitization during a long term chronic infection and to compare the results of immunosuppression with dexamethasone to that induced with cyclophosphamide treatment. Base line blastogenic response studies were conducted on 6 non-IBRV immune 6-month-old calves and 2 calves (positive controls) chronically infected with IBRV. All calves were exposed to IBRV by intranasal instillation. Blastogenic responses to PHA-M and inactivated IBRV antigen were conducted every other day for 2 weeks; then at 1-week intervals for 4 weeks and intermittently for 1 year. One year from the initial exposure 3 calves were treated with dexamethasone (Table 13) and 3 calves were treated with cyclophosphamide for 10 days. Cyclophosphamide was administered intravenously at the approximate rate of 4 mg/kg of body weight for the first 4 days and 2 mg/kg of body weight for the next 5 days. Nasal swabs for viral isolation were taken daily. Whole blood cultures and isolated lymphocyte microcultures were prepared every other day at 12-18 hours following immunosuppressive treatment. All animals except the positive controls were killed 10 days following the beginning of dexamethasone or cyclophosphamide treatment. Postmortem examinations were conducted and tissues were taken for viral isolation and histopathological examinations. Clinical hematological values were determined on each animal at the time blood cultures were established and B cell - T cell ratios of isolated lymphocyte suspensions were determined throughout the treatment period.

RESULTS

Results of each experiment are presented in the following tabular or narrative form as they relate to the objectives of the experimental design.

Experiment 1.

All steers in group I (dexamethasone treated group) began secreting IBRV in their nasal mucus on day 4 or 7 after dexamethasone treatment and continued to secrete virus until 8 days post treatment (Table 1). All four animals developed necrotic nasal mucosal plaques (Figs. 3 & 4) by 4 days post treatment and plaques were present for at least 1 week following the initial observation. The animal (#3031) that was killed and necropsied on day 8 had extensive mucosal plaques in the nasal mucosa and turbinates (Figs. 5 & 6). There were also necrotic plaques on the epiglottis (Fig. 7) and larynx. The suprapharyngeal lymph nodes were swollen, edematous and contained numerous petechial hemorrhages. The examination of H&E stained thin sections revealed numerous small foci of necrotic ulceration of the squamous epithelium of the internal nares, turbinates, epiglottis and larynx. Ulcers were covered by fibrin-containing neutrophils and cellular debris (Fig. 8), and the surrounding tissues were heavily infiltrated with neutrophils, lymphocytes and macrophages (Fig. 9). Necrotic mucosal plaques appeared histologically as large pedunculated, mushroom-shaped masses arising abruptly from the lesion interrupted stratified squamous epithelium (Fig. 10). These masses contained fibrin, necrotic inflammatory and epithelial cells, and a few scattered fibroblasts. Multifocal areas of ballooning degeneration were observed in the epithelium of the upper respiratory tract especially at the periphery of the necrotic plaques. Degenerating stratified squamous epithelium at the edge of the plaques contained eosinophilic intranuclear inclusion bodies (Figs. 11 & 12). Deep within the submucosa there were a

Table 1. Clinical, serological and virological evidence of IBRV recrudescence in cattle following dexamethasone and epinephrine treatment 90 days after experimental IBRV exposure.

Animal Number	Treatment	Virus Isolation	Lesions[a]	Antibody	
				Pre	Post
3031	DEX[b]	4, 7-8[c]	Yes	16[d]	ND[e]
5859	DEX	7-8	Yes	64	256
5253	DEX	7-8	Yes	32	512
7071	DEX	7-8	Yes	16	512
3637	EPI[f]	None	No	16	32
4647	EPI	None	No	32	32
6061	EPI	None	No	16	16
100	EPI	None	No	64	32
5455	None	None	No	64	64
2829	None	7	No	32	16
6465	None	None	No	16	16

[a]Necrotic nasal mucosal plaques observed.

[b]Dexamethasone (Azium) 30 mg. daily for 5 days.

[c]Days post treatment on which IBRV isolated from nasal secretions.

[d]Reciprocal of serum dilution neutralizing 50% of plaques in SVN test. Pretreatment sample was taken on day treatment was begun and post treatment samples taken 14 days post treatment.

[e]Not determined.

[f]Epinephrine, 1:1000, 10 ml daily for 5 days.

Figure 3. Necrotic nasal mucosal plaques that appeared on the nasal septum of a steer 4 days after dexamethasone treatment was begun.

IBRV virus was isolated from the nasal secretions the same day these plaques were first observed.

Figure 4. Close-up view of nasal mucosal plaque.

Notice raised appearance of the lesion.

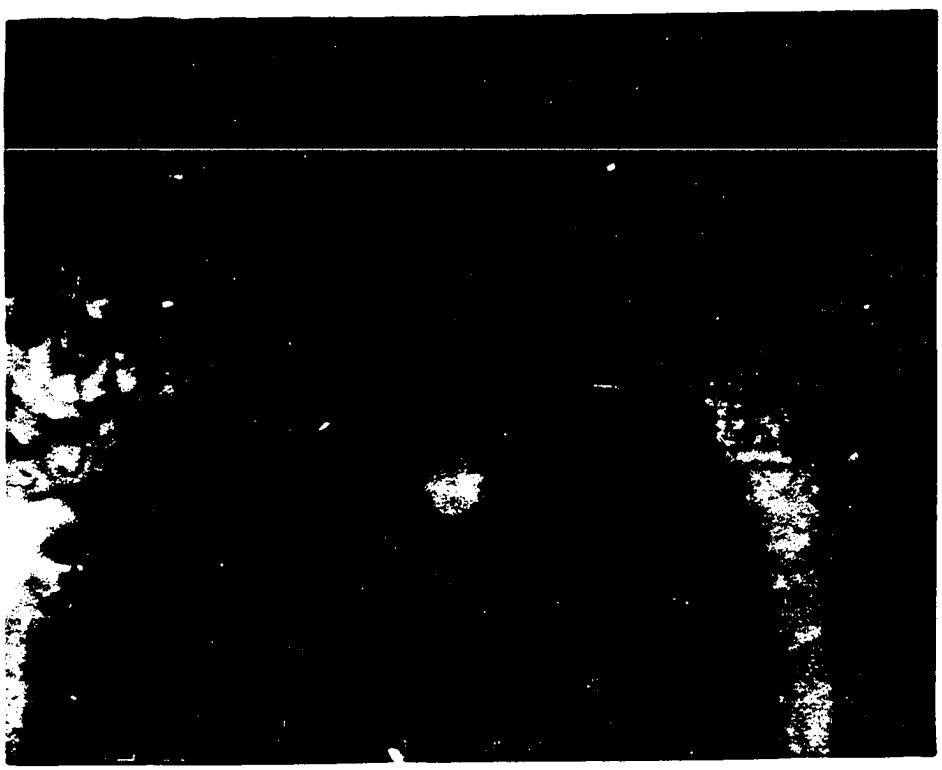


Figure 5. Raised necrotic mucosal plaques observed during postmortem examination of steer killed 8 days after dexamethasone treatment began.

Figure 6. Necrotic mucosal plaques observed on turbinates during postmortem examination of steer killed 8 days post dexamethasone treatment.

Notice multiple small papules.

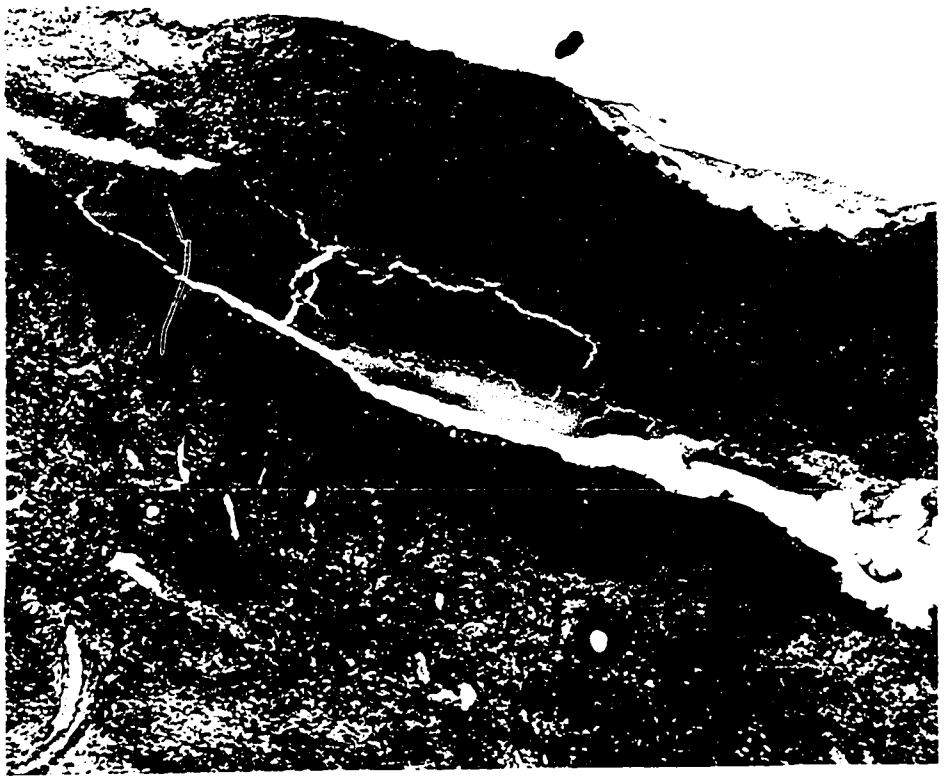
Figure 7. Epithelial ulceration and necrosis of epiglottis and larynx observed during postmortem examination of steer killed 8 days post dexamethasone treatment.

Figure 8. Tissue section of nasal mucosa from steer killed 8 days after dexamethasone treatment.

Edge of necrotic ulcer appears at lower right. Fibrin mass containing neutrophils and necrotic cellular debris covers ulcer and adjacent stratified squamous epithelium. Notice inflammatory cells in submucosal area. Hematoxylin and eosin stain.

Figure 9. Lymphocyte, macrophage and neutrophil accumulation in the submucosa of nasal tissue of a steer killed 8 days after dexamethasone treatment.

Hematoxylin and eosin stain.



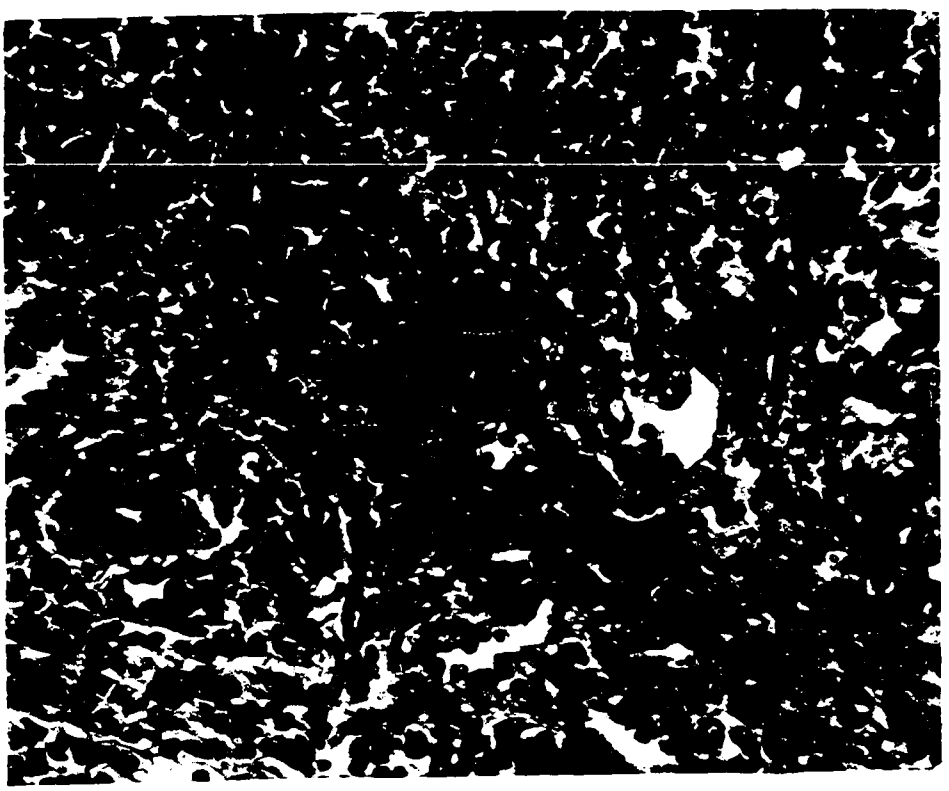


Figure 10. Section of tissue from pedunculated nasal mucosal plaque following dexamethasone-induced IBRV recrudescence.

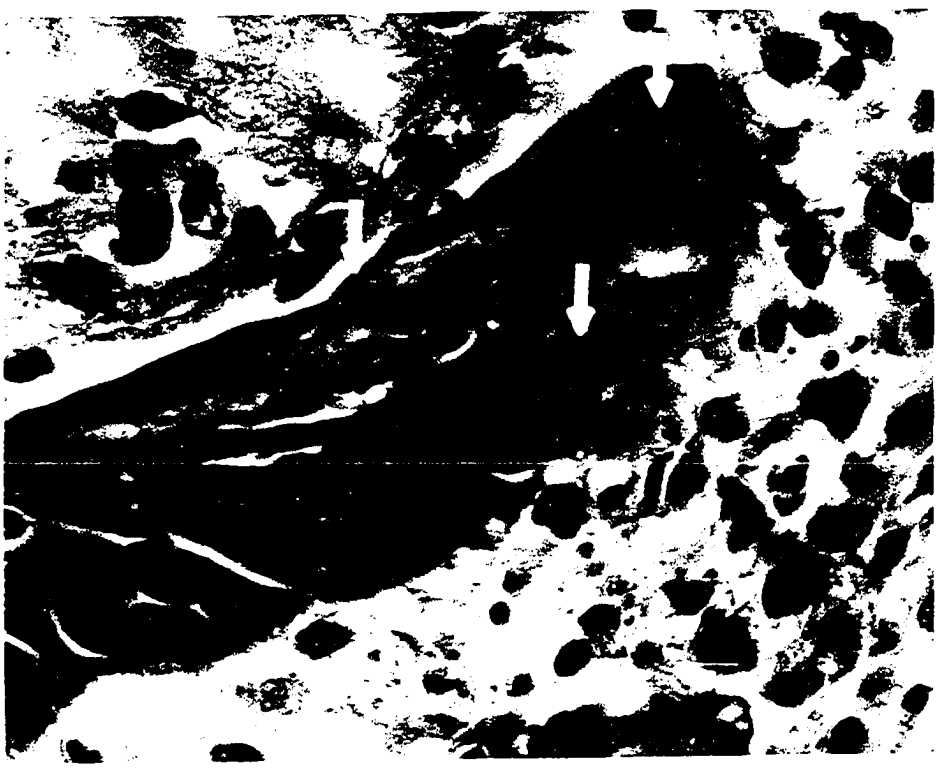
Notice abrupt interruption of stratified squamous epithelium and infiltration of the submucosa with inflammatory cells. Hematoxylin and eosin stain.

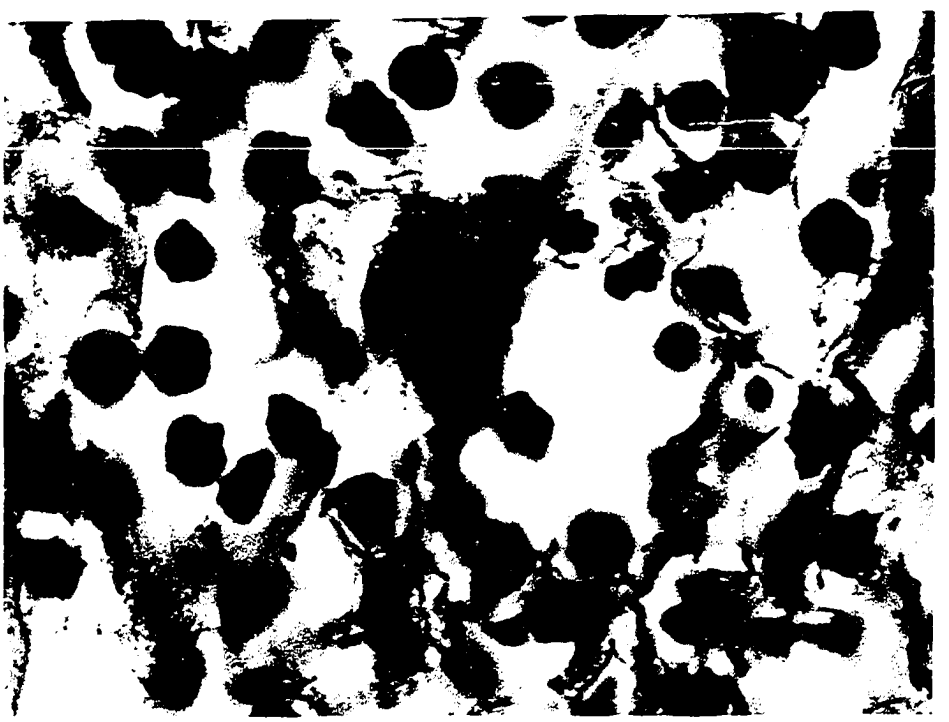
Figure 11. Ballooning degeneration of the stratified squamous epithelium at the periphery of necrotic plaque in nasal mucosa of calf following dexamethasone-induced IBRV recrudescence.

Note intranuclear inclusion bodies at arrows. Hematoxylin and eosin stain.

Figure 12. Large intranuclear inclusion body in a stratified squamous epithelial cell at periphery of necrotic plaque in nasal mucosa of a steer killed 8 days after dexamethasone treatment.

Hematoxylin and eosin stain.





few vessels with perivascular accumulations of plasmacytes and lymphocytes. Sub-epithelial tissues in these areas contained multiple foci of mononuclear cells, principally lymphocytes (Fig. 13).

Histopathological examination of the lymphatic tissue associated with the respiratory tract revealed diffuse necrosis of lymphoid cells and reticuloendothelial hyperplasia especially in the paracortical and follicular areas.

Frozen sections of necrotic mucosal plaques stained with anti-IBRV fluorescein-conjugated antibody preparations had intensive staining at the periphery of the lesions of the nasal septum and epiglottis (Fig. 14). Similar sections of the bronchial nodes were also positive for IBRV antigen (Fig. 15).

Even though virus was isolated from the nasal mucus taken the day before the animal was killed, 2 attempts to isolate virus from the nasal septum, larynx, bronchial and other regional lymph nodes were not successful.

All animals treated with dexamethasone had increased circulating anti-IBRV antibodies in serum samples taken 2 weeks after treatment (Table 1).

There were no viral isolations from daily nasal mucus samples taken from epinephrine treated or control animals except from 1 control animal (#2829) 7 days after other cattle had been treated (Table 1). No nasal mucosal plaques were observed and there were no increases in antibody titers in these 2 groups.

Experiment 2

The two 2-month-old calves infected with IBRV not treated with dexamethasone had mild rhinitis 1 day post exposure (DPE) and by 2 DPE had a high rectal temperature (Fig. 16) and distinct hyperemia of the mucus membranes of the nasal passages. Extensive necrosis and acute inflammation of the mucus membranes of the anterior nares and nasal septum was obvious 3 through 7 DPE. Severe respiratory distress and moist rales over the trachea were observed 3 to 6 DPE. These animals were anorectic from 3 to 5 days but showed marked clinical improvement at 10 DPE. Nasal lesions were healed by 14 DPE.

Both calves treated with dexamethasone and exposed to IBRV had less severe disease initially than those not treated. Body temperature elevations were generally lower and the initial rise was delayed by 48 hours (Fig. 16). Both calves developed severe respiratory distress and stenosis of nasal passages (Fig. 17) by necrotic debris often forced both calves to open-mouthed breathing (Fig. 18) by DPE 6. One calf (#7255) (Table 2) was found dying in the stall early on the morning of DPE 7. Postmortem and histopathological examination revealed extensive necrosis and severe multifocal fibrinopurulent rhinotracheitis. Most of the surface of the nasal turbinates was denuded of surface epithelium (Fig. 19). Much of the epithelium of the pharyngeal area was necrotic and sloughed (Fig. 20). Erosions of the ciliated epithelium (Fig. 21) occurred throughout the trachea. There were large areas of consolidation in the anterior ventral portion of all lobes of the lung (Fig. 22). Lymph nodes associated with the respiratory tract were

Figure 13. Focal area of mononuclear cell infiltration in the mucosa and submucosa of respiratory epithelium of the larynx of a steer killed 8 days after dexamethasone treatment.

Hematoxylin and eosin stain.

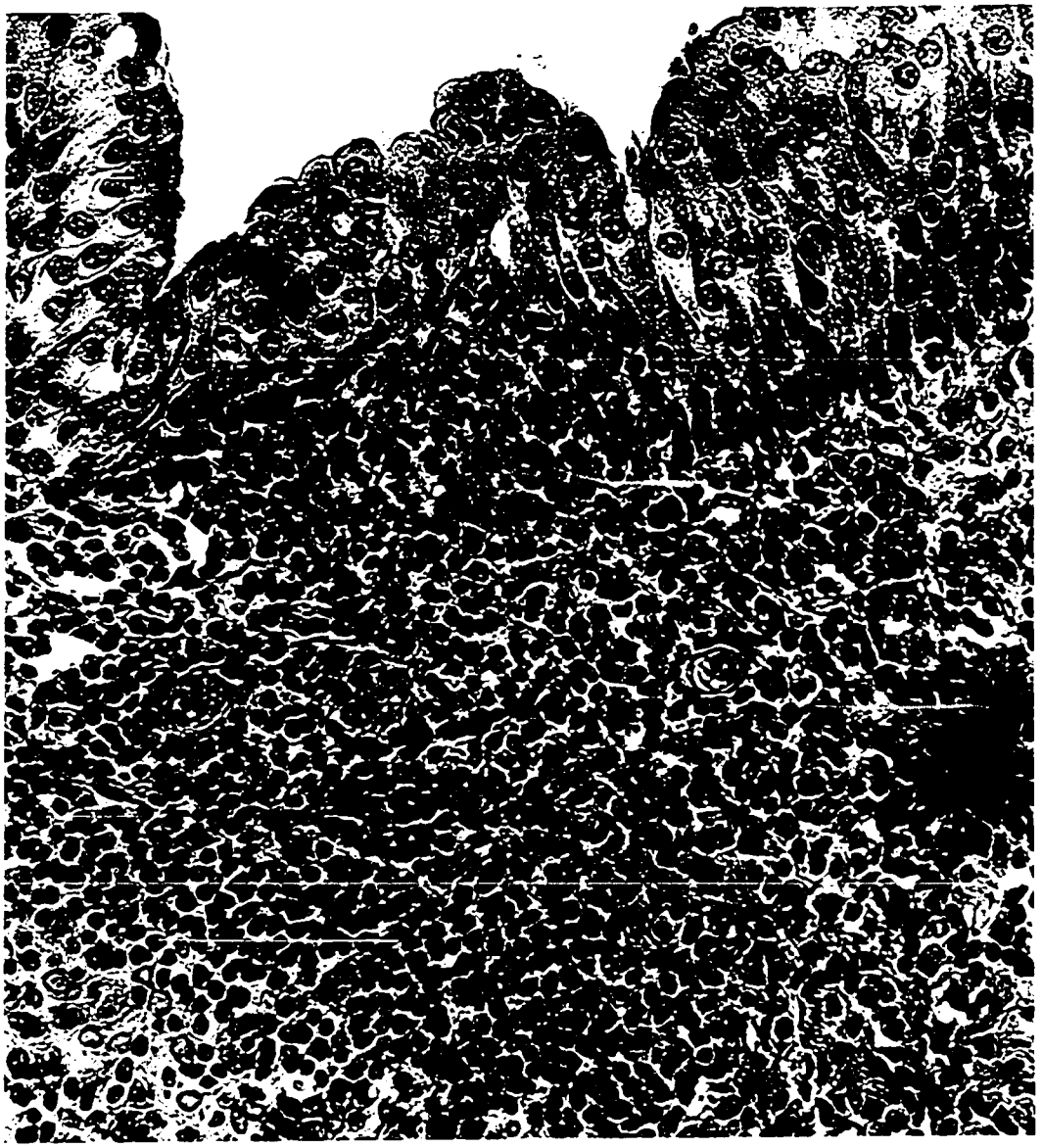
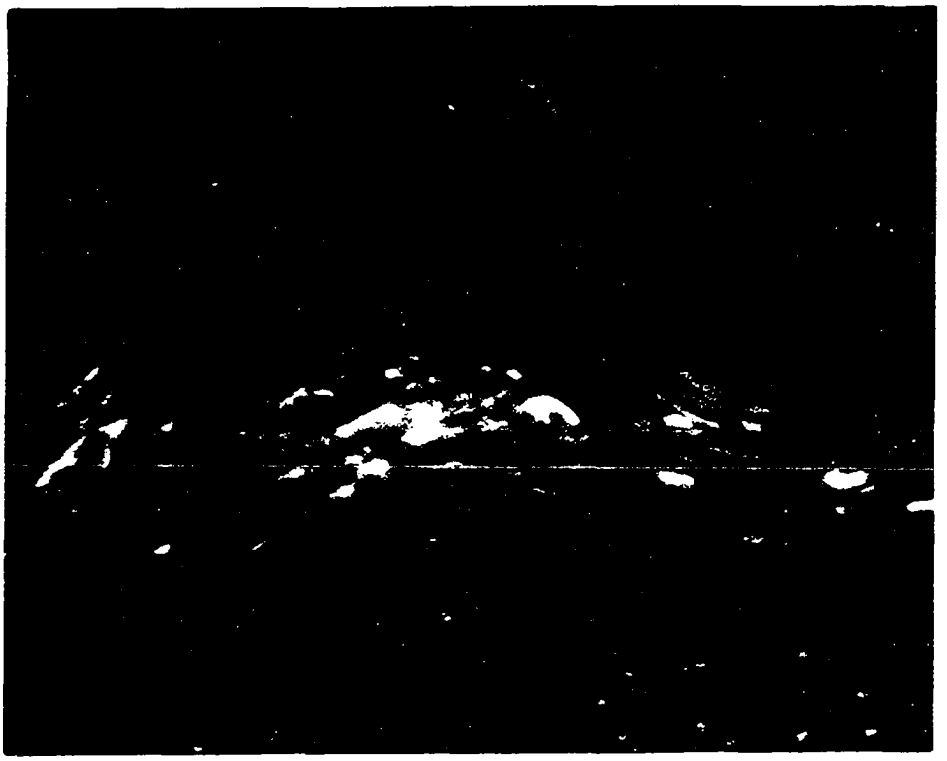


Figure 14. Section of tissue adjacent to necrotic lesion of pharynx stained with anti-IBRV fluorescein-conjugated antibody.

Note brightly stained area in the stratified squamous epithelium.

Figure 15. Section of tissue from the bronchial lymph node of steer killed 8 days after dexamethasone treatment.

Bright areas represent cells stained with anti-IBRV fluorescein-conjugated antibody.



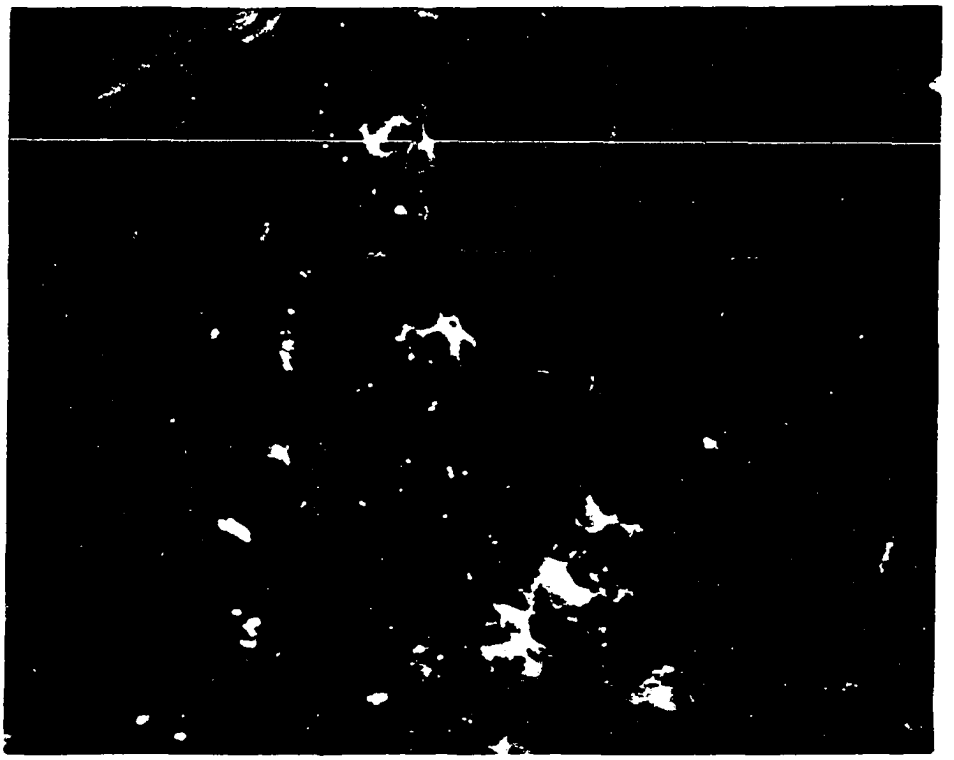


Figure 16. Rectal temperature of dexamethasone treated and nontreated calves exposed to IBRV by the aerosol route.


Daily average of two readings (A.M. and P.M.) of both dexamethasone treated calves.


Daily average of two readings (A.M. and P.M.) of both nontreated calves.


Exposed to IBRV.


1 of 2 treated animals died.

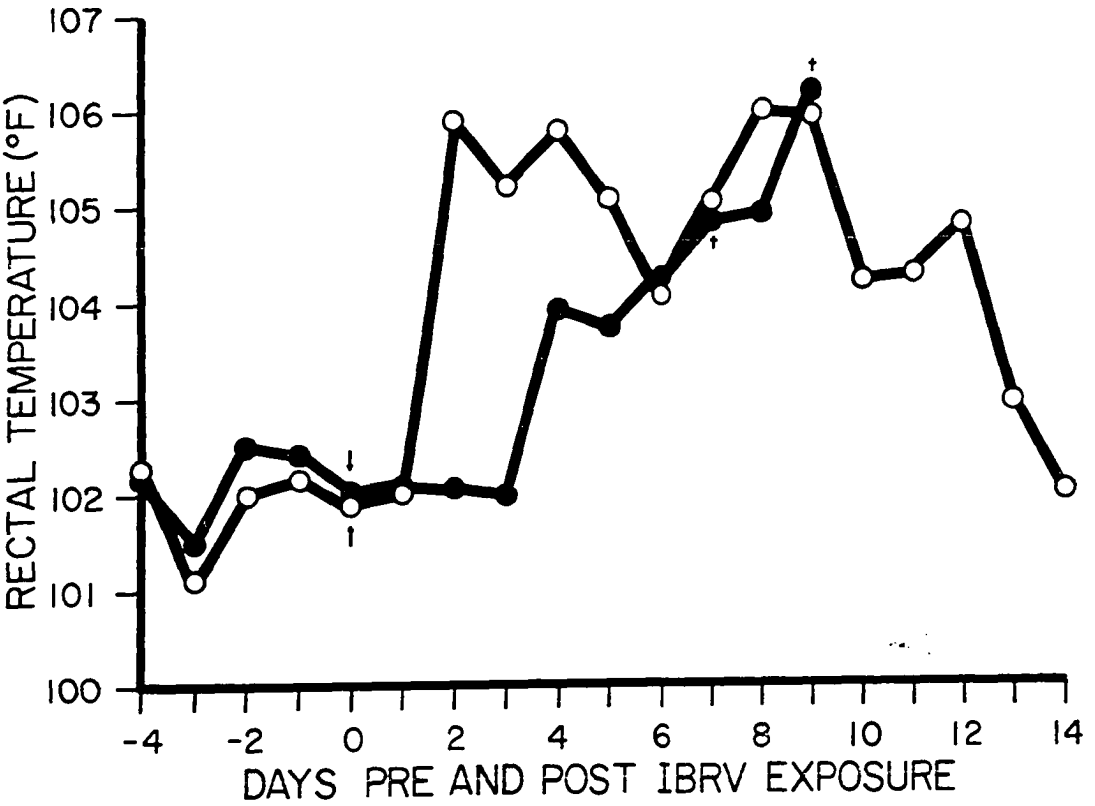
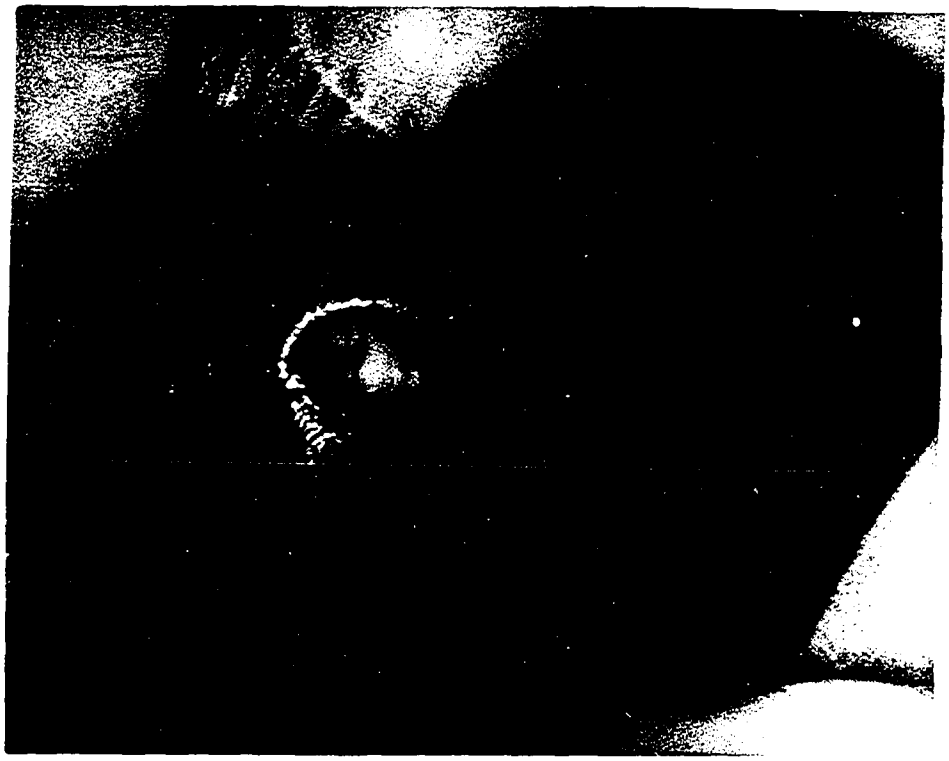

RECTAL TEMPERATURE (°F)
107
106
105
104
103
102
101
100
-4
-2
0
2
4
6
8
10
12
14
DAYS PRE AND POST IBRV EXPOSURE

Figure 17. External nares of calf with severe necrotic rhinitis following dexamethasone treatment and subsequent exposure to IBRV.

Note narrow slit-like opening into nasal passage.

Figure 18. Extension of head and neck and open-mouthed breathing by a calf following dexamethasone treatment and subsequent exposure to IBRV.



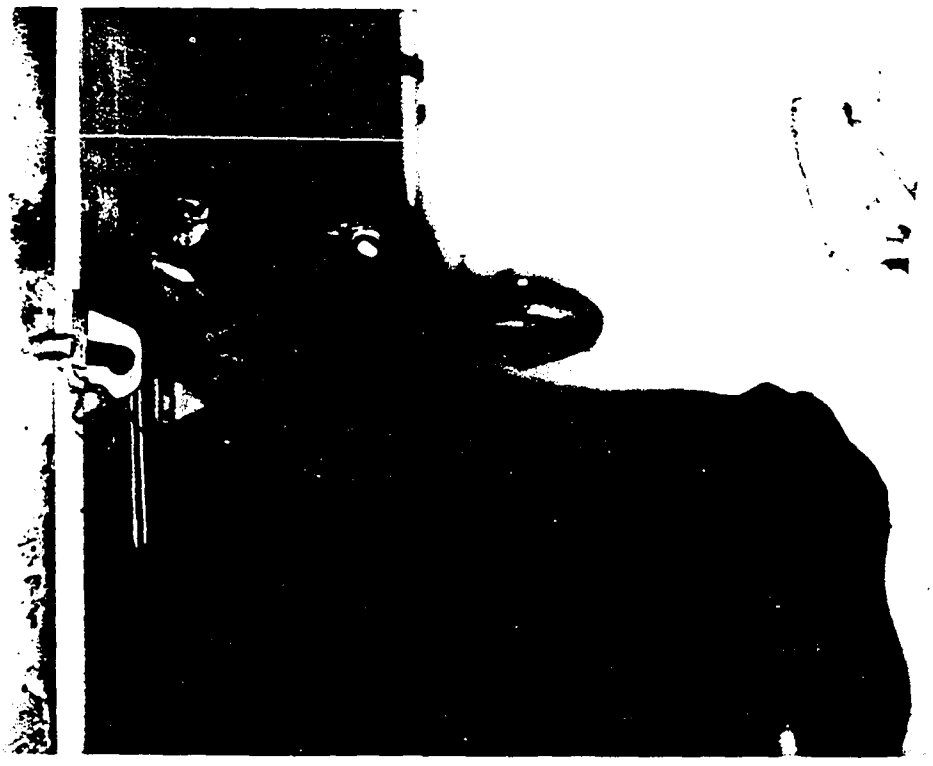
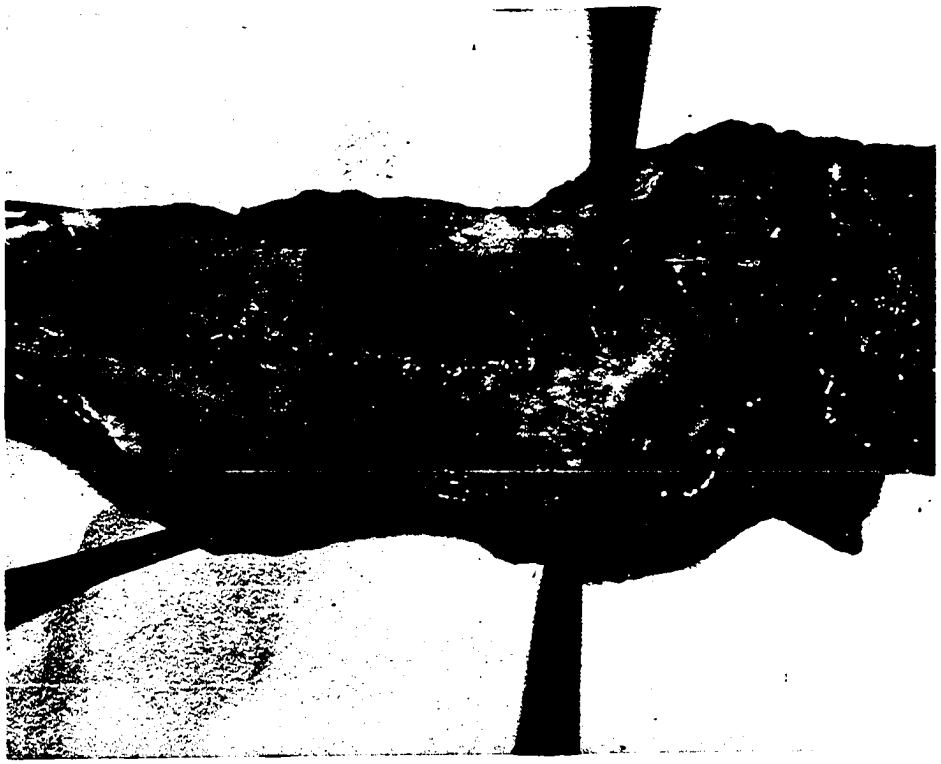


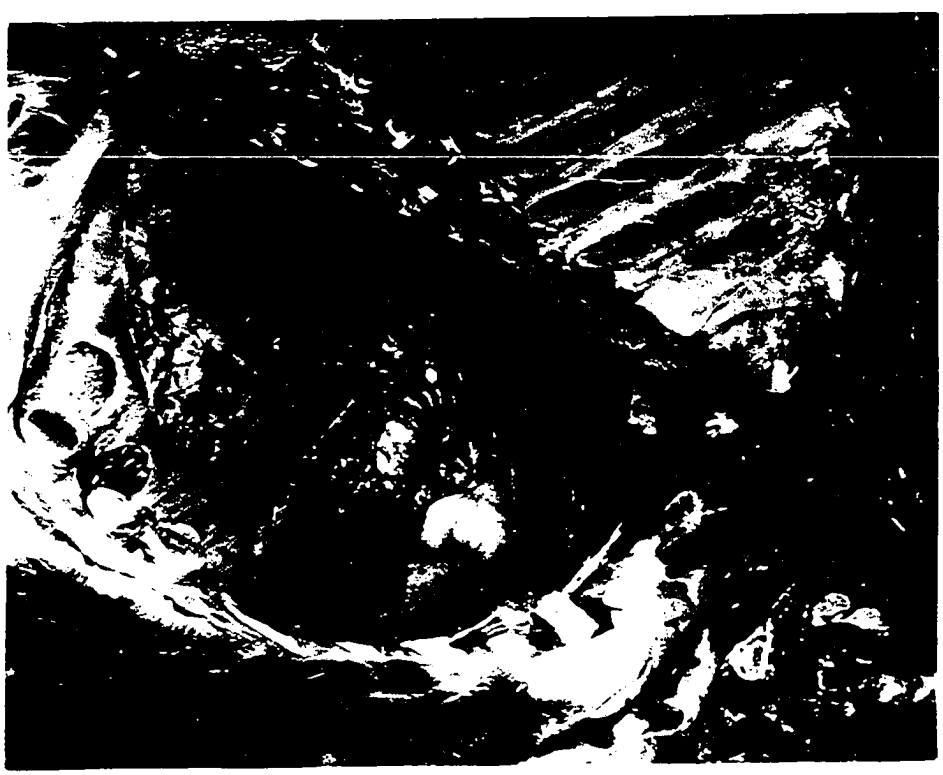
Figure 19. Necrosis and erosion of epithelium of turbinates in a calf that died 7 days after dexamethasone treatment and aerosol IBRV exposure.

Figure 20. Necrosis and erosion of epithelium of the larynx and pharynx of calf that died 7 days after dexamethasone treatment and aerosol IBRV exposure.

Figure 21. Necrosis and erosion of tracheal epithelium of a calf that died 7 days after dexamethasone treatment and IBRV aerosol exposure.

Figure 22. Consolidated areas of lungs of calf that died 7 days after dexamethasone treatment and IBRV aerosol exposure.





swollen, hemorrhagic and edematous. Ecchymotic hemorrhages were present on the greater omentum, mesenteric lymph nodes and the serosal surfaces of the caecum. Meningeal vessels were congested. Calf #7222 died during the night of DPE 9. Postmortem lesions were similar to those described for #7255 but less severe. Pulmonary consolidation was confined to the apical and cardiac lobes and involved much smaller areas than in the lungs of calf #7255.

IBRV was isolated from nasal swabs taken from all calves on DPE 1 and was continually recovered from daily swabs of dexamethasone-treated calves until their death (Table 2). Untreated calves shed virus in their nasal secretions until DPE 12 and virus was not recovered beyond that time. IBRV was recovered from all tissues taken for viral isolation from dexamethasone-treated calves (Table 3) but IBRV was not recovered from tissues taken during postmortem examination of nontreated calves killed DPE 17 (Table 3).

Histopathological examination of H&E stained sections of tissues taken from dexamethasone-treated, IBRV-infected calves revealed extensive acute to subacute fibrinopurulent rhinotracheitis and necrosis of the nasal septum, turbinates and external nares. The lesions were characterized by necrosis and sloughing of the stratified squamous epithelium, and infiltration of the submucosa by neutrophils and lymphocytes. Lymphocytes were especially prominent around small vessels and mucus glands. Acute to subacute laryngitis and pharyngitis characterized by epithelial necrosis and sloughing and the infiltration of inflammatory cells was present in both calves. Both treated calves had extensive tracheobronchitis and acute to subacute suppurative pneumonia. Alveolar

Table 2. IBRV isolations from nasal swabs taken from calves infected by the aerosol route. Calf numbers 7222 and 7255 were treated with dexamethasone (15 mg/day) 5 days before and 20 mg/day after exposure.

Animal Number	Days Pre- or Post-IBRV Exposure														
	-1	0	1	2	3	4	5	6	7	8	9	10	12	14	16
7222	-	-	+	+	+	+	+	+	+	+	+[a]				
7255	-	-	+	+	+	+	+	+	ND[b]						
7228	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-
7256	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-

[a]Calf died of acute respiratory disease.

[b]Calf died of acute respiratory disease.

Table 3. IBRV isolations from tissues taken during postmortem examinations.

Tissue	Calf Number			
	7222[a]	7255[b]	7228[c]	7256[c]
Adrenal	+	ND[d]	-	-
Cerebrum	+	+	-	-
Epiglottis	+	+	-	-
Larynx	+	+	-	-
Lung	+	+	-	-
Mediastinal LN[e]	ND	+	-	-
Prescapular LN	ND	+	-	-
Suprapharyngeal LN	+	+	-	-
Trachea	+	+	-	-
Tracheal Exudate	+	+	-	-

[a]Calf died of acute respiratory disease 9 days after aerosol exposure to IBRV.

[b]Calf died of acute respiratory disease 7 days after aerosol exposure to IBRV.

[c]Calf was killed 17 days after aerosol exposure to IBRV.

[d]Not determined.

[e]Lymph node.

macrophages and lymphocytes were also present in large numbers. Microscopic examination of sections of the cerebellum, cerebrum and brain stem revealed only mild changes. One area had perivascular cuffing of deep meningeal vessels with mononuclear cells.

Changes in the circulating leukocyte profiles following dexamethasone treatment were rapid increases in the total leukocyte counts (Fig. 23) of more than 100% in both treated calves. However, the total lymphocyte count (Fig. 23) continued to decrease until the day of death.

Experiment 3

Cattle exposed by aerosol to low doses of IBRV showed mild clinical responses. Rapid respiration and increased inspiratory sounds on auscultation were observed in 3 animals from 2 through 6 DPE. Occasional voluntary coughing was observed and coughing could be elicited in infected animals by physical manipulation of the trachea. The same techniques did not cause coughing in the control cattle. Increased amounts of nasal discharge, congestion of the nasal mucosa, and necrotic plaques on the nasal mucosa were not observed. Seven of eight exposed animals had a febrile response of 2 to 5 days duration beginning between 3 and 5 DPE (Table 4).

IBRV was isolated from 7 of 8 exposed animals (Table 4). Virus shedding began from 5 to 12 DPE and was isolated sporadically up to 21 days after exposure. No virus was isolated from 2 control animals.

Anti-IBRV antibody developed to near maximal levels 28 DPE in all exposed animals (Table 4). No anti-IBRV antibody developed in control animals.

Figure 23. Hematologic changes in calves treated with dexamethasone and subsequently exposed to IBRV by the aerosol route.

Data shown is average of 2 calves.


Total leukocytes in peripheral blood of dexamethasone treated-IBRV infected calves.


Total leukocytes in peripheral blood of nontreated IBRV infected calves.


Total lymphocytes in peripheral blood of dexamethasone treated-IBRV infected calves.


Total lymphocytes in peripheral blood of nontreated IBRV infected calves.


Dexamethasone treatment began.


All exposed to IBRV.


1 of 2 calves died.

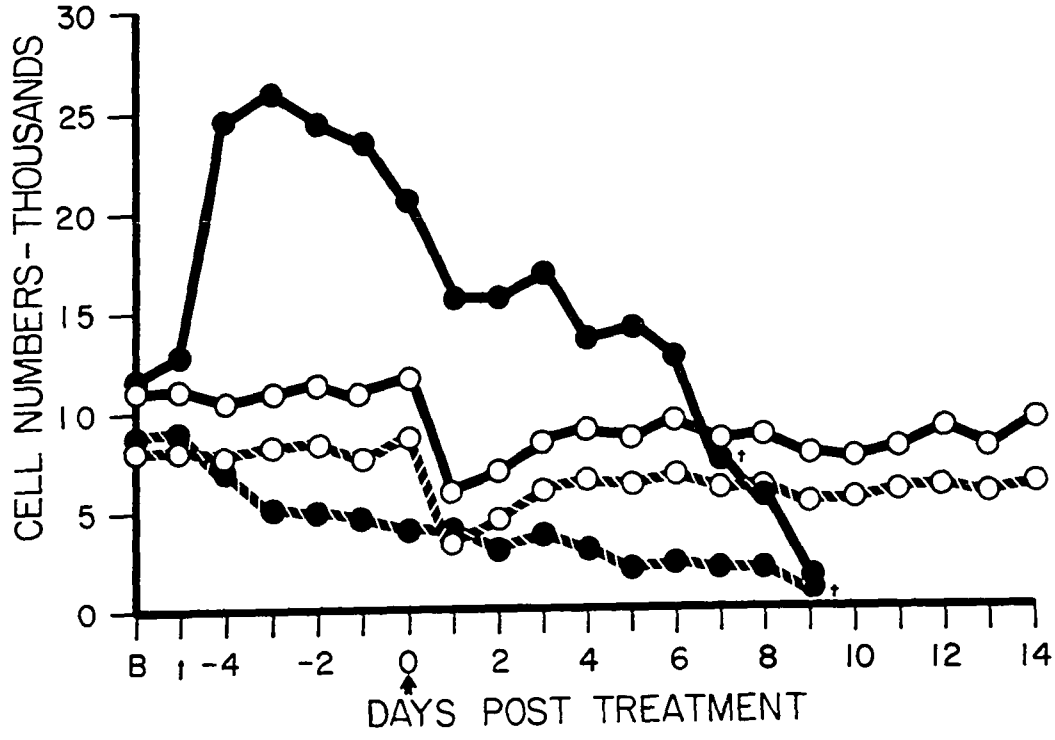

CELL NUMBERS - THOUSANDS
30
25
20
15
10
5
0
B
-4
-2
0
2
4
6
8
10
12
14
DAYS POST TREATMENT

Table 4. Clinical, serological and virological response of 6-month-old calves following aerosol exposure to IBRV Colo and LA strains.

Calf Number	IBRV Strain[a]	Fever[b]	Virus Isolation[c]	Antibody[d]
6973	LA	5-7	7-16,19,21	16
6975	LA	0	8,10,12	16
7018	LA	3,5,6,7,10	5,8,9	16
7025	LA	5,6,8	0	2
6966	Colo	3-10	9-16	16
6967	Colo	5-12	8-16	64
7016	Colo	5-9	12	32
7017	Colo	4-8	6,8,10,13	32
7019	None	0	0	>2
7020	None	0	0	>2

[a]LA = Los Angeles strain; Colo = Colorado strain (See text for strain history).

[b]Day or days post exposure when rectal temperature exceeded 39.4 C.

[c]Days post exposure when IBRV was isolated from nasal secretions.

[d]Reciprocal of serum dilution neutralizing 50% of plaques in SVN test. Samples taken 28 days after exposure.

Following challenge-IBRV-exposure, none of the previously exposed animals had clinical evidence of respiratory infection or shed virus in their nasal secretions during the two-week observation period. Challenge control animals showed evidence of disease similar to, but more severe than, that observed in the initial exposure (Table 5). All animals had an increase in antibody response by 14 days post challenge.

Cattle that had been exposed by aerosols of IBRV and challenged 6 months later by aerosol developed typical DTH skin test responses when injected intradermally with 0.05 ml of inactivated IBRV antigen prepared from IBRV-infected BT cells. Positive responses were recorded at 24 and 48 hours following injection of the antigen intradermally in the neck (Fig 24A) and caudal fold (Fig. 25A) sites. There seemed to be no direct correlation between the size of the DTH reaction and the anti-IBRV antibody titer in the serum of sensitized animals (Table 6). Dexamethasone treatment (30 mg intravenously for 10 days) caused a marked reduction in the DTH skin test response (Figs. 24B and 25B) in all animals and induced necrotic nasal mucosal plaques and virus secretion in nasal mucus of 5 of 8 animals treated. Humoral antibody titers increased following recrudescence of IBRV lesions (Table 6). Administration of the skin test by intradermal injection of 0.05 ml of inactivated IBRV antigen at 2 different sites (neck and caudal fold) caused a marked hyperthermia in all animals tested (Fig. 26). The 24-hour temperature increase in different individuals varied from 1.8 to 4.0 F above the average temperature computed from 9 days of temperature recordings prior to the test.

Table 5. Clinical, serological and virological response of calves previously exposed to IBRV (LA and Colo strains) and controls when challenge-exposed 6 weeks later to IBRV Colo by the aerosol route.

Calf Number	Fever[b]	Virus Isolation[c]	Antibody[a]	
			Pre-challenge	Post-challenge
6973	0[d]	0	32	64
6975	0	0	32	64
7018	0	0	16	32
7025	0	0	4	32
6966	0	0	32	64
6967	0	0	32	32
7016	9,10,13,14	0	32	ND[e]
7017	0	0	16	32
7019	2-6,9	6,7,10,11	>2	16
7020	2-6,9,10,14	2-11	>2	16

[a]Reciprocal of serum dilution neutralizing 50% or more of the plaques in SNV test. Pre-challenge serum sample taken 70 days after initial exposure and post-serum sample taken 14 days post-challenge.

[b]Day post-exposure when rectal temperature exceeded 39.4 C.

[c]Days post-exposure when IBRV was isolated from nasal secretions.

[d]No isolations.

[e]Not determined

Table 6. Serological and delayed-type hypersensitivity (DTH) responses to intradermal injection of inactivated IBRV antigen before and after dexamethasone treatment that caused recrudescence in chronically infected cattle.

Animal Number	DTH Response[a]		Antibody Response[b]		Virus Isolations[c]
	Pre-tr.	Post-tr.	Pre-tr.	Post-tr.	
7025	++++	+	32	128	8
6966	++++	±	64	256	6-12
6973	+++	±	64	≥256	6-15,18
6975	+++	+	64	256	0
7016	++	+	32	256	0
7019	++	±	16	128	10,11,13
7020	ND		32	64	4-8,9-13
2017	ND		64	64	0

[a]Each + represents approximately 1 cm. diameter by 0.5 cm. thickness of swollen skin at injection site.

[b]Reciprocal of serum dilution neutralizing 50% or more of the plaques in SVN test. Pre-treatment (Pre-tr.) taken 10 days before and post-treatment (Post-tr.) taken 6 days after dexamethasone treatment.

[c]Days after first day of dexamethasone treatment when IBRV was isolated from nasal secretion. 0 = no isolations.

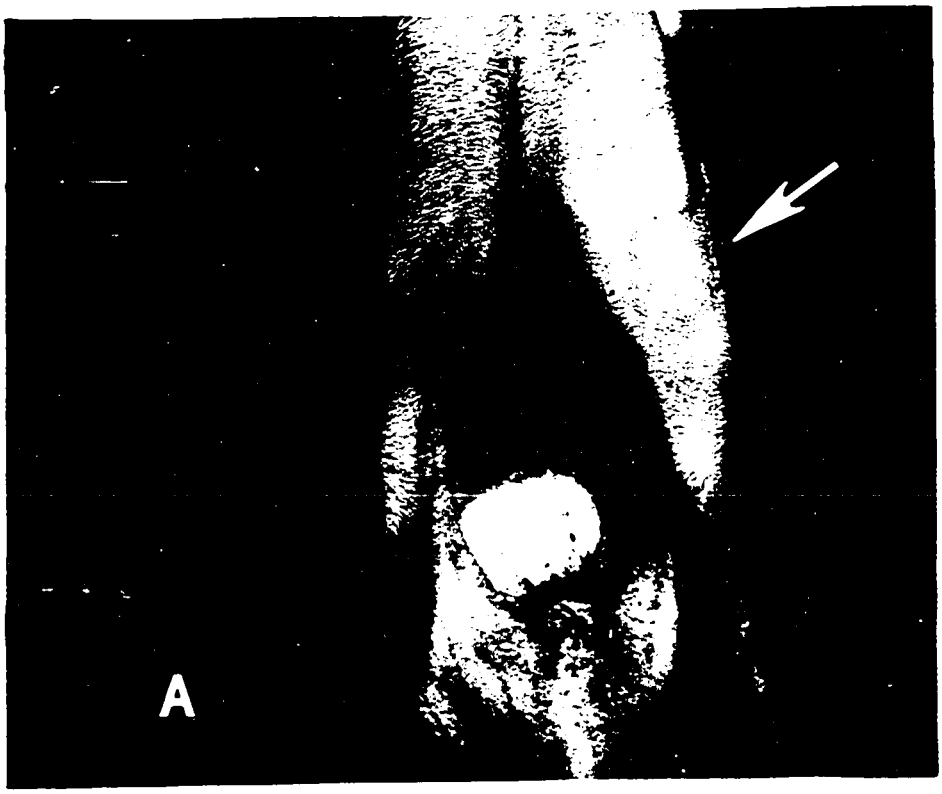

A


B

Figure 25. Delayed-type hypersensitive (DTH) response following intradermal injection of inactivated IBRV antigen into the skin of the caudal fold.

A. DTH response of chronically IBRV-infected steer before dexamethasone treatment. Arrow indicates area of IBRV antigen injection. Opposite side injected with cell control antigen.

B. Reduced DTH response in same steer after 10 days of treatment with dexamethasone. Arrow indicates area of IBRV antigen injection. Opposite side injected with cell control antigen.


A

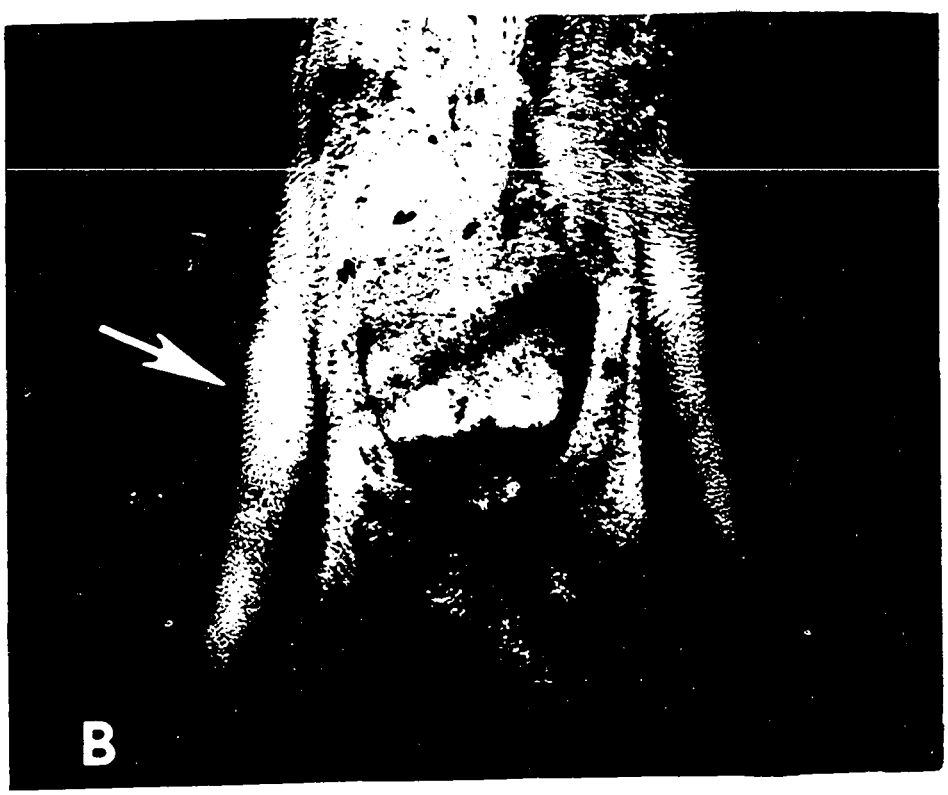
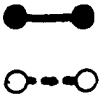
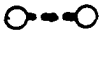
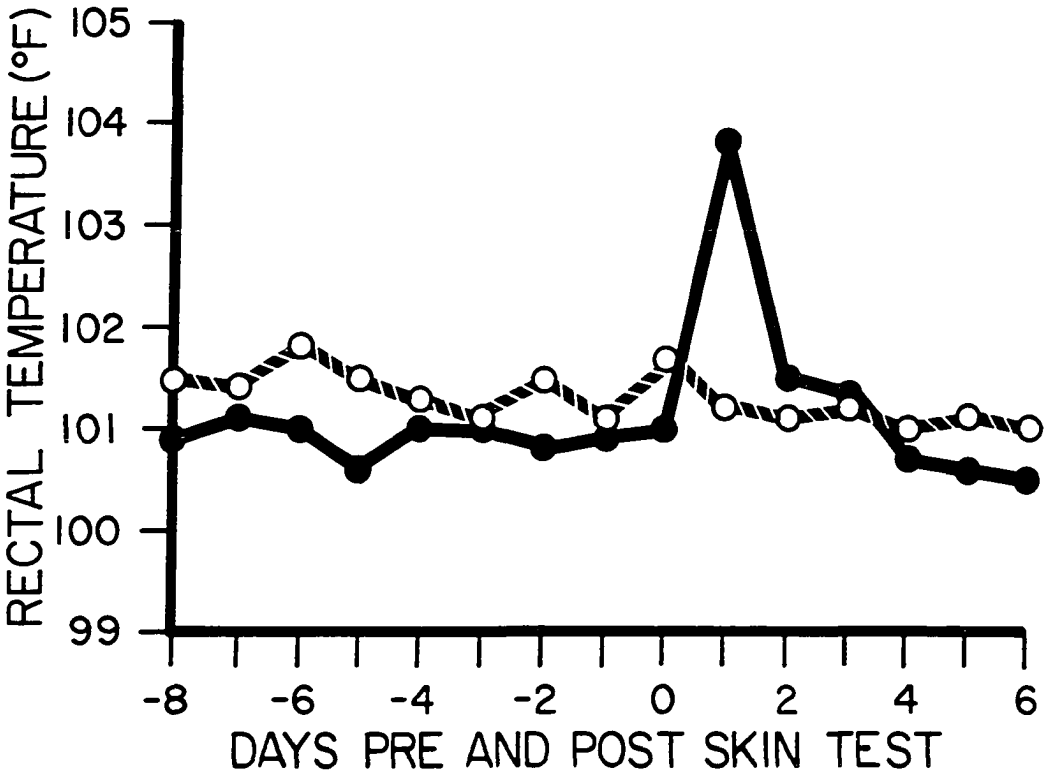

B

Figure 26. Rectal temperature of cattle following intradermal skin testing with inactivated IBRV antigen.






RECTAL TEMPERATURE (°F)
105
104
103
102
101
100
99
-8
-6
-4
-2
0
2
4
6
DAYS PRE AND POST SKIN TEST

Results of determinations for biochemical changes in plasma following dexamethasone treatment are recorded in Table 7. Only means of separate determinations on all 8 cattle at each specific time are recorded. Obvious increases in SGOT (serum glutamic oxalacetic transaminase), alkaline phosphatase, uric acid, and glucose began at 4-8 hours post treatment and remained elevated throughout the treatment period. Decreases in cholesterol and inorganic phosphorous were recorded during the same period of time. LDH (lactic dehydrogenase), total bilirubin, albumin, total protein, BUN (blood urea nitrogen) and calcium levels were not significantly changed.

Adrenal output of 17-dehydroxycortisone (DHC) was depressed in all 8 dexamethasone treated cattle (Table 8). Plasma samples drawn 1 hr after the initial intravenous injection of dexamethasone had a marked decrease of 17-DHC and by 8 hr post injection (P.I.) the natural hormone was depressed below detectable levels in all but one animal (Table 8). Three of 8 animals began a rebound effect by 12 hr P.I. This effect was more obvious in all eight animals 24 hr P.I.; however, 30 hr after the initial injection (6 hr after 2nd injection) all animals had little or no detectable 17-DHC in their plasma. Throughout the rest of the treatment period samples drawn 24 hr after the previous dexamethasone injection remained at less than 50% of the average base line, pretreatment levels (Fig. 27).

The blastogenic response of isolated lymphocyte microcultures established from IBRV infected cattle prior to, during, and following dexamethasone treatment was measured by the evaluation of tritiated

Table 7. Biochemical changes in bovine plasma during dexamethasone-induced IBRV recrudescence. Data is mean results of separate determinations on plasma drawn from 8 different cattle at times indicated

Plasma Constituent	Time - Days or Hours Pre-						
	-50	-30	0 hr.	1 hr.	2 hr.	4 hr.	8 hr.
SGOT	39.25	43.63	52.75	52.0	51.38	50.75	64.38
LDH	540.63	558.25	615.63	564.88	567.63	597.63	575.86
Alk. Phos.	56.25	52.75	54.0	55.13	52.38	53.25	68.25
T. Bili.	0.20	0.19	0.125	0.113	0.10	0.10	.125
Alb.	3.29	3.25	3.40	3.43	3.71	4.65	3.48
T.P.	6.3	6.45	6.50	6.51	6.34	6.3	6.79
Chol.	172.50	187.13	171.50	161.25	153.13	159.63	204.88
Uric Acid	0.58	0.48	0.64	0.54	0.65	0.61	0.99
BUN	11.5	12.0	12.0	13.50	13.50	12.63	13.13
Glu.	69.63	67.25	58.38	68.25	71.0	78.63	79.0
Inor. Phos.	6.2	6.8	7.19	6.58	5.99	5.49	5.56
Ca^{++}	8.5	8.49	8.54	8.39	8.2	8.31	8.49

[a]Total increase due to one unusually high value.

or Post Dexamethasone Treatment (30 mg I.V.)							
12 hr.	24 hr.	48 hr.	72 hr.	96 hr.	2 Day	9 Day	28 Day
60.63	53.13	51.13	51.0	42.14	100.38	225.25	56.86
513.75	544.13	483.38	534.38	372.14	392.86	615.75[a]	612.6
60.50	57.38	59.25	67.75	79.14	100.50	99.13	46.5
0.113	0.075	0.075	0.075	0.10	0.125	.325[a]	.125
3.99	4.10	4.13	4.19	3.99	3.91	3.88	3.86
6.66	6.50	6.50	6.63	6.47	6.43	6.36	6.46
167.63	142.63	138.25	137.0	122.14	103.0	111.63	135.5
.75	.64	.825	1.04	.86	1.03	1.01	.61
12.0	15.25	13.88	13.75	14.43	14.5	13.86	14.38
105.63	121.25	101.38	100.5	93.57	89.13	92.38	62.25
4.90	4.36	3.98	4.51	4.86	4.56	4.65	6.99
8.54	8.31	8.54	8.51	8.63	8.41	8.25	8.51

Table 8. Changes in bovine plasma levels of 17-hydroxycortisone[a] during dexamethasone-induced IBRV recrudescence.

Animal Number	Time - Days or Hours Pre- or Post-Dexamethasone Treatment														
	-6Da	-5Da	-3Da	0 Hr[b]	1 Hr	8 Hr	12hr	25hr	30hr	48hr	72hr	96hr	5 Da	6 Da	7 Da
6966	1.67	1.28	1.16	2.32	0.99	0.00	0.46	0.60	0.00	0.10	0.25	0.45	0.70	0.35	0.55
6973	1.72	0.70	0.78	0.65	0.57	0.00	0.34	0.30	0.00	0.20	0.50	0.70	0.55	0.40	0.30
6975	2.29	1.17	0.91	0.96	0.65	0.00	0.52	0.20	0.00	0.00	0.60	0.50	0.45	0.45	0.50
7016	0.99	0.65	0.65	1.48	0.78	0.00	0.00	0.03	0.00	0.00	0.70	0.55	0.50	0.00	0.55
7017	1.67	0.63	2.08	4.43	1.43	0.00	0.00	0.46	0.20	0.20	0.75	0.00	0.50	0.60	0.60
7019	1.61	0.56	1.54	1.17	0.42	0.00	0.00	0.34	0.00	0.00	0.60	0.80	0.50	0.55	0.50
7020	0.55	0.63	1.04	0.73	0.39	0.13	0.05	0.30	0.20	0.69	0.55	0.50	0.60	0.35	0.35
7025	1.56	0.70	0.47	1.69	0.52	0.00	0.00	0.20	0.20	0.25	0.45	0.50	0.70	0.50	0.20

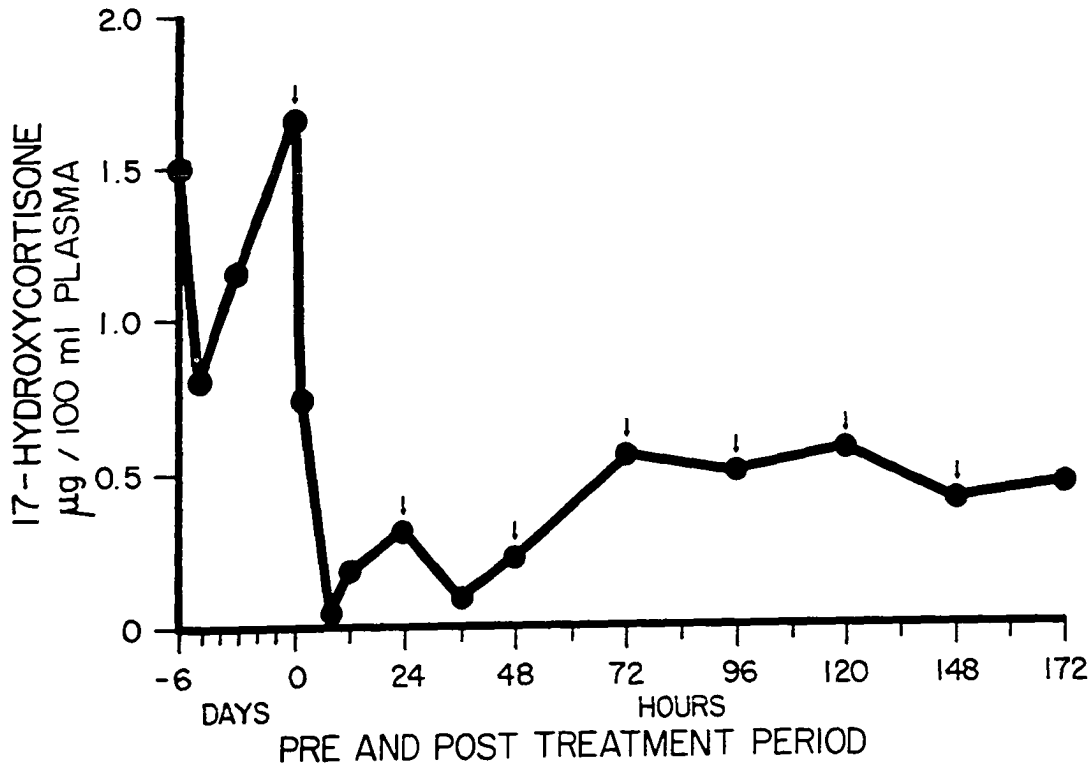
[a]Data recorded as µg % (micrograms/100 ml plasma). Analyses provided courtesy Dr. S. C. Whipp, National Animal Disease Center, Ames, Iowa. For method used see Whipp and Lyon (1970).

[b]Dexamethasone treatment begun.

Figure 27. Plasma 17-dehydroxycorticosterone levels before and during dexamethasone-induced IBRV recrudescence.

Data entered is mean of results of separate determinations on plasma taken from 8 different cattle at times indicated.

↓ Indicates time sequence of dexamethasone treatment.


17-HYDROXYCORTISONE
µg / 100 ml PLASMA
2.0
1.5
1.0
0.5
0
-6
0
24
48
72
96
120
148
172
DAYS
HOURS
PRE AND POST TREATMENT PERIOD

thymidine (^{3}HdT) uptake. The stimulation indices (SI's) of these cultures to PHA-M and inactivated IBRV antigen are recorded in Table 9. The PHA-M stimulation index (SI) was depressed from a pretreatment average of 30.0 to 6.9 within 24 hours of the beginning of dexamethasone treatment, remained at similar low levels for 72 hours and gradually returned to near pretreatment levels even during treatment (Table 9). IBRV SIs of cultures from chronically IBRV-infected cattle showed a distinct increase within 48 hours of the initial treatment (Table 9) and returned to pretreatment levels 7 days after dexamethasone treatment ceased. IBRV SIs of noninfected control animals were negative and showed no appreciable change during the treatment period. A composite histogram illustrating the mean PHA-SIs of all dexamethasone treated cattle and 2 noninfected controls is shown in Fig. 28. It should be noted that the increase in IBRV-SIs occurs rapidly after the PHA-SI depression. A more graphic illustration of this effect can be demonstrated by plotting the individual SIs of animal #7016 (Fig. 29).

Dexamethasone treatment decreased the percentage of T lymphocytes in the circulating lymphocytes of all cattle treated (Table 10). Pretreatment base line T lymphocyte percentages ranged from 73.2 to 94.6 and averaged 83.5%. The lowest group average of 67.8% occurred on post treatment day 6 and in one individual (#7138) only 52% of the lymphocyte pool were T lymphocytes. T lymphocyte depression became obvious 4 days post treatment (Fig. 30), reached a maximum at 6 days post treatment and had not returned to normal levels 12 days after treatment was stopped.

Table 9. Blastogenic response of microcultures of isolated lymphocytes from chronically IBRV-infected and control cattle to phytohemagglutinin-M (PHA-M) and inactivated IBRV-A antigen (Ag) before and after dexamethasone treatment. Each animal was given 30 mg. of dexamethasone intravenously for 10 consecutive days beginning March 18, 1974.

	3/4/74		3/11/74		3/18/74		3/20/74		3/22/74	
Animal Number	PHA[a] SI	IBR[b] SI	PHA SI	IBR SI	PHA SI	IBR SI	PHA SI	IBR SI	PHA SI	IBR SI
6966	96.3	15.3	33.5	8.5	27.7	10.4	2.1	5.5	5.5	32.5
6973	51.3	5.0	26.2	5.4	32.6	4.5	8.5	4.5	26.1	5.4
6975	32.2	4.7	22.9	3.9	17.4	3.9	9.9	4.2	29.0	5.7
7016	29.9	5.7	20.9	5.3	21.2	5.7	4.4	2.8	7.8	5.0
7019	28.1	.8	11.7	1.6	12.7	1.2	1.6	.9	7.0	1.7
7020	21.0	1.7	17.1	1.8	21.4	1.7	3.2	1.2	14.1	1.3
7025	32.2	4.1	5.3	.9	24.4	3.3	10.2	3.0	31.6	8.8
7126[c]	50.5	.4	19.4	.5	36.7	.7	12.0	.6	17.0	.6
7138[c]	70.0	.4	32.9	1.1	12.3	1.0	10.0	1.0	24.4	.8
$\bar{\Sigma}$IBR+[d]		5.33		4.0		4.8		3.2		8.6
$\bar{\Sigma}$IBR-[e]		.4		.8		.9		.8		.7
$\bar{\Sigma}$PHA-SI	45.7		21.1		22.9		6.9		18.1	

[a]PHA-SI = PHA-M stimulation index.

[b]IBR-SI = IBRV stimulation index

[c]Controls, noninfected.

[d]Mean IBRV-SI infected cattle

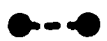
[e]Mean IBRV-SI controls

3/24/74		3/26/74		4/4/74		4/9/74		4/16/74	
PHA SI	IBR SI	PHA SI	IBR SI	PHA SI	IBR SI	PHA SI	IBR SI	PHA SI	IBR SI
13.3	31.0	18.4	23.0	27.8	12.0	22.5	8.1	16.1	2.4
27.3	4.1	44.1	3.8	35.7	7.4	40.9	6.9	58.9	2.8
16.3	3.7	13.3	2.3	32.3	6.6	20.4	6.8	74.5	3.9
4.6	9.7	4.2	12.4	29.2	15.0	20.4	5.1	NT	NT
2.0	1.1	47.5	.9	14.3	2.4	16.0	2.1	NT	NT
11.3	1.9	11.3	1.3	17.2	3.4	19.5	3.2	51.0	1.7
14.4	2.2	28.7	9.8	34.0	8.8	24.6	6.1	33.6	8.1
19.9	.8	28.5	.6	27.9	.8	36.2	.6	NT	NT
16.6	.9	18.1	.9	37.9	1.9	13.8	1.3	21.1	1.4
	7.7		7.6		7.9		5.5		3.8
	.9		.8		1.4		1.0		1.4
14.0		28.3		28.5		23.8		36.9	

Figure 28. Blastogenic response of microcultures of isolated lymphocytes from chronically IBRV infected and control cattle to phytohemagglutinin-M (PHA-M) and inactivated IBRV antigen following intravenous dexamethasone treatment (30 mg I.V./animal/day).


PHA-M stimulation index.


IBRV stimulation index, chronically infected cattle.


IBRV stimulation index, noninfected controls.


Period of dexamethasone treatment.

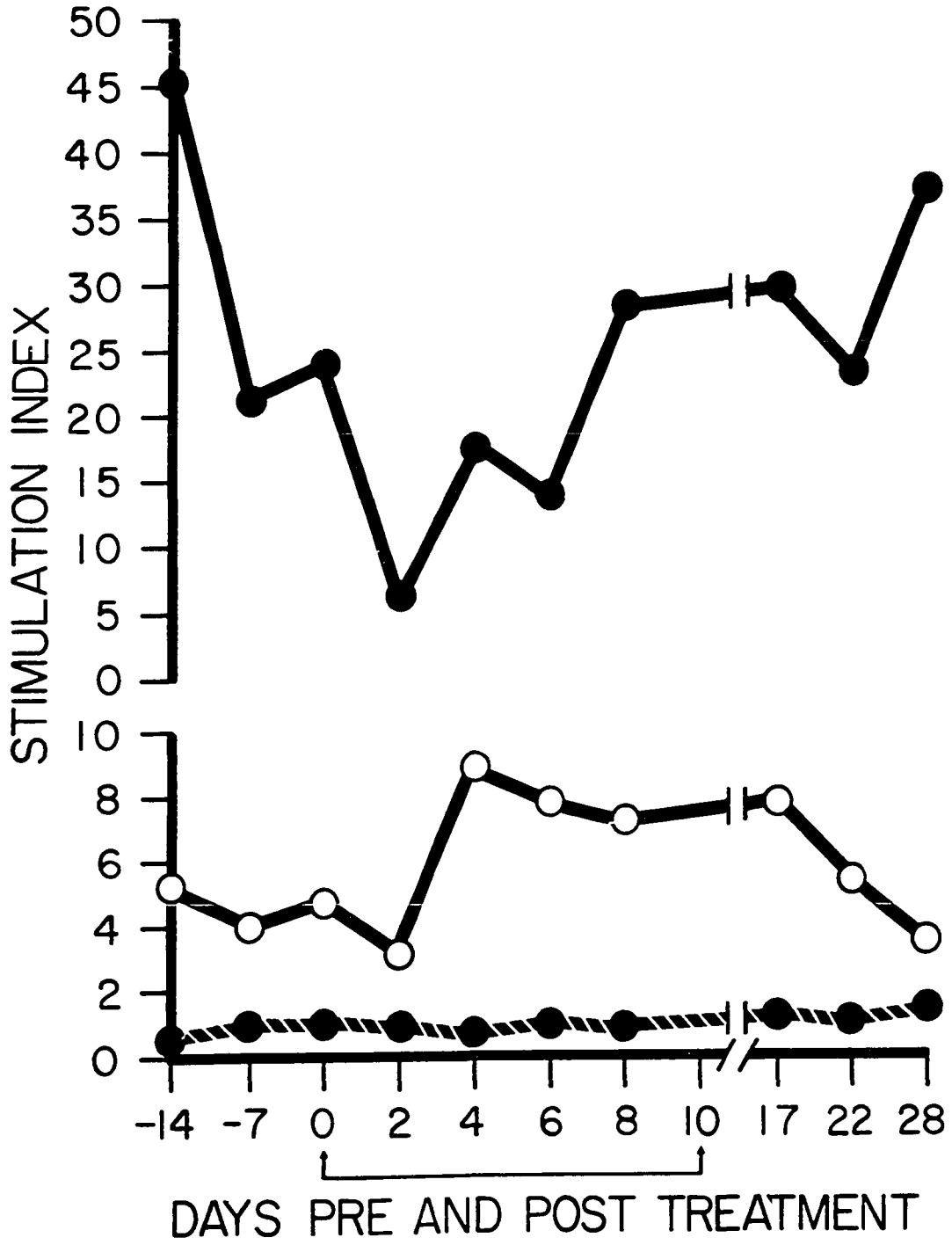

STIMULATION INDEX
50
45
40
35
30
25
20
15
10
5
0
10
8
6
4
2
0
-14
-7
0
2
4
6
8
10
17
22
28
DAYS PRE AND POST TREATMENT

Figure 29. Blastogenic response of microcultures of isolated lymphocytes from one animal (#7016) to PHA-M and IBRV antigen following dexamethasone treatment.

The period of treatment was 0 through 10 days.

●—● PHA-M stimulation index.

○—○ IBRV stimulation index.

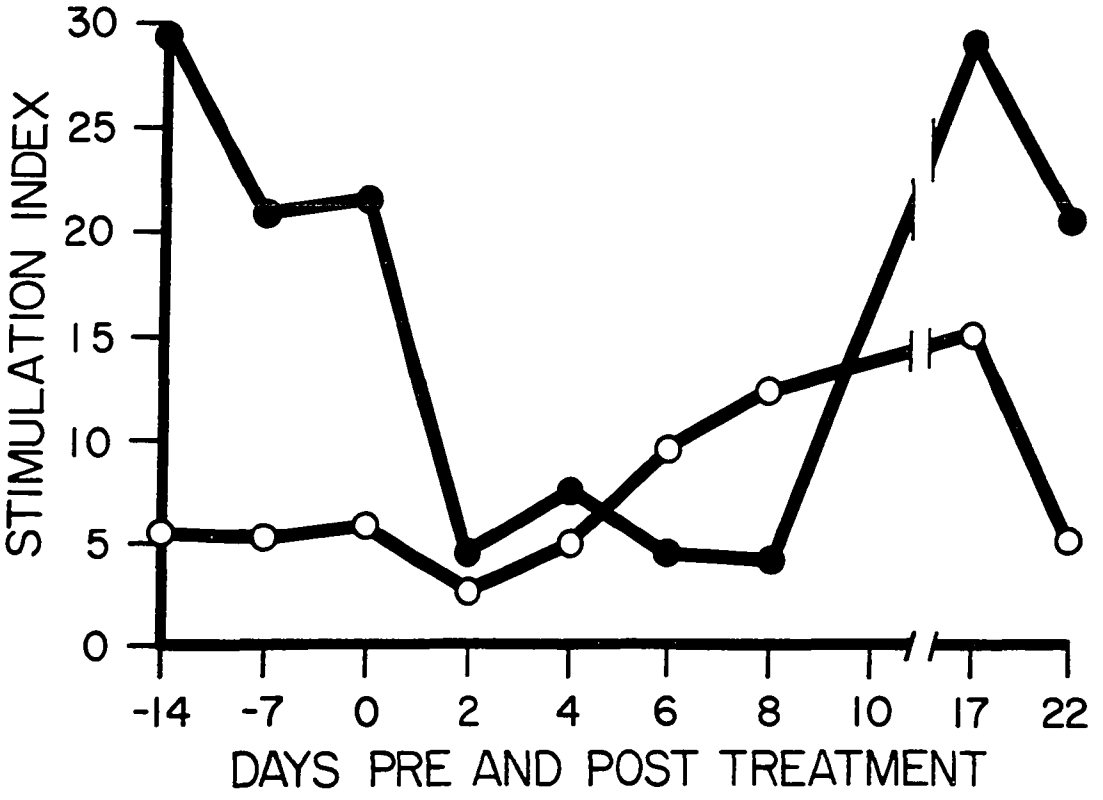

STIMULATION INDEX
30
25
20
15
10
5
0
-14
-7
0
2
4
6
8
10
17
22
DAYS PRE AND POST TREATMENT

Table 10. Effect of dexamethasone treatment on percentage of T-lymphocytes in Ficoll-Hypaque isolated portions of circulating lymphocyte pools of cattle. Each animal was given 30 mg. of dexamethasone intravenously for 10 consecutive days beginning 3/18/74

Animal Number	Date T lymphocyte percentage was determined										
	2/12/74	2/25/74	3/4/74	3/11/74	3/18/74[a]	3/20/74	3/22/74	3/24/74	3/26/74[b]	4/4/74	4/9/74
6966	79.0	85.1	83.2	84.5	82.2	75.0	83.0	75.0	69.0	86.0	79.0
6973	82.5	86.0	81.5	84.9	81.3	82.0	74.0	61.0	63.0	78.0	76.0
6975	89.6	92.2	81.1	81.9	90.7	80.0	80.0	58.0	77.0	84.0	78.0
7016	ND[c]	88.3	75.6	86.7	88.9	79.0	67.0	72.0	82.0	66.0	82.0
7019	85.4	87.4	80.0	92.6	85.0	76.0	80.0	76.0	83.0	74.0	76.0
7020	76.2	85.3	80.9	90.0	87.5	80.0	81.0	76.0	80.0	70.0	83.0
7025	84.4	88.6	81.5	86.1	87.8	79.0	76.0	65.0	71.0	76.0	78.0
7126	86.2	94.6	76.7	77.8	84.7	84.0	77.0	74.0	68.0	71.0	72.0
7138	83.2	ND	79.5	79.6	87.4	81.0	71.0	52.0	66.0	65.0	72.0
Mean	82.1	88.4	80.0	84.9	86.2	79.6	76.6	67.8	73.2	77.4	77.3

[a]Dexamethasone treatment began 3/18/74.

[b]Dexamethasone treatment stopped 3/27/74.

[c]ND = Not Determined.

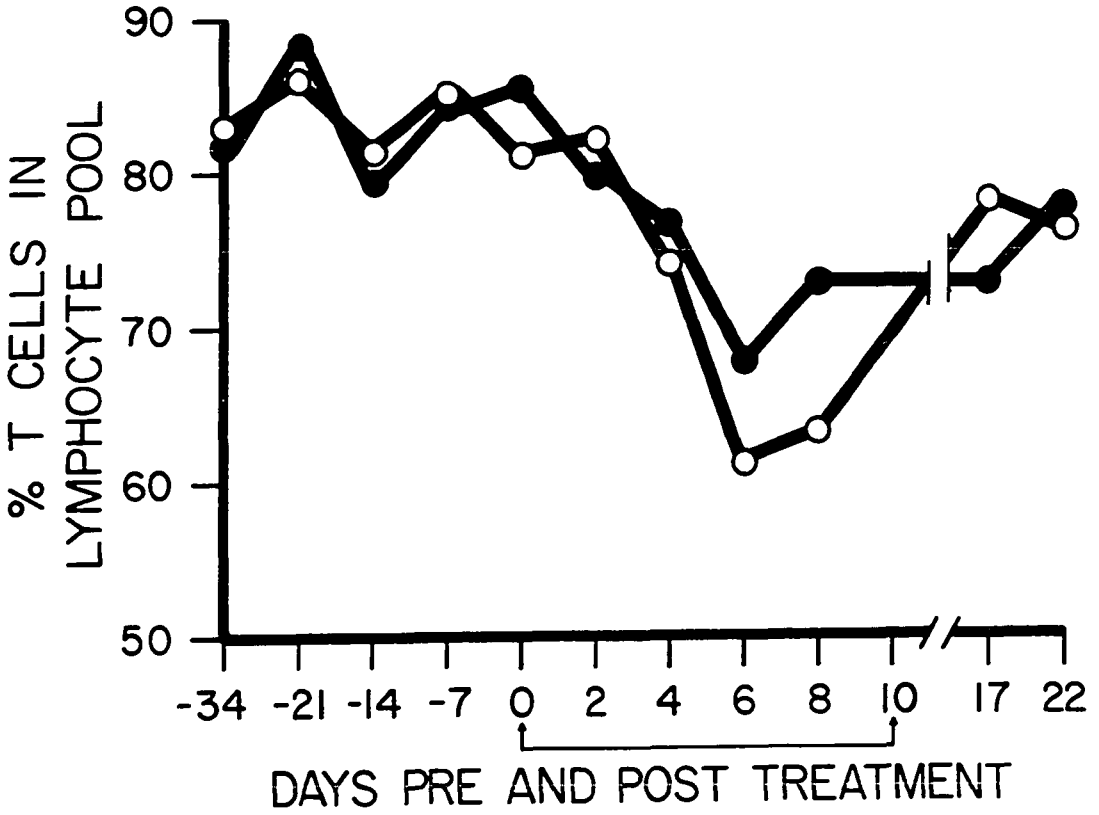
Figure 30. Effect of dexamethasone treatment of cattle on percentage of T lymphocytes in their circulating lymphocyte pools.

Data plotted are mean of separate determinations on 9 cattle. Data from 1 (#6973) of 9 are plotted separately.


Mean of separate determinations of 9 cattle.


Determinations of 1 individual (#6973).


Period of dexamethasone treatment.


90
80
70
60
50
% T CELLS IN LYMPHOCYTE POOL
-34 -21 -14 -7 0 2 4 6 8 10 17 22
DAYS PRE AND POST TREATMENT

In experiment 3 it was interesting to compare the results of IBRV isolation attempts from nasal secretions. Cattle that secreted virus for several days in nasal mucus following initial exposure also secreted virus for several days following dexamethasone treatment (Table 11). Conversely, cattle from which little or no virus was isolated after initial exposure also secreted little or no virus from the nasal passages following dexamethasone treatment even though they had high levels of anti-IBRV antibodies in their serum.

Histopathological examination of skin taken by surgical biopsy 12 hr post intradermal injection of IBRV antigen revealed early necrotic changes in the stratified squamous epithelium of the dermis and edema of the subcutaneous tissues. Accumulation of inflammatory cells in the interstitial areas were composed primarily of neutrophils and lymphocytes (Fig. 31A). Small blood vessels had perivascular accumulations of lymphocytes (Fig. 31B). The histopathologic changes in the 24 hr biopsy section were similar to those of the 12 hr specimen. Necrosis of the stratified squamous epithelium was prominent over the injection site in the 24 hr biopsy sample (Fig. 31C). Perivascular accumulations of small lymphocytes around the small vessels were more pronounced and more widely disseminated (Fig. 31D). Neutrophil accumulations were not as prominent in the 48 hr biopsy (Fig. 32A) sections as in the 12 and 24 hr sections. The lymphocyte and macrophage accumulation was the predominant inflammatory cell present. The 72 hr biopsy section (Fig. 32C) had few neutrophils but the perivascular accumulation of lymphocytes and macrophages was not markedly changed from that seen in earlier biopsies.

Table 11. Comparisons of IBRV shedding in nasal secretions during initial (IE), first dexamethasone induced recrudescence (R1) and second recrudescence (R2).

Animal Number	Event	Dyas Pre- or Post-Treatment of Exposure[a]																		
		-1	-2	-3	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
6966	IE	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
	R1	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-
	R2	-	-	-	-	-	-	+	+	+	+	+	+	ND	+	ND	ND	ND	ND	ND
6973	IE	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
	R1	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-
	R2	-	-	-	-	-	-	-	+	+	+	+	+	ND	+	ND	ND	ND	ND	ND
6975	IE	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-
	R1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R2	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	ND	ND	ND	ND	ND
7016	IE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
	R1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R2	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	ND	ND	ND	ND	ND
7019	IE	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-
	R1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
	R2	-	-	-	-	-	-	+	+	+	+	+	+	ND	+	ND	ND	ND	ND	ND
7020	IE	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	R1	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-
	R2	-	-	-	-	-	-	-	+	+	+	+	+	ND	-	ND	ND	ND	ND	ND
7025	IE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R1	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	R2	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	ND	ND	ND	ND	ND

[a]-Virus isolations attempted but none isolated, +virus isolated from nasal secretions, ND Not determined.

Figure 31. Tissue sections made from surgical biopsies of skin test reaction to intradermal injection of inactivated IBRV antigen.

A. 12 hr post injection (PI) biopsy.

B. Perivascular accumulation of mononuclear cells 12 hr PI biopsy.

C. 24 hr PI biopsy. Note necrosis of stratified squamous epithelium (top).

D. Perivascular accumulation of mononuclear cells 24 hr PI. Hematoxylin and eosin stain.

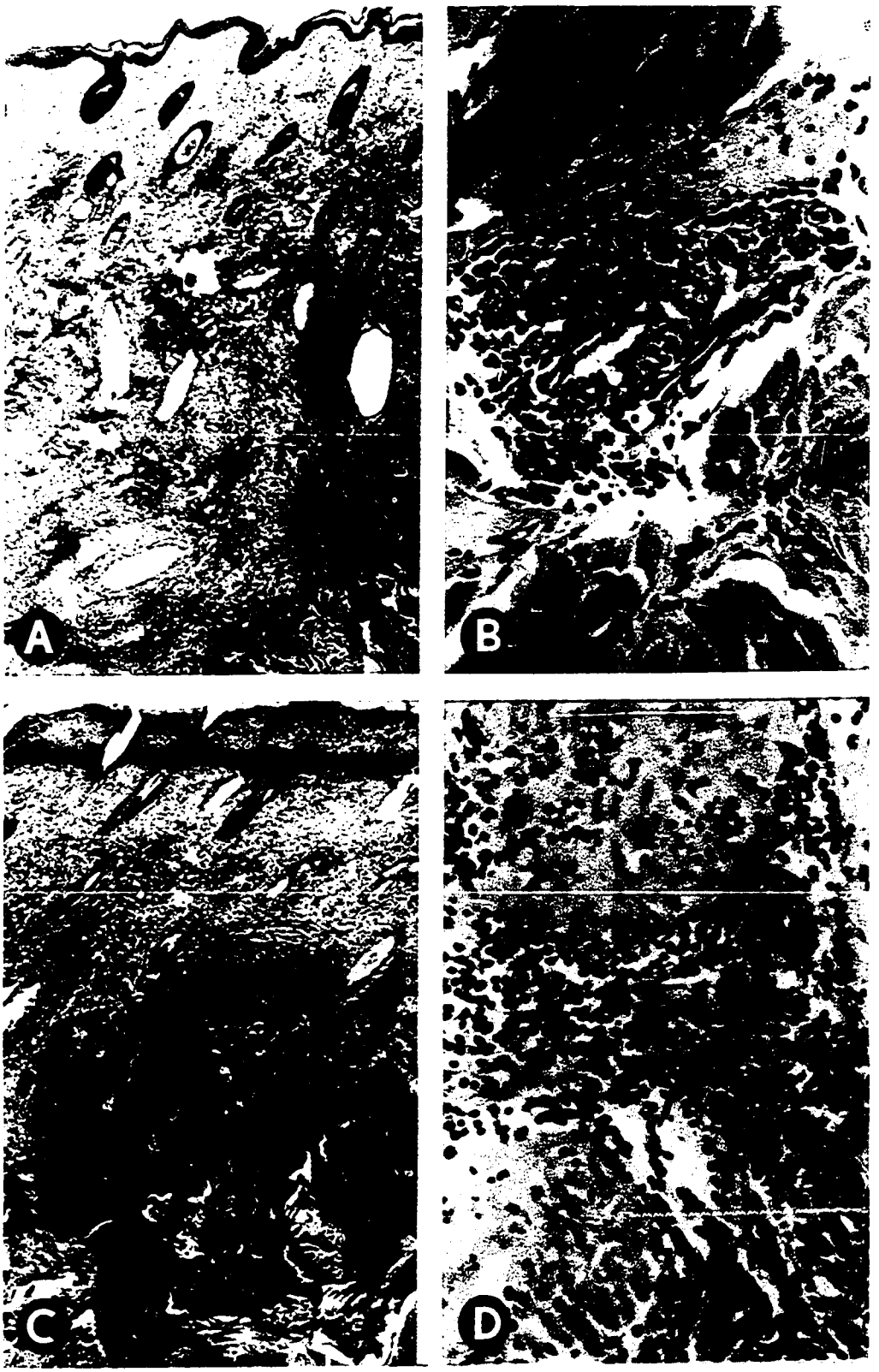

A
B
C
D

Figure 32. Tissue sections made from surgical biopsies of skin test reaction to intradermal injection of inactivated IBRV antigen.

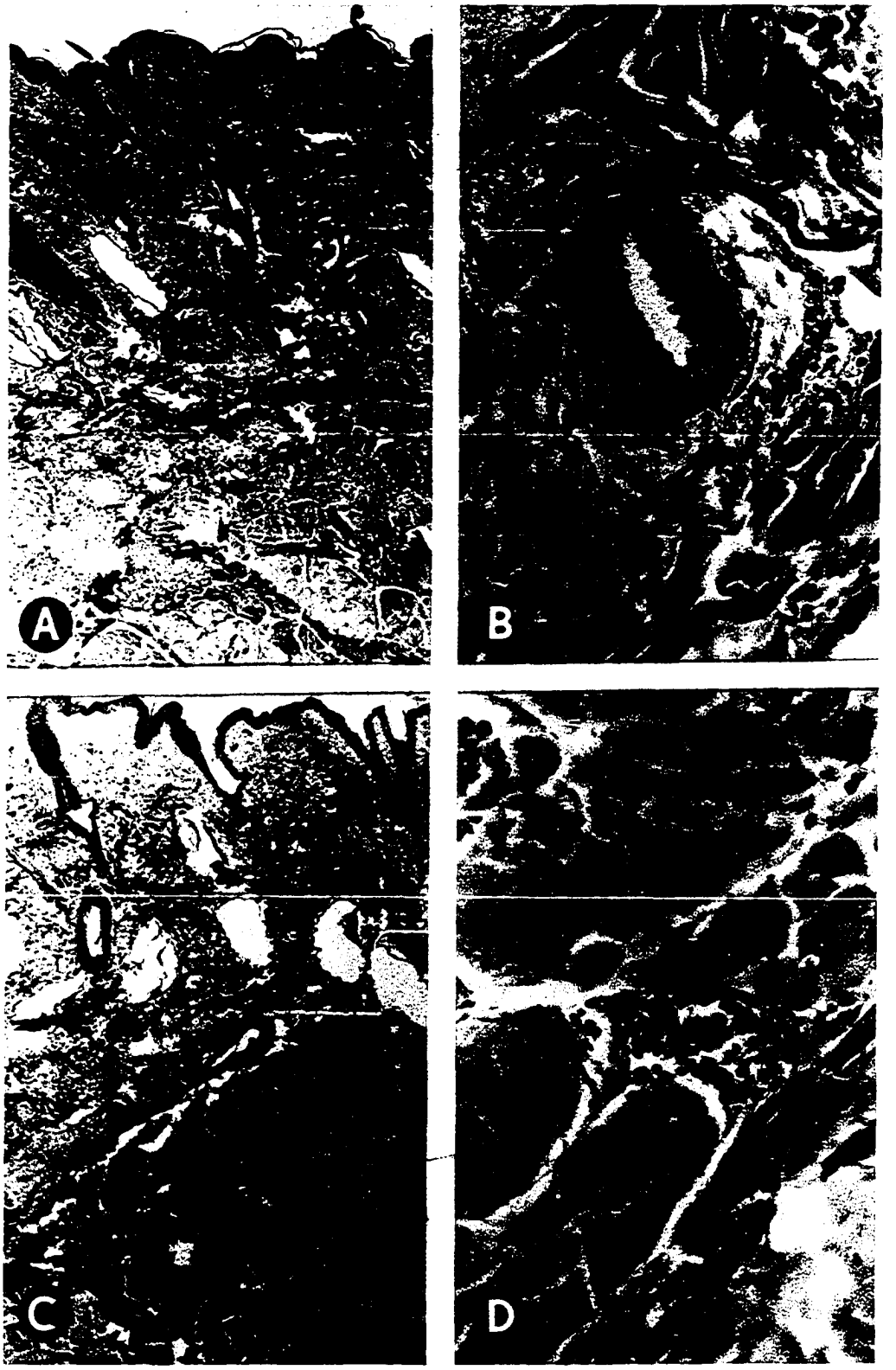
A. 48 hr post injection (PI).

B. 48 hr PI.

C. 72 hr PI.

D. 72 hr PI.

Hematoxylin and eosin stain.


A
B
C
D

Dexamethasone treatment suppressed the development of necrosis, swelling and edema and virtually eliminated the infiltration of the area with neutrophils (Fig. 33). However, the mononuclear cellular infiltrate did not appear to be markedly reduced (Figs. 33 and 34).

Experiment 4

All calves exposed to IBRV, Colo, Jensal, and K-22 strains by the intranasal route developed acute respiratory disease characterized by hyperthermia (Fig. 35), rapid respiration, anorexia and early development of focal necrotic lesions of the respiratory epithelium in the anterior nares. There were no obvious differences in the extent or degree of clinical illness except that IBRV Colo strain-infected calves had an elevated rectal temperature for a longer period of time than those exposed to other strains (Fig. 35). All calves secreted virus in their nasal mucus from 1 to 8-14 days after exposure. Only one calf (#7472) secreted virus for more than 10 days post exposure (Table 12).

The blastogenic response to inactivated IBRV antigen as measured by the uptake of tritiated thymidine was not detectable by the method used through 3 weeks post exposure. No differences in response to homologous or heterologous antigens were detected. Only lymphocyte cultures from calves having had repeated skin tests with inactivated IBRV antigen gave a stimulation index more than 2.0 (Fig. 36).

Dexamethasone treatment (30 mg I.V. daily) 6 months after initial exposure caused IBRV reactivation in all 5 calves. One calf died of bloat 5 months post exposure. No major differences in clinical illness nor secretion of virus in nasal mucus could be detected between strains (Table 12).

Figure 33. Tissue sections made from surgical biopsies of skin test reaction to intradermal injection of inactivated IBRV antigen after 10 days of dexamethasone treatment.

A and B. 12 hr post injection (PI)

C and D. 24 hr PI.

Hematoxylin and eosin stain.

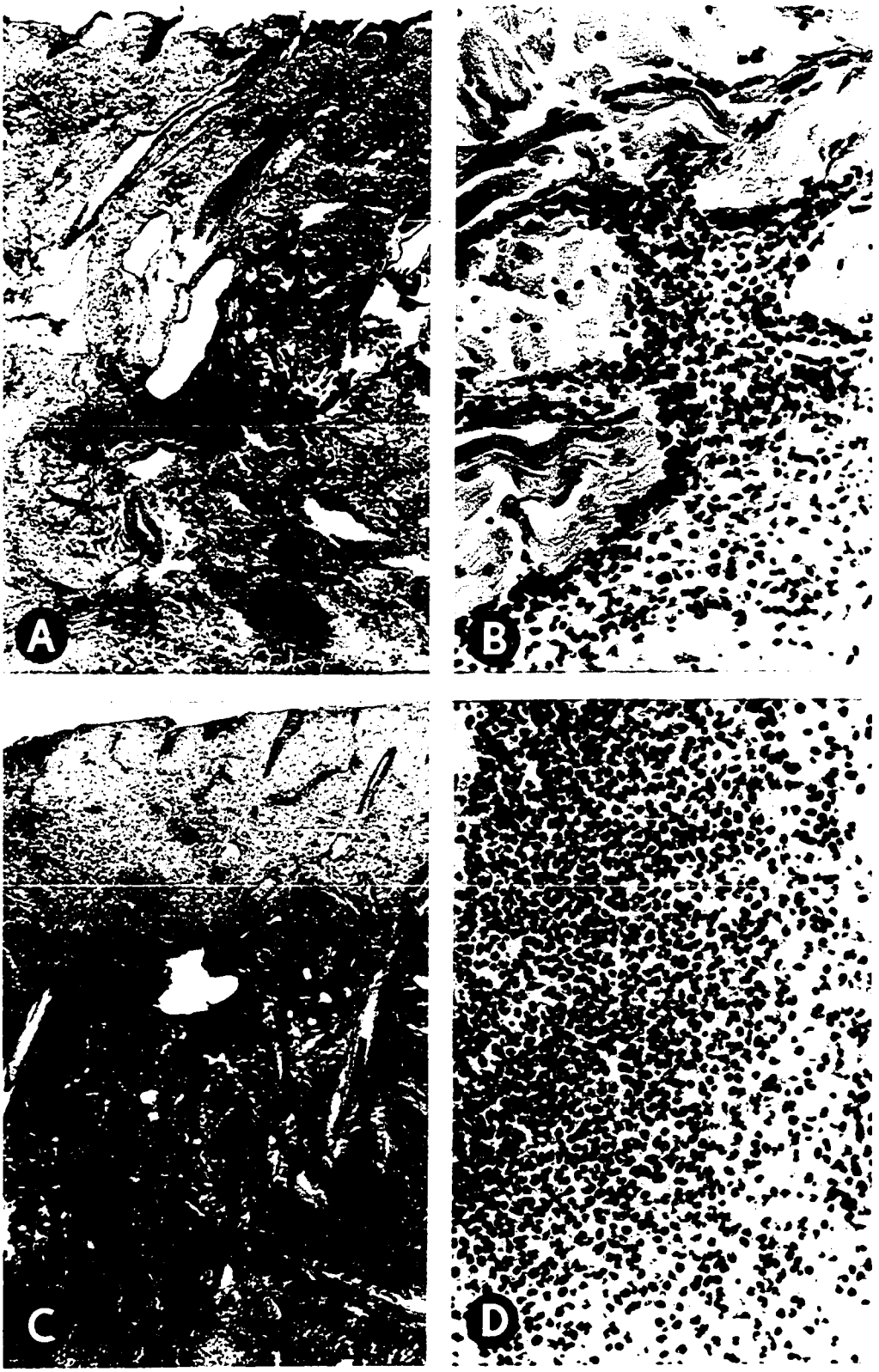

A
B
C
D

Figure 34. Tissue sections made from surgical biopsies of skin test reaction to intradermal injection of inactivated IBRV antigen after 10 days of dexamethasone treatment.

A. 48 hr PI.

B. 48 hr PI.

C. 72 hr PI

D. 72 hr PI

Hematoxylin and eosin stain.

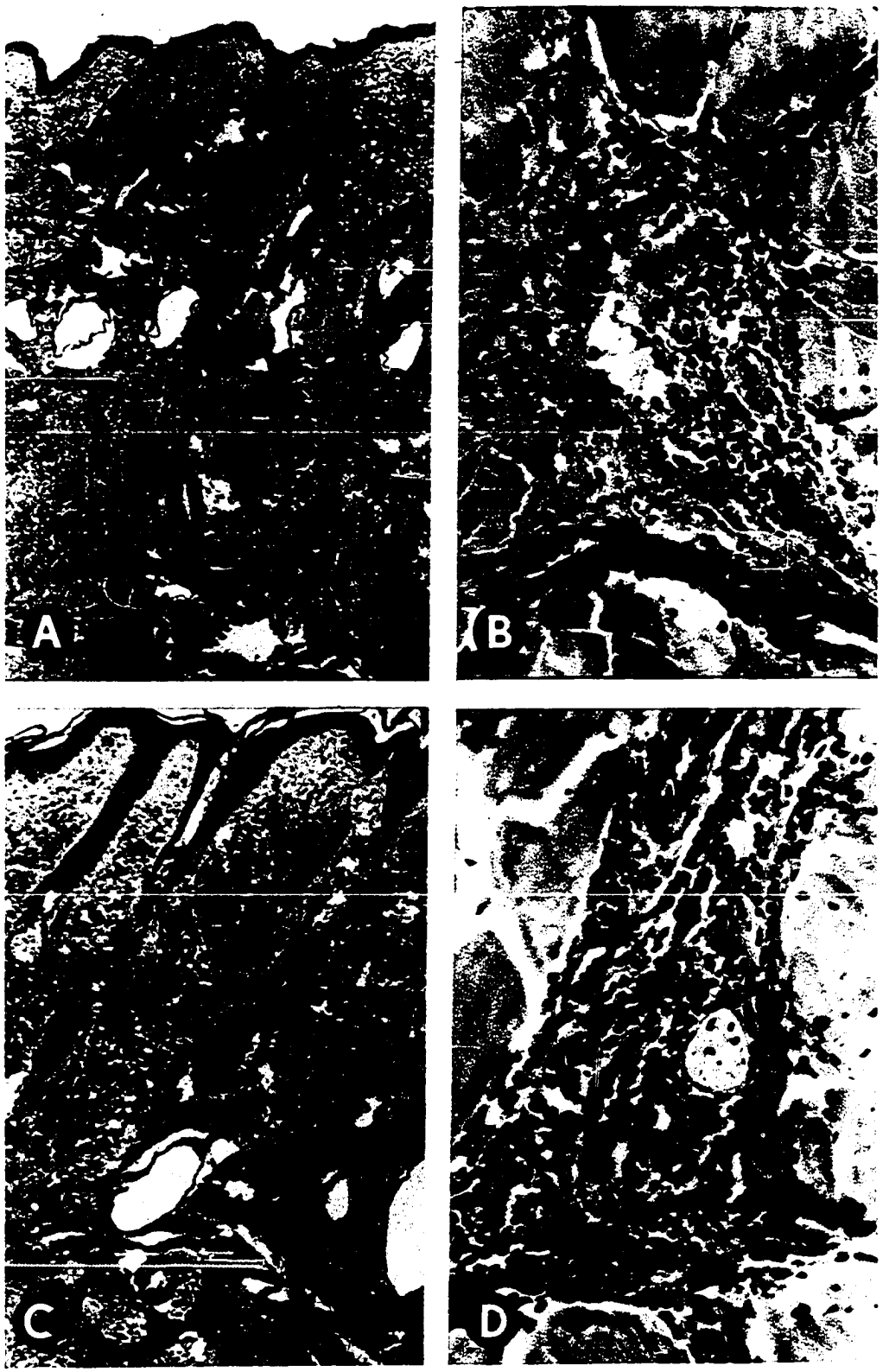

A
B
C
D

Figure 35. Rectal temperature of calves following aerosol exposure to IBRV Colo, Jensal and K-22 strains by the intranasal route.

Data plotted are means of daily temperatures of 2 animals exposed to each IBRV strain.

 Two calves exposed to Jensal strain IBRV.

 Two calves exposed to K-22 strain IBRV.

 Two calves exposed to Colo strain IBRV.

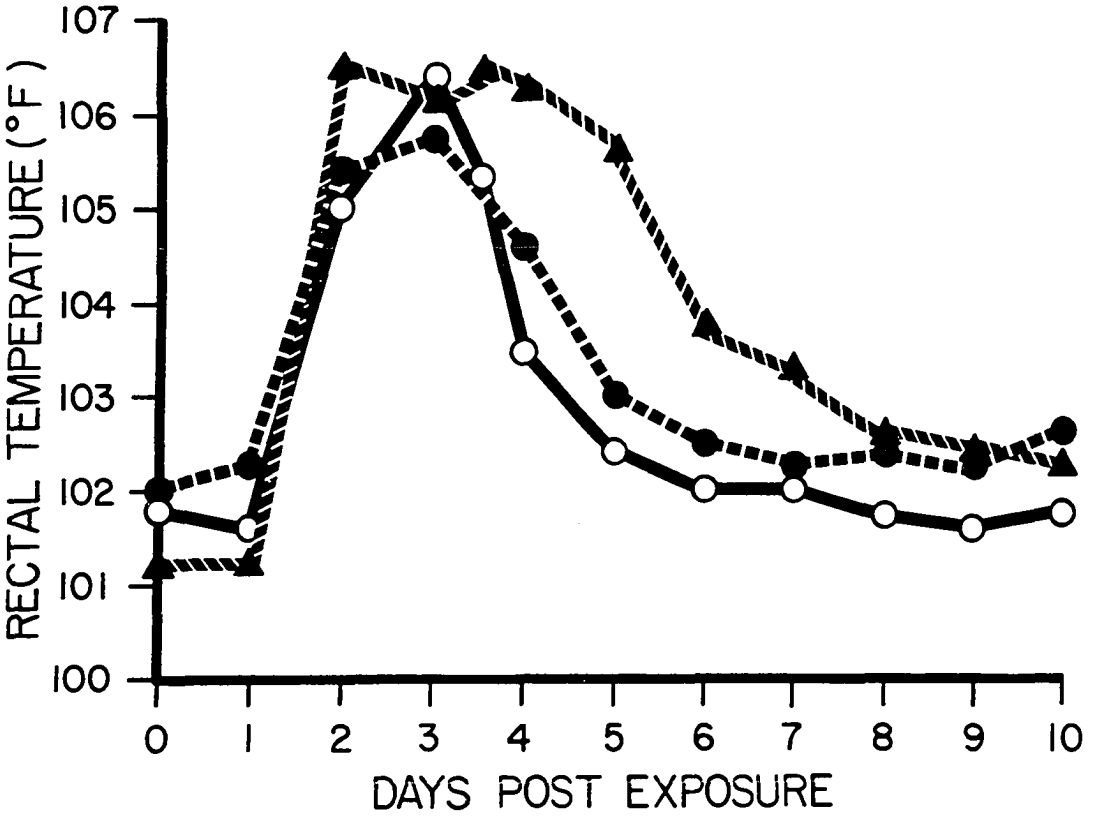

RECTAL TEMPERATURE (°F)
107
106
105
104
103
102
101
100
0
1
2
3
4
5
6
7
8
9
10
DAYS POST EXPOSURE

Figure 36. Immunostimulation of microcultures of isolated lymphocytes due to exposure to inactivated IBRV antigen following IBRV exposure and skin testing with inactivated IBRV antigen.

Data are results from one animal (#7466).

↓ IBRV skin test administered.

⇓ IBRV recrudescence due to dexamethasone treatment.

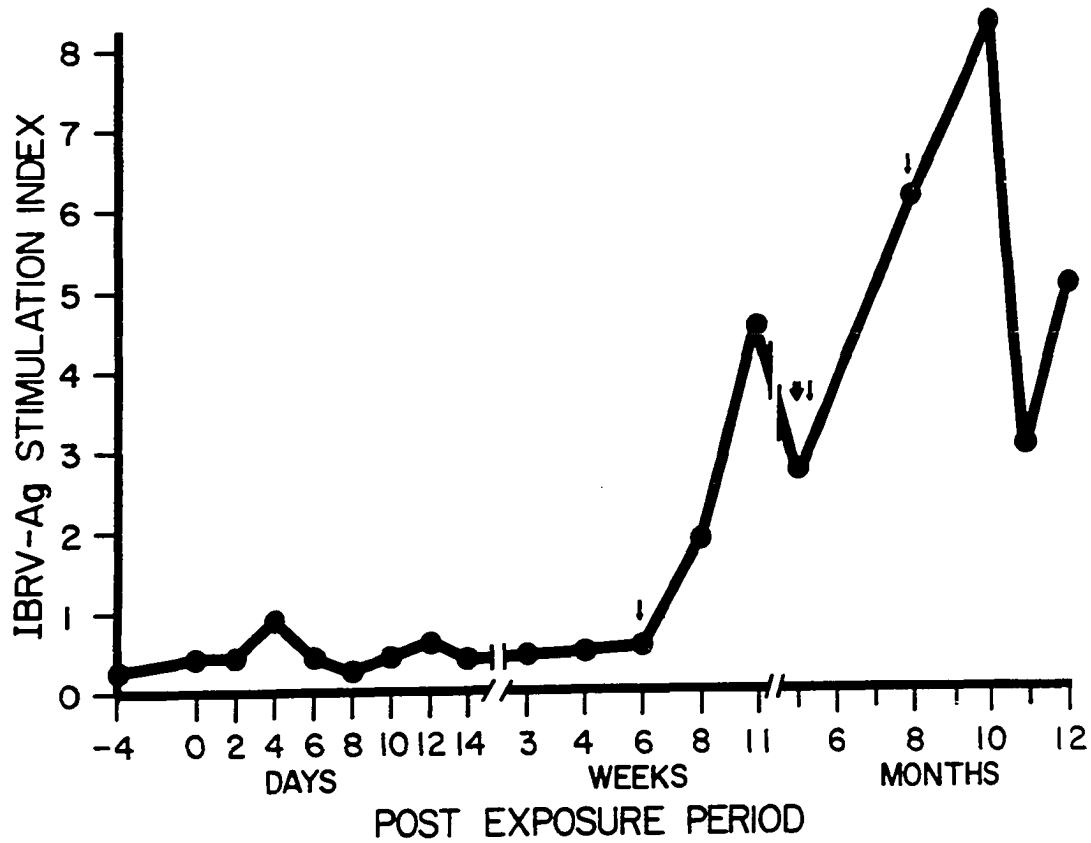

IBRV-Ag STIMULATION INDEX
0
1
2
3
4
5
6
7
8
-4 0 2 4 6 8 10 12 14 3 4 6 8 11 6 8 10 12
DAYS
WEEKS
MONTHS
POST EXPOSURE PERIOD

Table 12. IBRV isolations from nasal secretions following intranasal inoculation of Colo, Jensal, and K-22 strains of IBRV and reactivation of infection 6 months later with dexamethasone treatment (30 mg I.V./day for 10 days).

Animal Number		Days Post Exposure of Post Dexamethasone Treatment													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
7465[a]	IE	+	+	ND	+	ND	+	ND	+	ND	+	ND	-	ND	-
	R1	-	-	-	-	+	+	+	+	+	+	+	-	ND	-
7472[a]	IE	+	+	ND	+	ND	+	ND	+	ND	+	ND	+	ND	+
	R1				+	+	+	+	+	+	+	ND	-	ND	-
7471[b]	IE	+	+	ND	+	ND	+	ND	+	ND	-	ND	+	ND	-
	R1[c]														
7469[b]	IE	+	+	ND	+	ND	+	ND	+	ND	-	ND	-	ND	-
	R1	-	-	-	-	-	+	+	+	+	-	+	-	ND	-
7454[d]	IE	+	+	ND	+	ND	+	ND	+	ND	-	ND	-	ND	-
	R1	-	-	-	+	+	+	+	+	+	+	+	-	ND	-
7466[d]	IE	+	+	ND	+	ND	+	ND	+	ND	+	ND	-	ND	-
	R1	-	-	-	+	+	+	+	+	+	+	+	-	ND	-

[a]Initially exposed to IBRV-Colo strain.

[b]Initially exposed to IBRV IPV-K22 strain.

[c]Animal died from bloat.

[d]Initially exposed to IBRV-Jensal strain.

Experiment 5

All calves exposed to IBRV by aerosol became severely ill with acute upper respiratory disease except the two controls. All 6 of the nonimmune calves experienced extreme difficulty in breathing from 4-10 days post exposure to such an extent that penicillin-streptomycin was administered intramuscularly to 2 animals for 3 consecutive days. Anorexia, hyperthermia and depression were more prominent in these calves than is usual in experimentally IBRV-exposed calves. However, all calves were making rapid recovery by 12-14 days post exposure.

IBRV was isolated from 1 to 12 days post exposure from all non-immune calves, but only on 1 day from immune controls.

The blastogenic response of IBRV-Ag stimulated microcultures of isolated lymphocytes taken from the nonimmune calves during the period of illness failed to reach levels considered to be positive. Cultures prepared every other day for 2 weeks, weekly thereafter through 8 weeks and at variable intervals thereafter for 178 days failed to produce stimulation indices above 2.0 (Fig. 37). Positive controls remained high throughout the same period. The blastogenic response to PHA-M was at usual high levels throughout the entire test period.

Whole blood cultures from nonimmune calves began showing positive IBRV-Ag blastogenic responses early and reached maximum levels by 6 days post exposure (Fig. 38). Although the whole blood culture specific mitogen-induced response fell slightly at 12 to 14 days post exposure it remained relatively high throughout the 6 month test period. Toward the

Figure 37. Blastogenic response of isolated lymphocyte microcultures to IBRV inactivated antigen (IBRV-Ag) following aerosol exposure of cattle to IBRV.


Positive controls, average of 2 cattle.


IBRV initial exposure, average of 6 cattle.

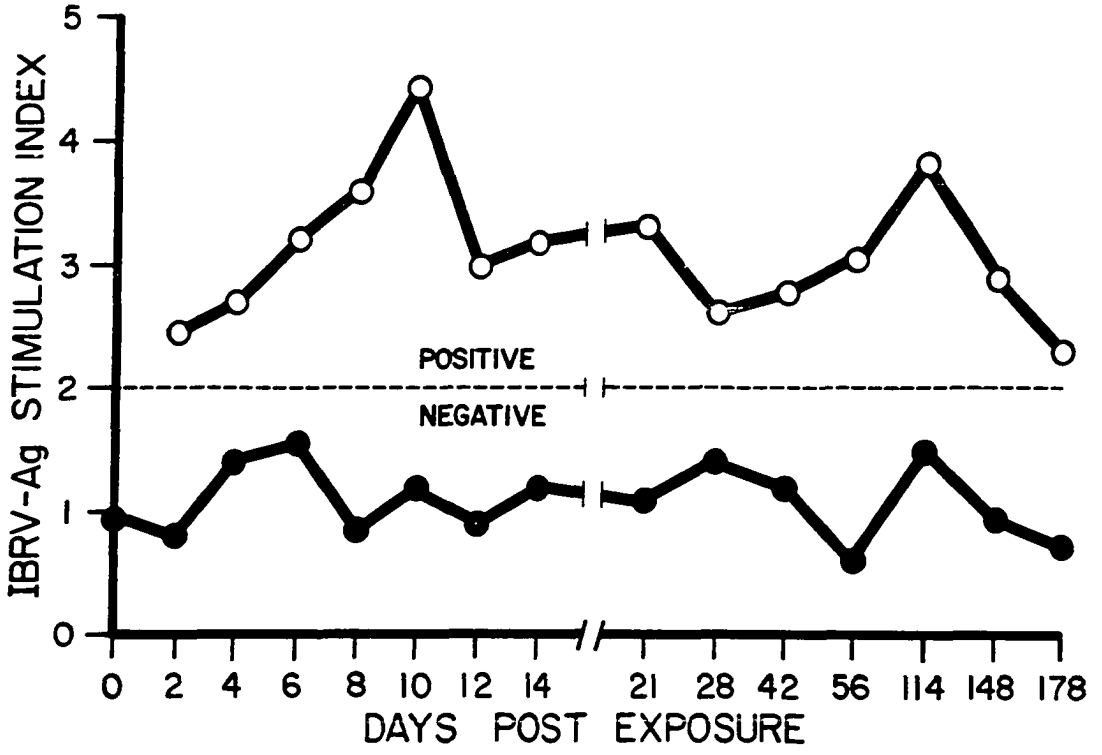
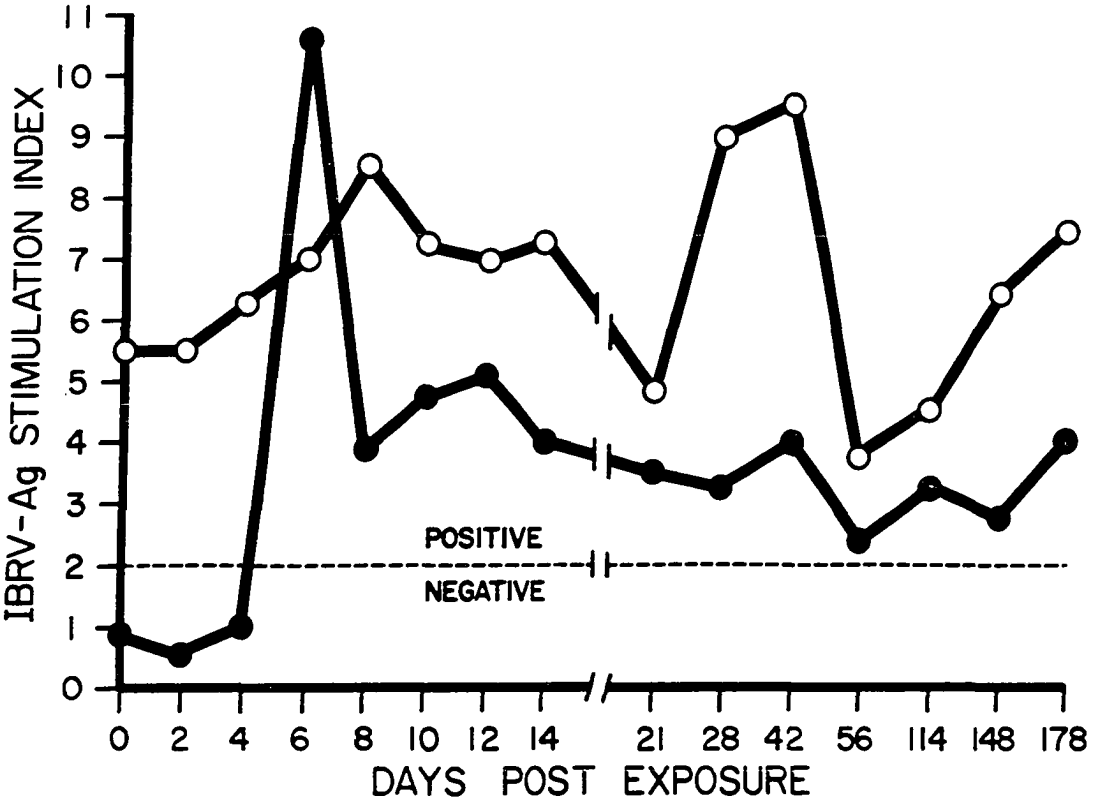

IBRV-Ag STIMULATION INDEX
5
4
3
2
1
0
POSITIVE
NEGATIVE
0 2 4 6 8 10 12 14 21 28 42 56 114 148 178
DAYS POST EXPOSURE

Figure 38. Blastogenic response of whole blood cultures to IBRV inactivated antigen (IBRV-Ag) following aerosol exposure of cattle to IBRV.


Positive controls, average of 2 cattle.


IBRV initial exposure, average of 6 cattle.


IBRV-Ag STIMULATION INDEX
0
1
2
3
4
5
6
7
8
9
10
11
POSITIVE
NEGATIVE
0
2
4
6
8
10
12
14
21
28
42
56
114
148
178
DAYS POST EXPOSURE

end of the test period (Fig. 38) the IBRV stimulation indices appeared to wane slightly. No skin testing or chemical recrudescence were conducted in this period of time.

Approximately 1 year following the initial exposure base line evaluations of specific antigen and phytomitogenic responses were evaluated in whole blood cultures and isolated lymphocyte microcultures prepared from all eight original animals in this experiment and one other nonimmune calf that was to be used as a negative control.

Dexamethasone treatment 1 year following initial exposure to IBRV caused recrudescence of nasal mucosal plaques and secretion of IBRV from the nasal mucus of 3 of 3 calves from 4 days post treatment (DPT) until calves were killed on DPT 9 (Table 13). Cyclophosphamide treated cattle were anorectic and showed evidence of dehydration from day 2 or 3 post treatment until the animals were killed on DPT 9. There was no clinical evidence of IBRV infection. Focal erosions of mucosa of the buccal cavity were obvious from day 6-9 post cyclophosphamide treatment. IBRV was isolated from the nasal secretion of 1 steer (#7736) on DPT 8 (Table 13). Control animals had no evidence of clinical illness nor secreted virus in the nasal mucus during this period.

Total leukocyte numbers in the peripheral blood were elevated markedly in all dexamethasone treated calves beginning DPT 1 (Fig. 39). Conversely leukocyte numbers in the peripheral blood of cyclophosphamide treated animals was depressed beginning on the 3rd day post treatment (Fig. 39). Leukocyte numbers were within normal limits in nontreated animals throughout the experiment (Fig. 39).

Table 13. IBRV isolations from nasal secretions of chronically infected cattle following immunosuppression with dexamethasone and cyclophosphamide.

Animal Number	Treatment	Days Pre- or Post-Treatment									
		-5	1	2	3	4	5	6	7	8	9
7736	Cy[a]	-	-	-	-	-	-	-	-	+[b]	-
7737	Cy	-	-	-	-	-	-	-	-	-	-
7738	Cy	-	-	-	-	-	-	-	-	-	-
7741	Dex[c]	-	-	-	-	+	+	+	+	+	+
7746	Dex	-	-	-	-	+	+	+	+	+	+
7748	Dex	-	-	-	-	+	+	+	+	+	+
7465[d]	None	-	-	-	-	-	-	-	-	-	-
7466[d]	None	-	-	-	-	-	-	-	-	-	-
7436[e]	None	-	-	-	-	-	-	-	-	-	-

[a]Cyclophosphamide injected intravenously (4 mg/kg 1st 4 days, then 2 mg/kg for 5 days)

[b]+ Viral isolate positively identified as IBRV by serological methods.

[c]Dexamethasone injected intravenously (30 mg/animal/day, 0-9).

[d]IBRV immune nontreated controls.

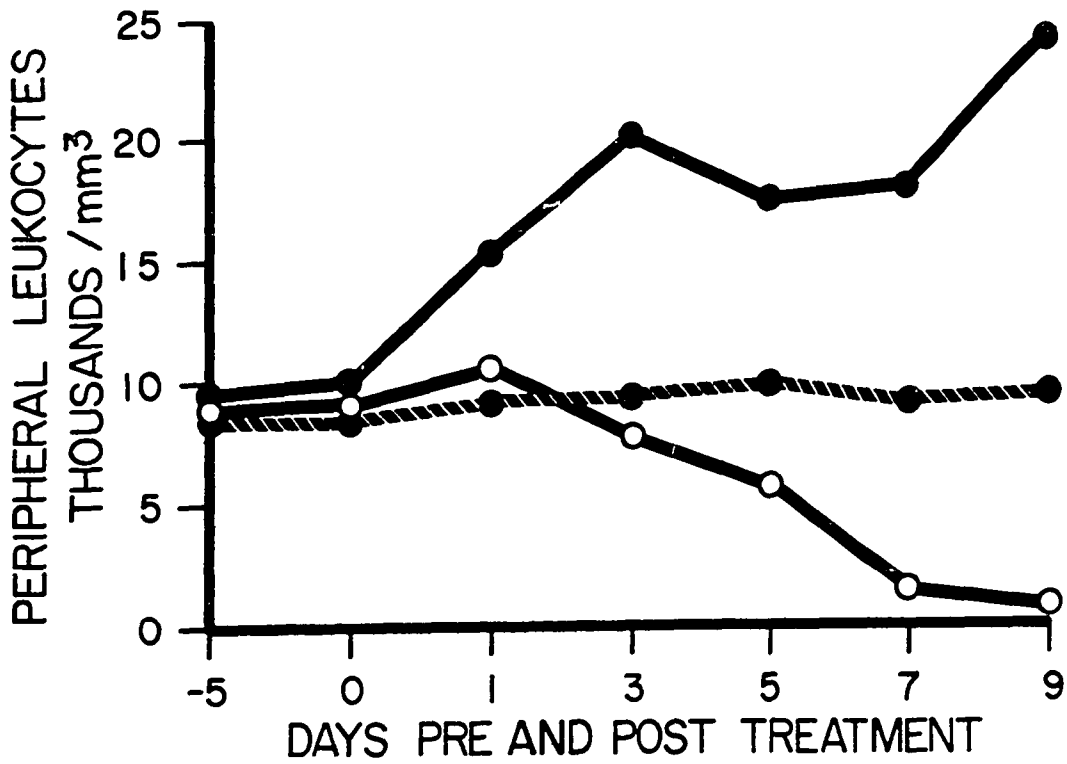
[e]IBRV nonimmune, nontreated controls.

Figure 39. Effects of dexamethasone and cyclophosphamide treatment on peripheral leukocytes of cattle with chronic IBRV infections.

● Average of 3 dexamethasone treated cattle.

○ Average of 3 cyclophosphamide treated cattle.

● Average of 2 nontreated control cattle.


PERIPHERAL LEUKOCYTES
THOUSANDS / mm3
25
20
15
10
5
0
-5
0
1
3
5
7
9
DAYS PRE AND POST TREATMENT

The dexamethasone-induced leukocytosis was due to a distinct neutrophilia in treated cattle (Fig. 40). Neutrophil numbers rose sharply at exactly the same intervals as total leukocytes. Lymphocytopenia developed during the concurrent leukocytosis and neutrophilia (Fig. 40) in dexamethasone treated cattle.

Cyclophosphamide-induced leukopenia in treated cattle was due to a distinct drug-induced neutropenia (Fig. 41). Neutrophil numbers decreased gradually during the treatment period.

Results of the determination of B cell - T cell ratios of isolated lymphocyte populations indicate that dexamethasone caused a pronounced depression of the percent of T cells in this lymphocyte population (Fig. 42). Cyclophosphamide caused an increase in the percentage of T cells because of its selective toxic effect on B cells (Fig. 42). B cell - T cell ratios remained relatively constant in nontreated controls.

The blastogenic response of isolated lymphocyte microcultures to phytomitogens (PHA-M) during dexamethasone and cyclophosphamide treatment is presented in Fig. 43. The PHA-M blastogenic response reflected in the stimulation index rose sharply from pre-treatment base line levels in cyclophosphamide treated cattle. The PHA-M induced blastogenic response in isolated lymphocyte microcultures showed a depression due to dexamethasone treatment whereas those of control animals remained relatively constant.

IBRV-Ag induced blastogenic responses of isolated lymphocyte microcultures in cyclophosphamide-treated cattle rose slightly following treatment whereas dexamethasone treatment apparently caused a slight

Figure 40. Effects of dexamethasone treatment on neutrophil and lymphocyte segments of the peripheral leukocyte population of cattle with chronic IBRV infections.

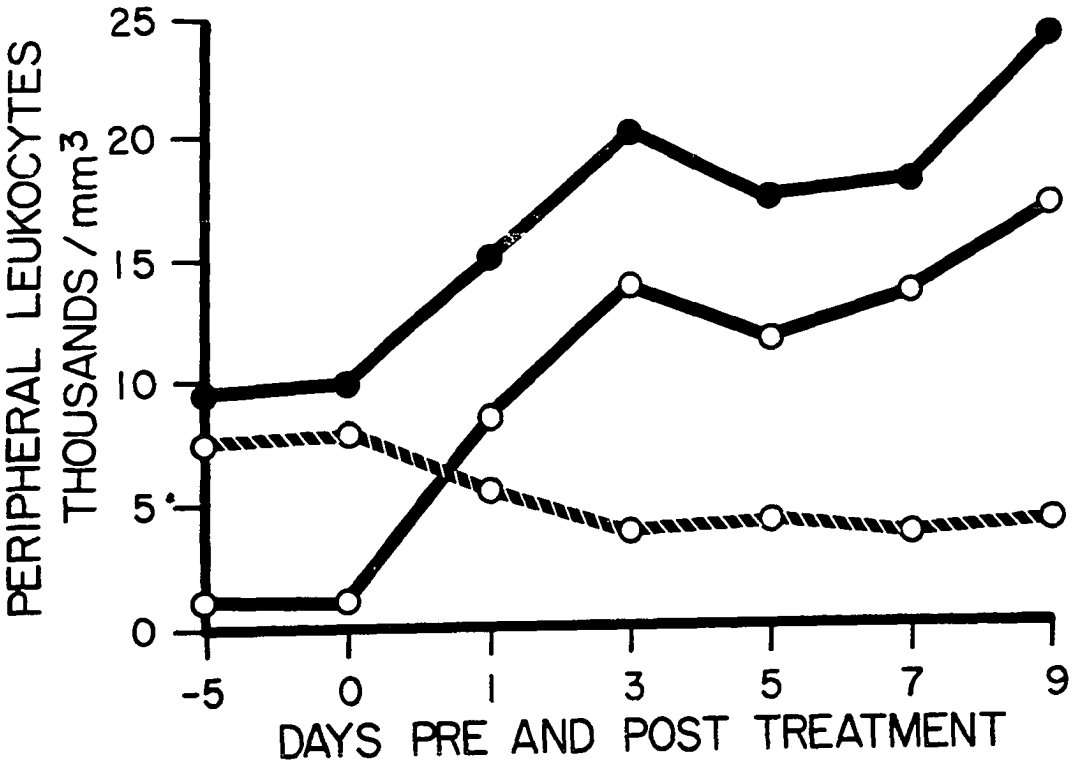
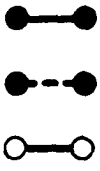

PERIPHERAL LEUKOCYTES
THOUSANDS / mm3
25
20
15
10
5
0
-5
0
1
3
5
7
9
DAYS PRE AND POST TREATMENT

Figure 41. Effects of cyclophosphamide treatment on the neutrophil and lymphocyte segments of the peripheral leukocyte population of cattle with chronic IBRV infections.



Total leukocytes, average of 3 cattle.

Total neutrophils, average of 3 cattle.

Total lymphocytes, average of 3 cattle.

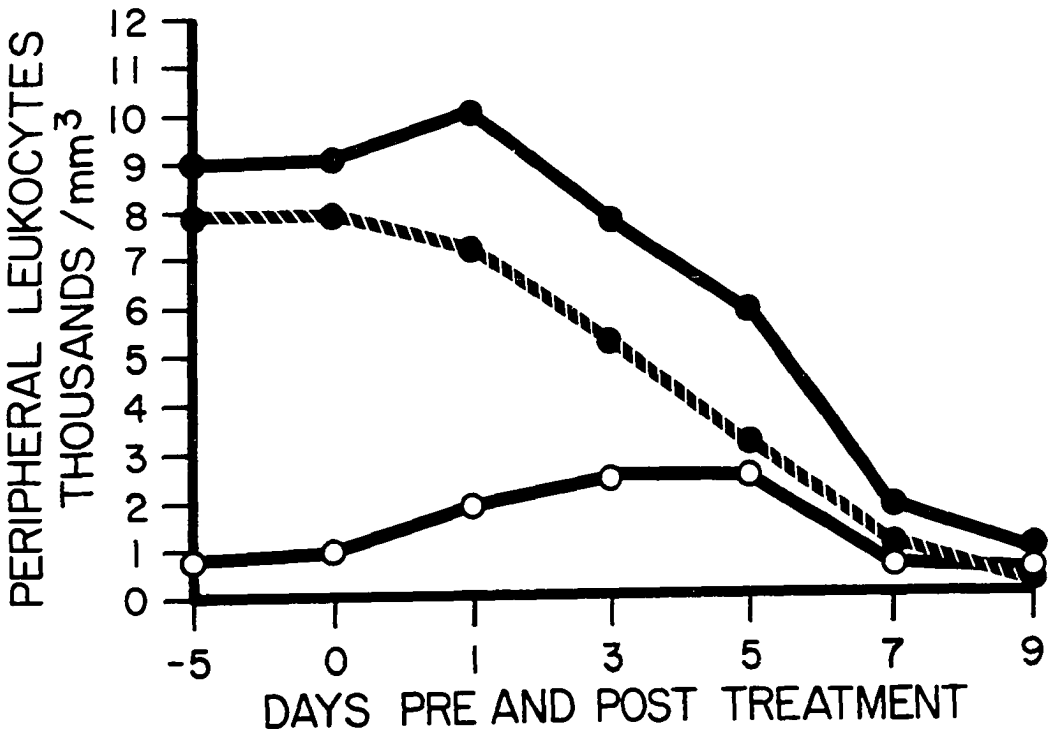

PERIPHERAL LEUKOCYTES
THOUSANDS /mm3
0
1
2
3
4
5
6
7
8
9
10
11
12
-5
0
1
3
5
7
9
DAYS PRE AND POST TREATMENT

Figure 42. Effects of dexamethasone and cyclophosphamide treatment on percent of T cells in isolated lymphocytes of cattle.


Percent T cells in lymphocytes of dexamethasone treated cattle (average of 3).


Percent of T cells in lymphocytes of cyclophosphamide treated cattle (average of 3).


Percent of T cells in lymphocytes of nontreated cattle (average of 2).

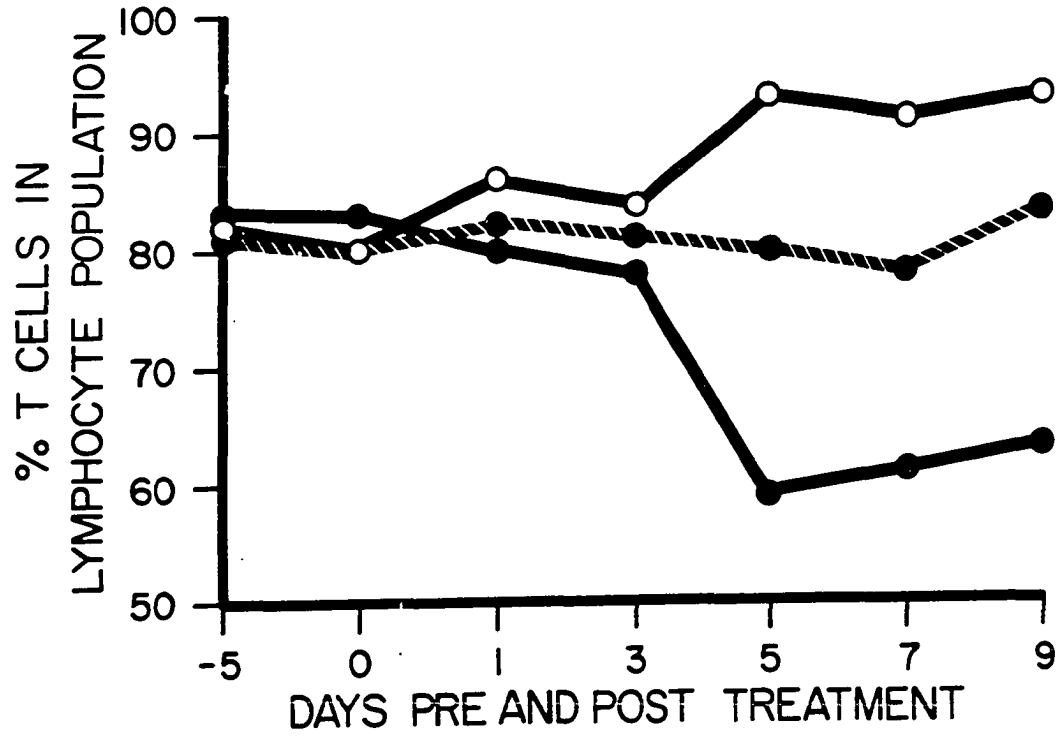
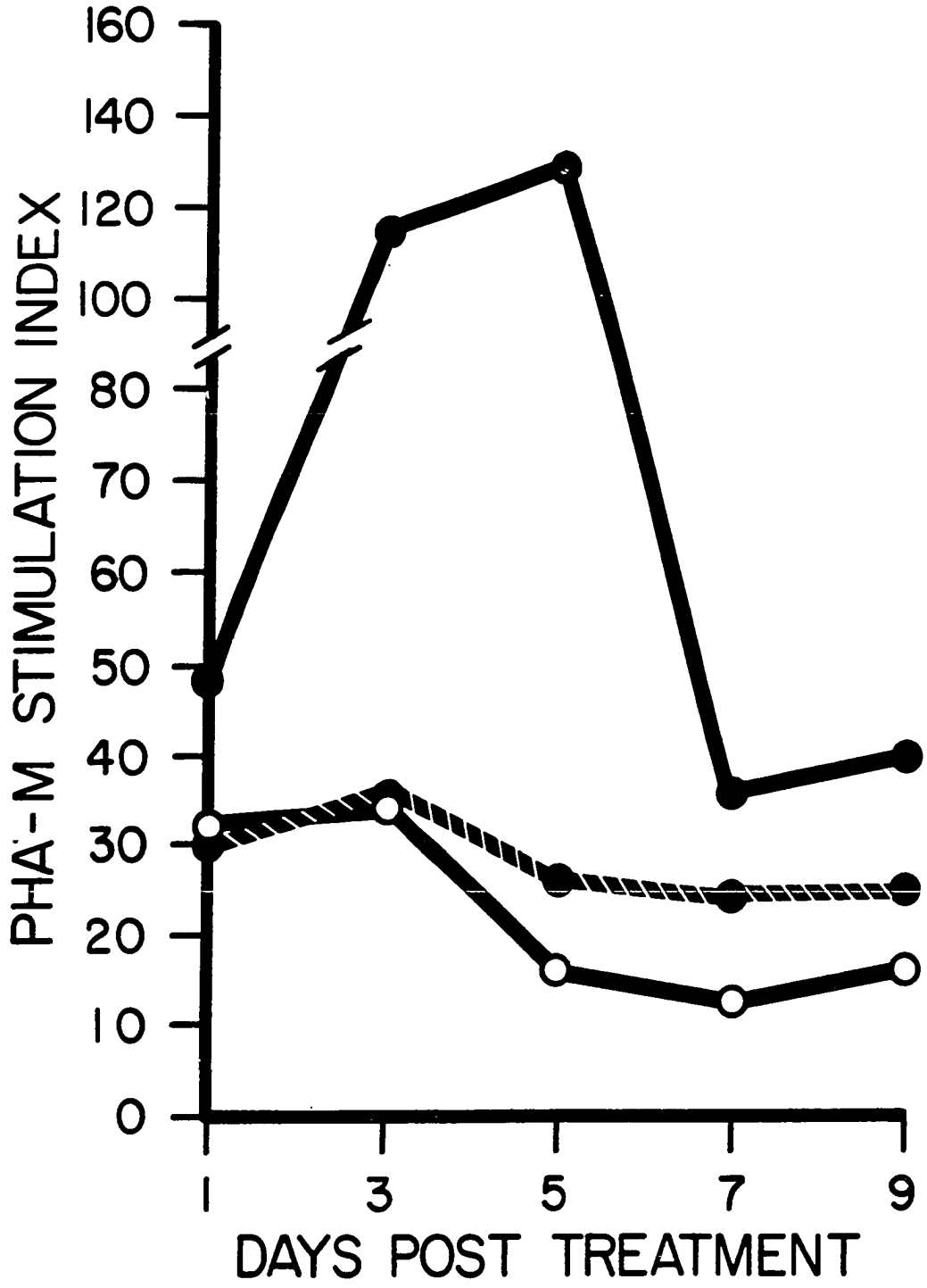

% T CELLS IN
LYMPHOCYTE POPULATION
100
90
80
70
60
50
-5
0
1
3
5
7
9
DAYS PRE AND POST TREATMENT

Figure 43. Blastogenic response of isolated lymphocyte microcultures to PHA-M following dexamethasone and cyclophosphamide immunosuppression in chronically IBRV infected cattle.

● Average of 3 cyclophosphamide treated cattle.

○ Average of 3 dexamethasone treated cattle.

● Average of 3 nontreated control cattle.


160
140
120
100
80
70
60
50
40
30
20
10
0
PHA-M STIMULATION INDEX
1
3
5
7
9
DAYS POST TREATMENT

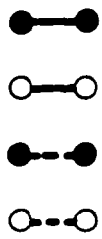
decrease in treated cattle. The negative and positive responding controls remained at relatively constant levels throughout the entire experiment (Fig. 44).

The blastogenic response of whole blood cultures to phytomitogen (PHA-M) is presented in Fig. 45. Cultures from cyclophosphamide treated cattle responded with a dramatic increase in stimulation indices when exposed to PHA-M. A slight depression of PHA-M stimulation was evident in cultures from dexamethasone treated cattle and control nontreated animals showed little or no change.

IBRV-Ag induced blastogenic responses in whole blood cultures established from cyclophosphamide treated cattle were markedly decreased following treatment (Fig. 46). Dexamethasone treated cattle had less marked decrease except for post treatment day 5. Cultures from positive nontreated controls showed a rise in IBRV-Ag SIs but the nonimmune negative control stayed fairly constant.

Dexamethasone and cyclophosphamide treated, chronically IBRV-infected cattle killed and necropsied 9 days following the initial treatment were examined for gross pathologic lesions. All three dexamethasone treated cattle had multifocal necrotic plaques and erosions in the mucosal surfaces of the anterior nares, nasal septum, and pharyngeal area. Two cattle (#7741 and #7746) had moderate areas (3 x 6 cm) of consolidation in the ventral portion of the anterior and cardiac lobes of the lungs. IBRV was isolated from pharyngeal lesion tissues of 2 of 3 cattle, but not from lung, trachea, or associated lymphatic tissue. An interesting incidental cytopathic agent was

Figure 44. Blastogenic response of isolated lymphocyte-microcultures to IBRV inactivated antigen (IBRV-Ag) following dexamethasone and cyclophosphamide immunosuppression in chronically IBRV infected cattle.



● Average of 3 cyclophosphamide treated cattle.

○ Average of 3 dexamethasone treated cattle.

● Average of 2 positive nontreated control cattle.

○ Negative, nonimmune, nontreated control.

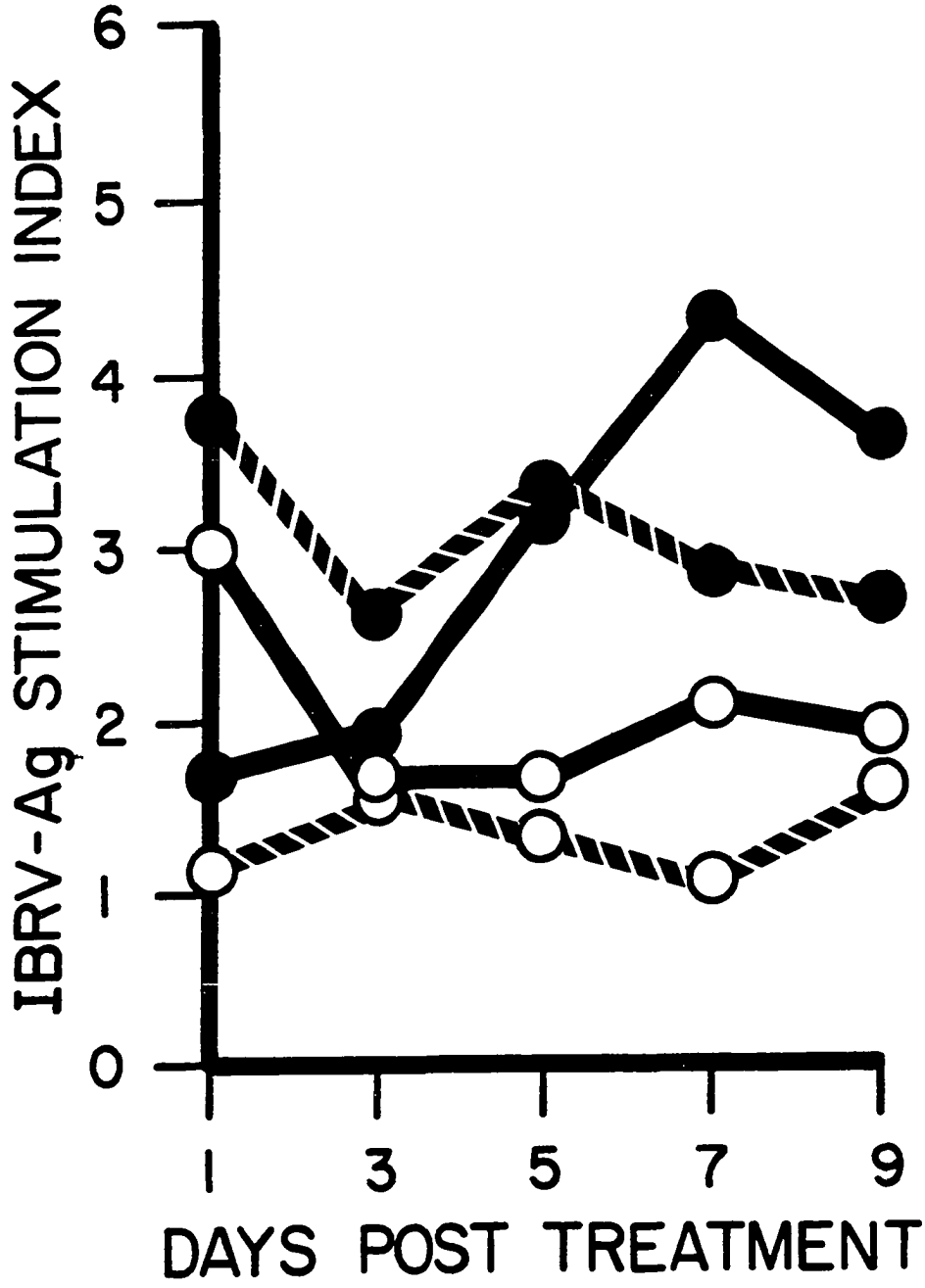

IBRV-Ag STIMULATION INDEX
0
1
2
3
4
5
6
1
3
5
7
9
DAYS POST TREATMENT

Figure 45. Blastogenic response of whole blood cultures to PHA-M following dexamethasone and cyclophosphamide immunosuppression in chronically IBRV infected cattle.


Average of 3 cyclophosphamide treated cattle.


Average of 3 dexamethasone treated cattle.


Average of 3 nontreated control cattle.

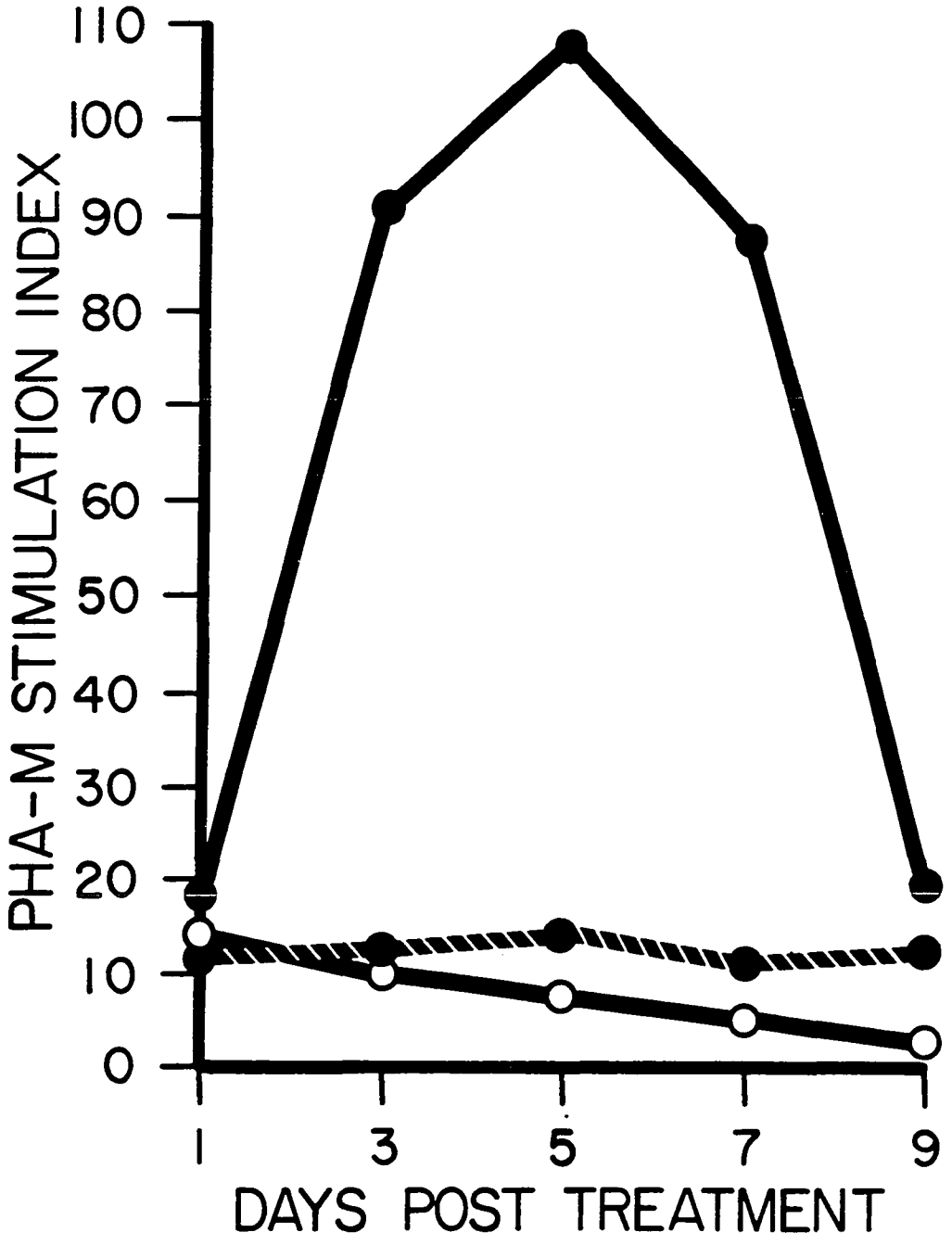

PHA-M STIMULATION INDEX
110
100
90
80
70
60
50
40
30
20
10
0
1
3
5
7
9
DAYS POST TREATMENT

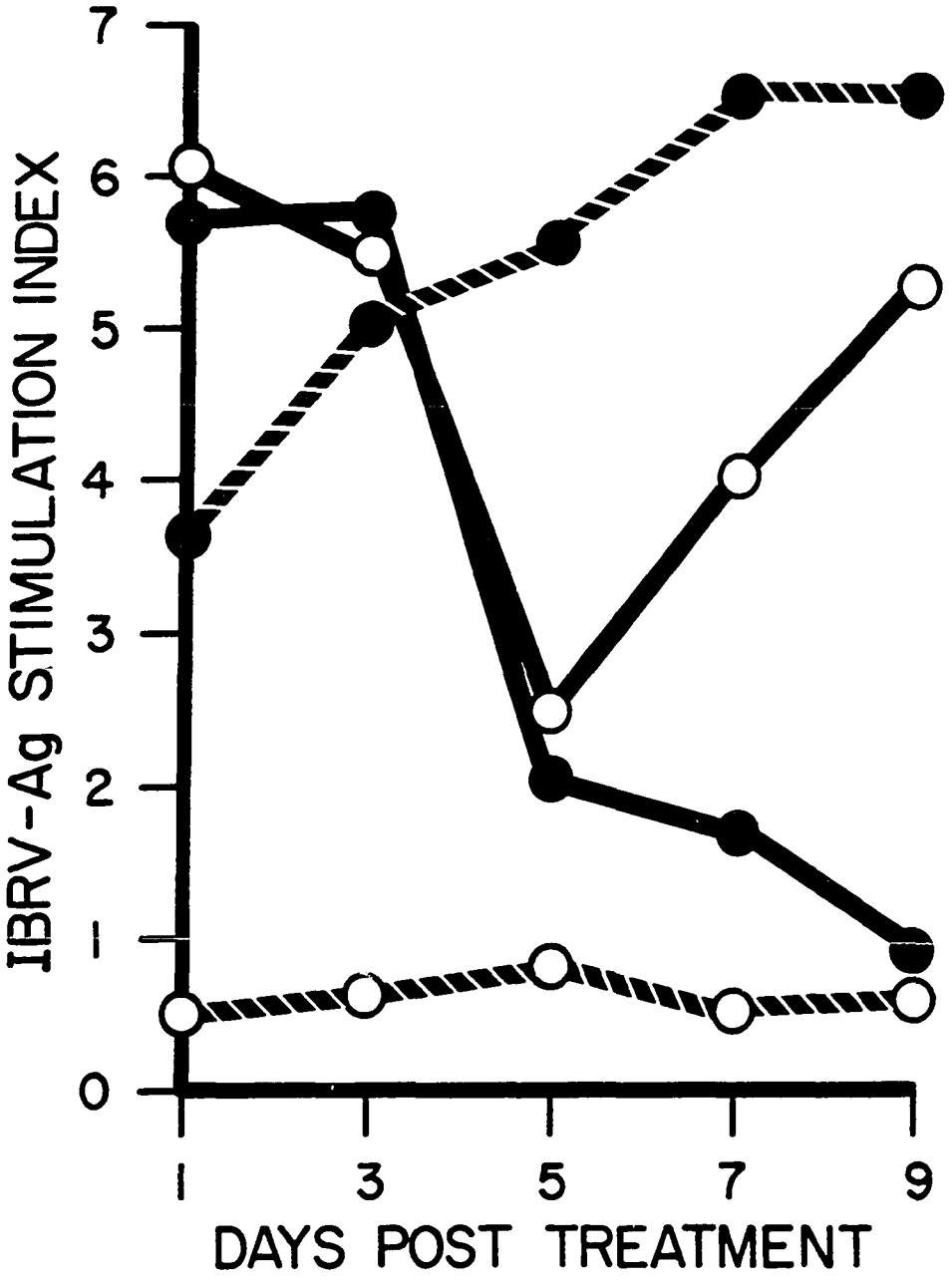
Figure 46. Blastogenic response of whole blood cultures to IBRV inactivated antigen (IBRV-Ag) following cyclophosphamide immunosuppression of chronically IBRV infected cattle.


Average of 3 cyclophosphamide treated cattle.


Average of 3 dexamethasone treated cattle.


Average of 2 positive, nontreated control cattle.


Negative, nonimmune, nontreated control.


IBRV-Ag STIMULATION INDEX
0
1
2
3
4
5
6
7
1
3
5
7
9
DAYS POST TREATMENT

isolated from the consolidated lung tissue of calf #7741. This agent was isolated in EBT cells and identified by serological and electron microscopic methods as belonging to the suggested bovid herpesvirus 5 group (Smith 1977).

Erosive noninflammed lesions of the buccal mucosa especially around the gingiva were observed in 2 of 3 cyclophosphamide-treated cattle. Urinary and gall bladders seemed to be distended in all 3 animals.

Lymphatic tissues from all dexamethasone and cyclophosphamide treated cattle were enlarged and edematous.

Histopathological examination of hematoxylin and eosin stained sections of tissues taken at necropsy from cattle treated with dexamethasone revealed an acute to subacute necrotic rhinotracheitis and pharyngitis characterized by multifocal areas of epithelial ulceration and necrosis with fibrinopurulent exudate over the affected surfaces. The stratified squamous epithelium at the edge of the ulcers were undergoing ballooning degeneration and occasionally contained small intranuclear inclusion bodies. The epithelium of the ducts and acini of the serous glands were also undergoing degenerative necrosis. The submucosal tissues were infiltrated with lymphocytes, plasma cells and macrophages and a few scattered neutrophils. Perivascular cuffs of lymphocytes and macrophages were common.

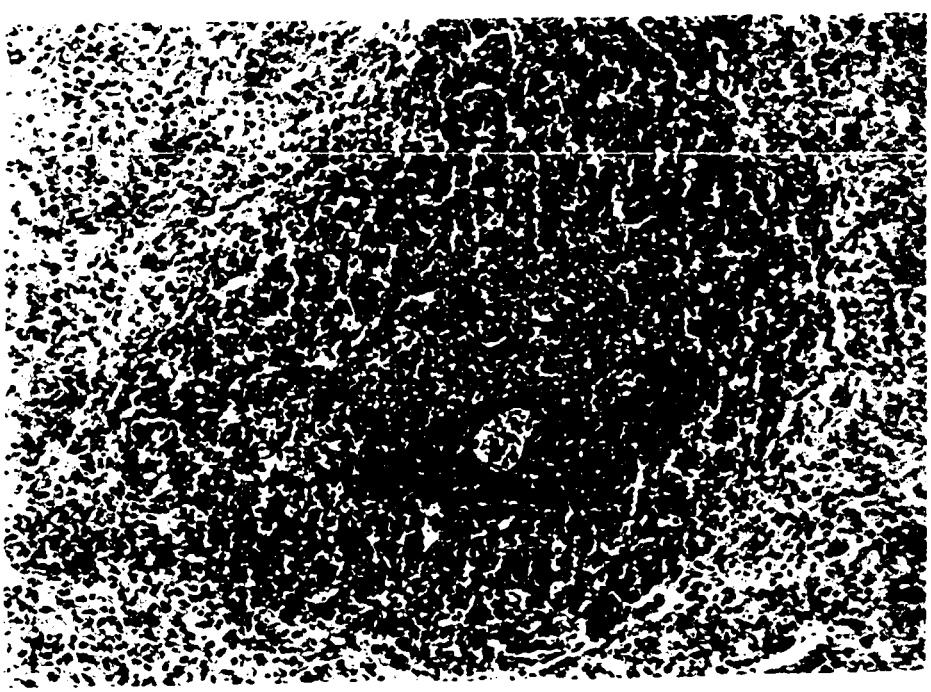
The tonsils and pharyngeal tonsillar tissues had lymphoid depletion and reticular cell hyperplasia. There was a moderate lymphoid depletion of splenic tissues from dexamethasone-treated cattle (Fig. 47). A small

Figure 47. Histological section of spleen from dexamethasone treated steer.

Note lymphoid depletion and reticuloendothelial hyperplasia. Hematoxylin and eosin stain.

Figure 48. Histological section of spleen from dexamethasone treated steer.

Notice light staining central artery and small dark staining periarteriolar sheath of lymphoid cells. Large, less dense stained circular sheath is composed of neutrophils.



collar of periarteriolar lymphoid sheaths was surrounded by a distinct well-defined area of necrotic cells and neutrophils (Fig. 48). The splenic cords were fibrosed and large areas of collagen had been deposited in this area. Two of the 3 animals had several areas of chronic focal nephritis. The inflammatory cells around the kidney tubules were predominantly lymphocytes and plasmacytes.

Bone marrow sections taken from the 4th rib seemed mildly depleted of blast cells and megalokaryocytes. There seemed to be an overabundance of progranulocytes in the bone marrow (Fig. 49).

Lymph nodes were moderately depleted of lymphoid germinal centers (Fig. 50) and the paracortical zones normally considered to be populated with T lymphocytes were devoid of lymphoid cells. Blast type lymphoid cells were not present in the germinal centers and had been replaced with reticular cells (Fig. 51).

Tissues taken from the area of the buccal ulcers of cyclophosphamide-treated cattle had multifocal areas of well-defined epithelial necrosis (Fig. 52). All tissue in this area was denuded to the area of the basement membrane. Large filamentous organisms and coccoid shaped bacteria were present in large colony type masses in the denuded area (Fig. 53). However, there was little or no evidence of inflammatory cells in the mucosal or submucosal area. One animal (#7736) had several small areas of subacute tracheitis in the anterior portion of the trachea.

Spleens from cyclophosphamide treated cattle showed evidence of severe lymphoid depletion (Fig. 54) but small collars of lymphoid

Figure 49. Histologic section of bone marrow of 4th rib taken from dexamethasone treated steer.

Hematoxylin and eosin stain.

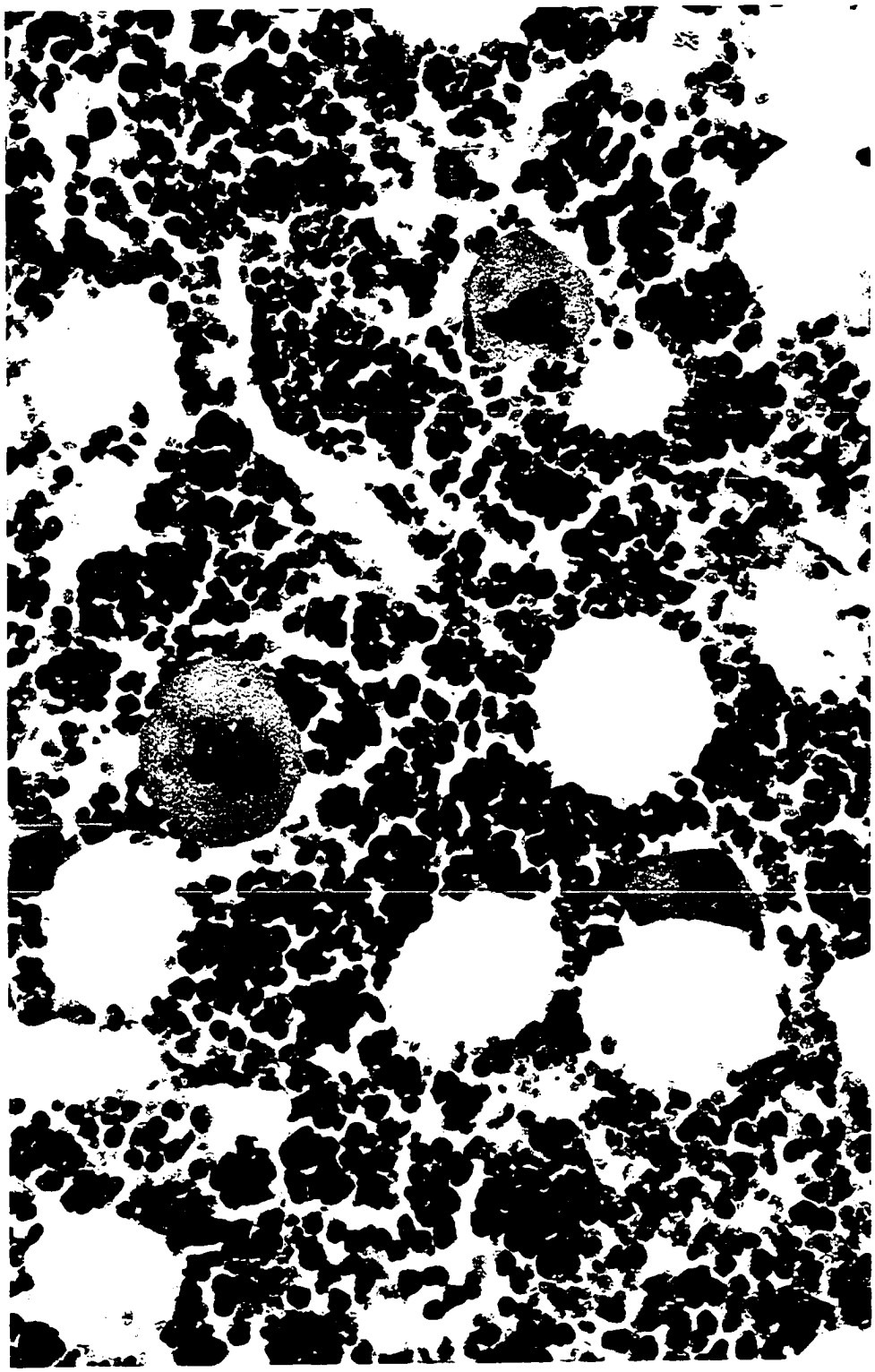
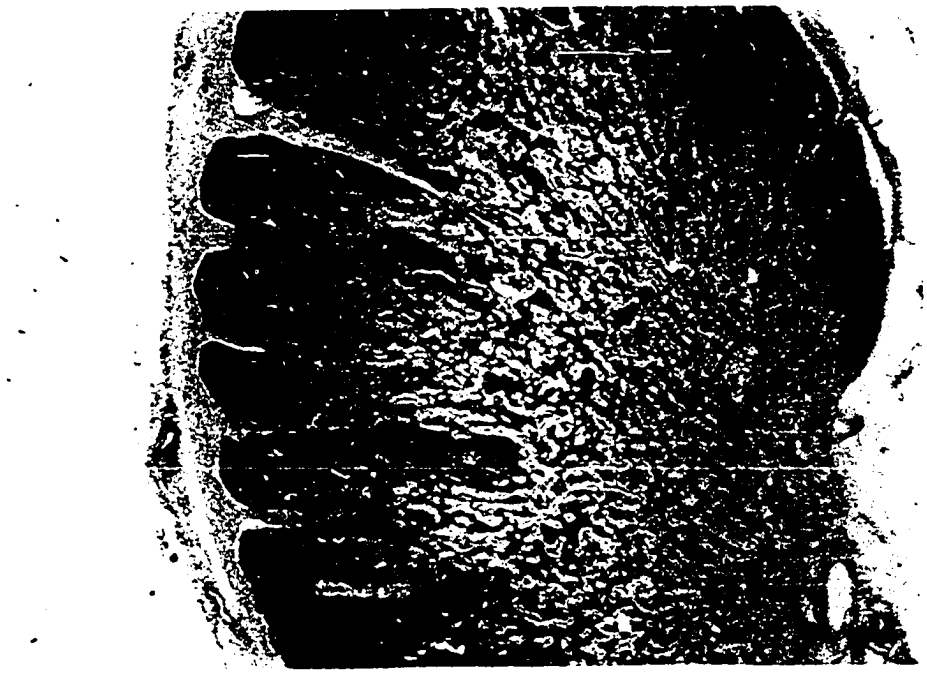


Figure 50. Histologic section of a suprapharyngeal lymph node from a dexamethasone treated steer.

Note moderate lymphoid depletion. Hematoxylin and eosin stain.

Figure 51. Lymphoid follicle from suprapharyngeal lymph node of dexamethasone treated steer.

Note reticulo-hyperplasia in center of germinal follicle.



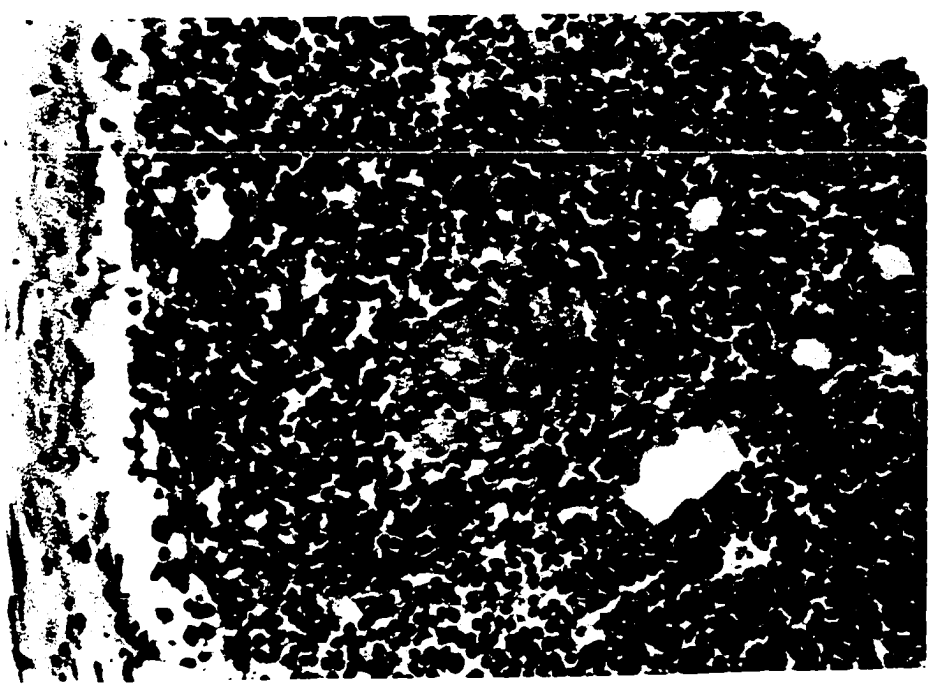


Figure 52. Epithelial necrosis in the buccal mucus membrane of cyclophosphamide treated cattle.

Hematoxylin and eosin stain.

Figure 53. Epithelial necrosis in the buccal mucus membrane of cyclophosphamide treated steer.

Note large filamentous organisms. Hematoxylin and eosin stain.

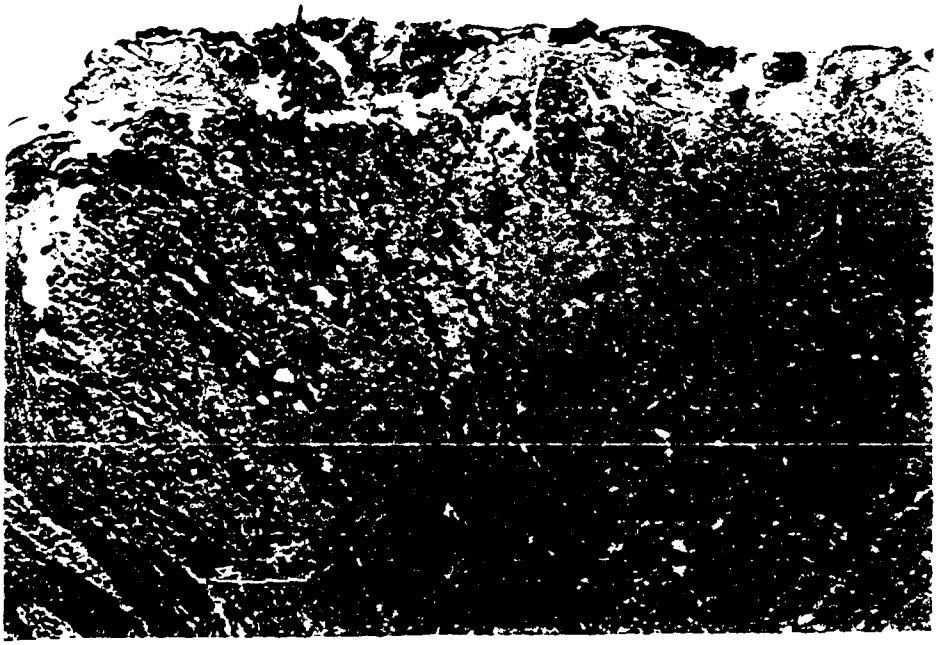
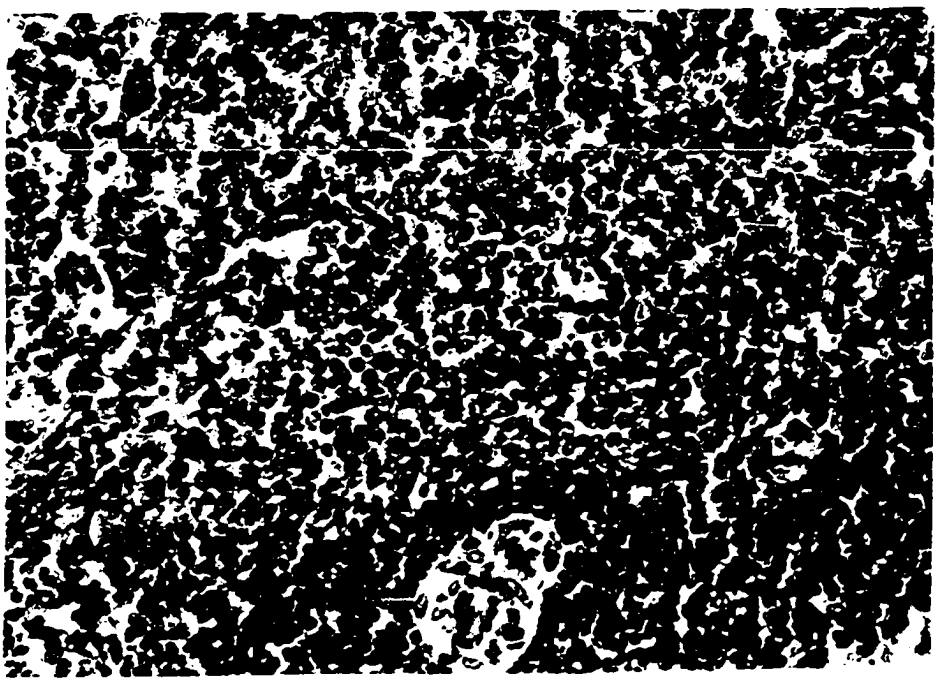


Figure 54. Histologic section of spleen from cyclophosphamide treated steer.

Note the severe lymphoid depletion. Hematoxylin and eosin stain.

Figure 55. Depleted germinal center in spleen of dexamethasone treated steer.

Note few lymphoid cells in periarteriolar sheath. Hematoxylin and eosin stain.



cells remained in the periarteriolar lymphoid sheaths (Fig. 55). Areas normally considered to be populated with B cells were remarkably absent.

All lymph nodes taken from the cyclophosphamide-treated group showed evidence of severe lymphoid depletion (Fig. 56) and reticulo-endothelial hyperplasia. Germinal centers were almost nonexistent. Many macrophages had also accumulated at the cortico-medullary junction of the lymph node (Fig. 57). These cells lay in large closely packed sheets of morphologically homogenous cells.

The thymus, surprisingly, had little or no evidence of thymic depletion or other histopathologic change.

Bone marrow from cyclophosphamide-treated cattle was depleted of blast type cells and megalokarocytes (Fig. 58). The bone marrow was markedly depleted from progranulocyte-type cells.

Figure 56. Histologic section of a suprapharyngeal lymph node from a cyclophosphamide treated steer.

Note severe lymphoid depletion. Hematoxylin and eosin stain.

Figure 57. Reticuloendothelial hyperplasia and macrophage accumulation in suprapharyngeal lymph node of cyclophosphamide treated steer.

Hematoxylin and eosin stain.

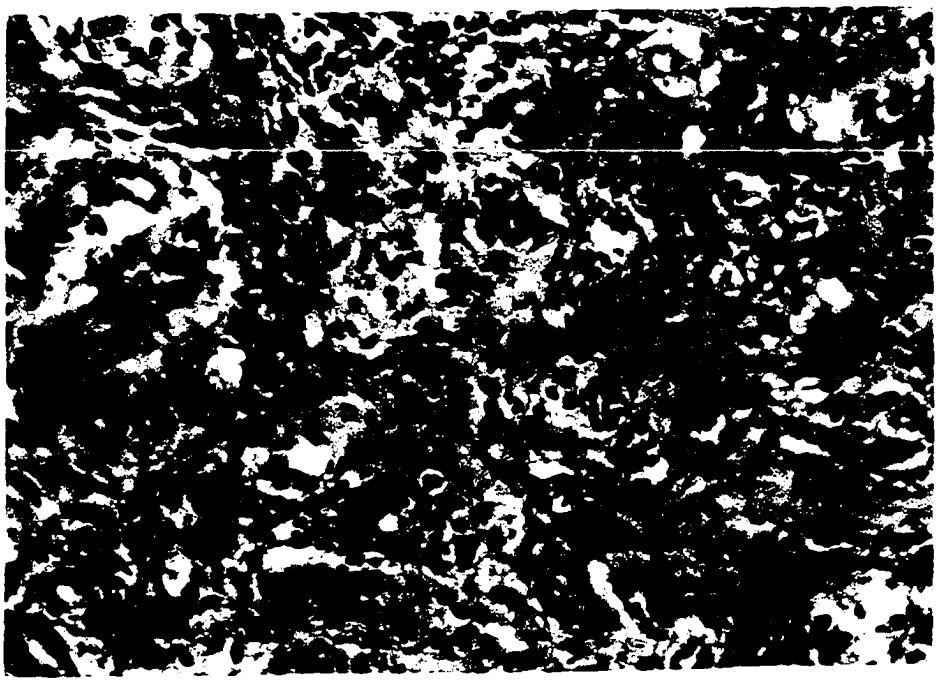
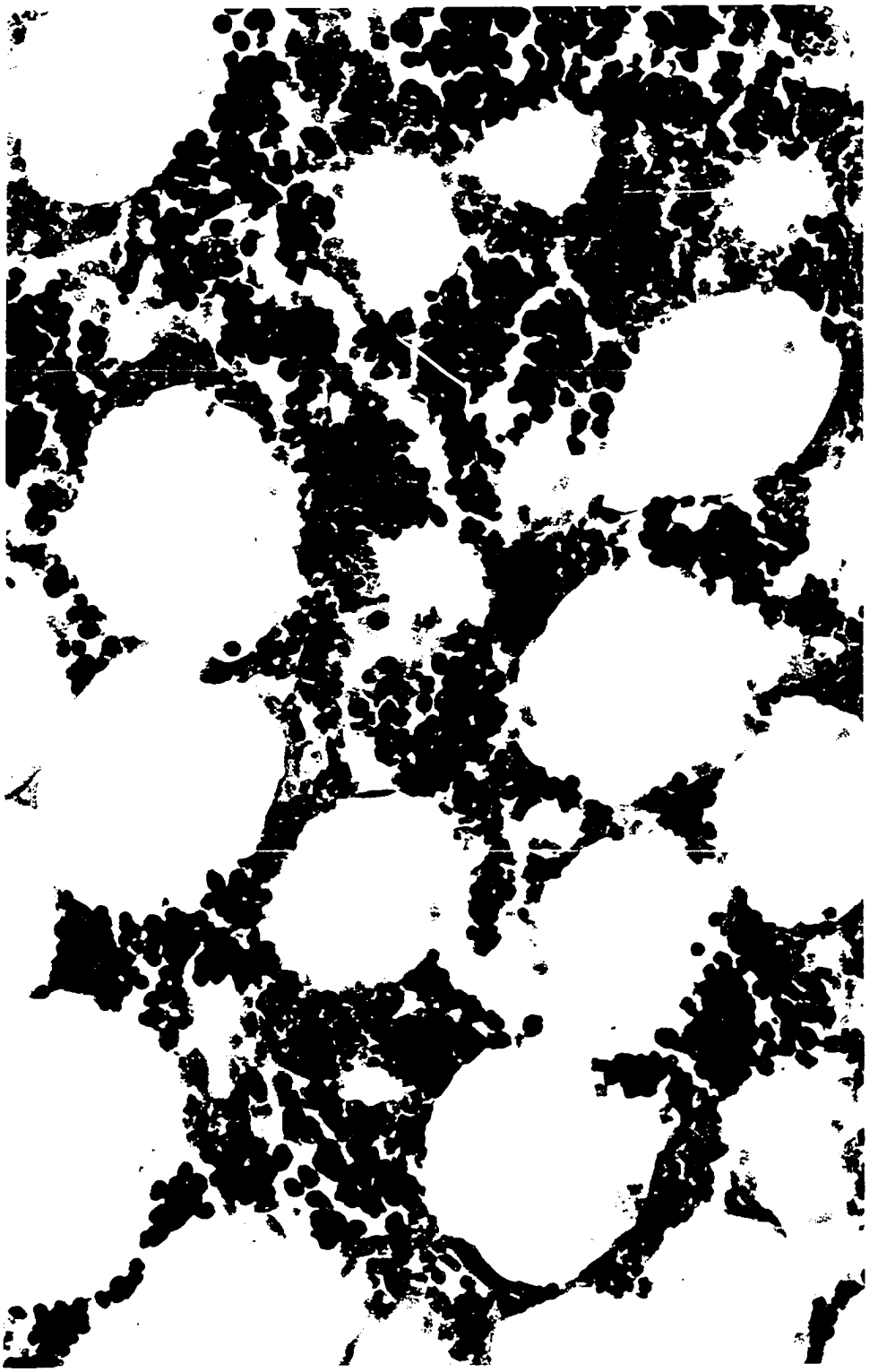


Figure 58. Bone marrow section from steer treated with cyclophosphamide.

Note absence of megalokaryocyte and progranulocytes.
Hematoxylin and eosin stain.



DISCUSSION

Dexamethasone-induced immunosuppression of calves prior to and during acute IBRV infection transforms a nonlethal illness into a disseminated infection causing death. The recovery of IBRV from the brains of two dexamethasone treated calves raises the question of whether the idea of specific tissue tropism of certain strains of IBRV for central nervous tissue may actually reflect specific genetic or environmental immunodeficient states of the neonatal calves so affected. However, the biological differences between neurotropic and nonneurotropic strains of IBRV appear real (Bagust 1972) even though other reports indicate no biochemical or antigen difference can be detected (Black and Slack 1972). The disseminated human herpesvirus 2 infections that occur in neonates born of infected mothers may be analogous.

Dexamethasone treatment of cattle with chronic IBRV infections caused recrudescence of clinical disease, viral shedding in the nasal mucus, and elicited necrotic lesions in the upper respiratory mucosal surfaces at 3, 6, and 12 months after initial infection. It is interesting to note that cattle having only very mild signs of disease during the initial exposure (experiment 3) had obvious necrotic mucosal plaques following dexamethasone treatment. However, the length of time that virus was recovered from the nasal secretions following initial exposure seemed to correlate well with IBRV recovery patterns following drug-induced recrudescence (Table 11). This observation indicates that the anti-IBRV immune mechanism of calves vary widely in individuals and that the competence to eliminate the initial invading virus may be

reflected in decreased viral shedding during recrudescence. It seems possible that this same mechanism may not be sensitive to dexamethasone treatment. It is interesting to speculate that this might be related to the individual's ability to produce specific humoral and secretory antibody since dexamethasone seems to be less detrimental to B cells than to T cells.

These data indicate that different field strains and modified live virus vaccine strains establish chronic infections in exposed cattle and can be reactivated by immunosuppressive factors. They are compatible with those of Davies and Duncan (1974) and heightens the suspicions of others (Kiesel et al. 1972; Curtis and Angulo 1974) that challenge the wisdom of widespread use of modified live virus vaccines in feeder cattle. The chronic infection established with modified live virus vaccines in young calves may be reactivated by environmental stress-induced immunosuppression during shipment.

Distinct biochemical changes in blood plasma occurred soon after the administration of dexamethasone. The regulatory feed-back mechanism of 17-dehydroxycorticosterone production by the adrenal cortex is apparently activated by dexamethasone and this activation is reflected in the rapid decrease in the natural hormone following dexamethasone treatment. Stress related fluctuations of natural hormones may play an immunosuppressive role during the shipment and post shipment period causing IBRV recrudescence in chronically infected cattle. The changes recorded for other plasma constituents are apparently related to the gluconeogenic effect of the synthetic corticosteroid.

The metabolic changes induced by dexamethasone treatment of chronically IBRV-infected cattle are probably directly related to the immunosuppressive changes responsible for the reactivation of IBRV-induced necrotic mucosal plaques and IBRV secretion in nasal mucus.

The rapid increase in neutrophils in the peripheral circulation after dexamethasone treatment and the continued neutrophilia during periods of viral shedding seems to indicate that total numbers of neutrophils play no significant role in maintaining the chronic or latent state of IBRV infection. However, the increase in total numbers of cells, probably brought about by premature release of neutrophils from the bone marrow, does not necessarily imply that the functional capabilities have not been impaired. These data do not support the suggestion by Grewal *et al.* (1977) that neutrophils play a predominant role in maintaining IBRV latency.

Dexamethasone-induced lymphocytopenia precedes recrudescence of clinical IBRV in cattle and coincides with the severity of illness in drug-modified acute disseminated infections in young calves. Furthermore, the more specific detrimental effect of dexamethasone upon the T lymphocyte and a relative sparing of the B lymphocyte population indicates that the T lymphocyte plays a major role in maintaining the latent IBRV infection in cattle. Further evidence to support this contention is provided by the profound B lymphocyte related immunosuppressive effect induced by cyclophosphamide treatment without IBRV recrudescence. The marked effect upon the B cell population and relative sparing effect of the T cell population supports this hypothesis.

T lymphocytes are known to be the principal immunocytes involved in delayed hypersensitivity. Results of these experiments showed that dexamethasone treatment of IBRV antigen-sensitized cattle caused a marked reduction in the DTH response during the reactivation of chronic IBRV infections. However, since the number of cells involved in the inflammatory response were not obviously diminished it can be assumed that the normal cell functions of lymphokine and interferon production had been impaired. This hypothesis is supported by reports of others (Rhinehart et al. 1974; Claman 1975; Wahl et al. 1975) concerning the detrimental effect of dexamethasone upon lymphokine production and macrophage function.

The use of isolated lymphocyte microcultures in this study has provided several observations that seem to conflict with results reported by other investigators. Rouse and Babiuk (1974a) indicated that specific IBRV antigen immunostimulation of isolated lymphocytes occurred at 4-6 days post IBRV infection and waned to undetectable levels by 14 days post infection. Their report was supported by the investigations of Reggiardo (1975). I was not able to detect such early stimulation in isolated lymphocyte microcultures when stimulation indices of less than 2.0 were discarded as being nonsignificant. My decision to use such a breaking point was based upon the fact that cultures from nonsensitized animals often approached such index levels due to day to day variations. It should be noted that the use of an index level below 2.0 as being no indication of stimulation would essentially eliminate the early stimulatory effect reported in their

studies. Rouse and Babiuk (1974a) reported high SIs of 10-18 in cattle with long-standing immunity, a situation that we were able to confirm following repeated skin testing or recrudescence with corticosteroids.

If the specific antigen immunostimulation represents an in vitro cell mediated immune response then one would suspect that the evidence of sensitivity would not rise as early as these investigators have indicated and would remain well beyond the 14-day time indicated as undetectable. Furthermore, continued presence of the antigens and intradermal injections should heighten the response, a situation confirmed by our results.

The use of specific IBRV antigens to stimulate whole blood cultures prepared from IBRV infected cattle was first reported by Davies and Carmichael (1973) to be an acceptable method of studying cell mediated immunity and its role in primary and recurrent infections. Their report indicated that the early response (4-6 days) after primary infections waned to nondetectable levels by 14 days post exposure. The results of experiments I conducted indicate that even though the initial immunostimulation occurs as early as 6 days post exposure the specific immunostimulation remained positive throughout an entire year without restimulation. Whether the system I adapted for harvesting cultures was responsible or not, the pattern noted in these results is more compatible with the long-standing duration of other indications of cell-mediated immunity such as DTH response. The reasons for the difference in experimental results are not obvious. One possible explanation may be that the isolated lymphocyte technique may not be sensitive enough to detect subtle changes and are, therefore, only detecting the peak

response soon after infection. Another possibility is that the response detected early in both lymphocyte and whole blood cultures may not be directly related to CMI but may be evidence of some other immune response such as the Jones-Mote reaction.

An overall view of the specific antigen-induced and nonspecific plant mitogen-induced (PHA-M) immunostimulation of whole blood and isolated lymphocyte cultures during dexamethasone and cyclophosphamide treatment provides a tool for dissecting the immune mechanism responsible for maintaining latent IBRV infections in cattle. If we can assume that nonspecific immunostimulation of lymphocyte and whole blood cultures with PHA-M is due principally to the blastogenic response of T lymphocytes in the culture then the decreased PHA-M mitogenic response following dexamethasone treatment as it occured in these experiments is to be expected. The unexpected response in these experiments is the fact that specific IBRV antigenic blastogenesis rises during the period of dexamethasone-induced IBRV recrudescence. Even though several times the normal amount of whole blood had to be processed to allow for adequate lymphocyte harvest, the sensitivity of the cells to respond to the specific mitogen remained. Shortman and Jackson (1974) have shown that there are at least 2 sub-populations of T lymphocytes in mice. One, the long-lived sub-population, is cortisone-resistant whereas the other, a short-lived population, is cortisone-sensitive. Others (Greaves et al. 1973) indicate that evidence for T lymphocyte sub-populations in other animals is strong. It appears that in cattle the PHA-M induced blastogenic response resides in the short-lived or

corticosteroid sensitive population whereas the specific IBRV antigen sensitivity apparently resides in the corticosteroid resistant sub-population.

These findings and the data suggested assumptions can then be integrated into a plausible theory of the mechanisms involved in maintaining the delicate host cell-virus relationship in chronically IBRV infected cattle. This theory coupled with the knowledge of the action of corticosteroids upon phagocytes and immunocytes can be used to explain potential mechanisms involved in dexamethasone-induced IBRV recrudescence.

The chronic infection represented by slow replication of IBRV in slowly metabolizing cells produce herpesvirus-directed surface membrane antigens as viral replication proceeds. These antigens attract specifically sensitized T lymphocytes (the dexamethasone resistant subpopulation) which subsequently attract macrophages and other T lymphocytes (nonsensitized, PHA-M immunoresponsive, dexamethasone sensitive lymphocytes) of the cytotoxic type. Through lymphokine and interferon production and the combined effects of T lymphocyte and activated macrophage accumulation, the infected cell is destroyed and releases infective viral particles that are immediately neutralized by specific humoral antibody. Such a theory becomes more palatable when combined with the previously reported results that indicate that the principal mode of immunosuppressive action of corticosteroids is upon the macrophage, whose digestive capacity is depressed by the drug. The inhibition of interferon and lymphokine production and the inhibition of the cytotoxic

or (killer) T lymphocyte action is also impaired by corticosteroids perhaps by destroying biologically active substances produced by the macrophage that play a major role in T lymphocyte-macrophage interactions.

The known mode of action of corticosteroids may fit into our proposed theory conveniently not by depressing the IBRV antigen sensitive cells but by inhibition of lymphokine (MIF, MAF) production in cells after they have been attracted to the area. (This contention would also explain the findings of numerous mononuclear cells in the skin test site even though the DTH response is severely depressed.) The dexamethasone-induced depletion of the cytotoxic T lymphocytes (the PHA-M responsive sub-population) and the depression of macrophage digestive function and interferon production would allow for contiguous spread to adjacent cells without the extracellular exposure to antibody.

The failure of severe cyclophosphamide-induced immunosuppression to cause recurrent IBRV lesions and nasal mucus secretion is logically explained by this theory. The primary effect of the drug seemed to be upon the neutrophil and B lymphocyte, thus basic cell mediated immune responses were not interrupted.

These studies emphasize the need to focus scientific attention on the cell-mediated immune response as a measure of IBRV resistance rather than the classic idea of the presence of serum-virus neutralizing antibody being equated with resistance. They further seem to emphasize the need to seek to enhance the cell mediated immune response in immunizing procedures. They should also serve to remind us that we can expect recurrent infections with modified live virus vaccines in immunosuppressed conditions.

SUMMARY

Dexamethasone treatment of chronically IBRV-infected cattle resulted in recrudescence of necrotic lesions of the respiratory tract and the secretion of IBRV in the nasal mucus. Reactivation of latent infections was produced with dexamethasone treatment at 3, 6, and 12 months after initial IBRV exposure.

The pretreatment of calves with dexamethasone and subsequent intranasal exposure to IBRV and continued dexamethasone treatment caused death due to widely disseminated IBRV infection. The drug caused neutrophilia and lymphopenia and had a distinct depressive effect upon the percent of T lymphocytes in the peripheral blood. Data indicated that two distinct T lymphocyte sub-populations existed, one sensitive and one more resistant to dexamethasone treatment. The dexamethasone sensitive sub-population was more responsive to PHA-M and the severe depletion of this sub-population coincided with the time of IBRV recrudescence.

Whole blood cultures were more useful than isolated lymphocyte microcultures in detecting the cell mediated immune response after acute IBRV infections. Specific IBRV antigen immunostimulation of whole blood cultures rose to high levels by 6 days post treatment. The response was sustained in chronically infected animals for 1 year without further stimulation, a finding that refutes the contention of other investigators that it drops to undetectable levels within 14 days following initial exposure.

The techniques used in isolated lymphocyte microcultures failed to provide evidence of specific IBRV antigen-induced blastogenesis following initial exposure and could only be demonstrated following skin testing or drug-induced IBRV recrudescence.

Cyclophosphamide treatment failed to cause IBRV recrudescence in chronically infected cattle. The failure of development of acute inflammation in response to microbial invasion of the epithelium and widespread histopathological evidence of lymphoid depletion indicated that the drug caused severe immunodepression. The principal effect of the drug seemed to be upon the neutrophil and the B lymphocyte.

The use of different immunosuppressants, dexamethasone and cyclophosphamide, provided a mechanism whereby the immune mechanisms of cattle could be segmented and evaluated separately.

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