INFLUENCE OF MANAGEMENT STRESS ON IMMUNITY IN FARMED DEER

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INTRODUCTION

In 1878, Claude Bernard first recognised that physiological changes occurred in animals exposed to environmental stimuli. He described the *milieu interieur* of the animal; characterised by its constancy, and the external environment; characterised by its variability. Cannon (1914) further refined this concept as 'homeostasis', where a steady state is obtained by the optimum interaction of counteracting processes within the host. He also defined the 'flight-fight' reflex of animals exposed to severe environmental change (stress) and identified the potentially damaging effects of stress, involving the activation of endocrinological factors in the sympathetic adrenomedullary axis (Cannon, 1935). In the next decade, Selye (1946) described the response of laboratory animals exposed experimentally to noxious stimuli, which he classified as the 'general adaptation syndrome' (GAS). The syndrome was classified as having three distinct phases: first, an alarm reaction: second, a resistance phase: and finally, if stress was not dissipated, exhaustion of the biological system. Because different noxious stimuli produced similar physiological responses, involving corticosteroid production through activation of the pituitary-adrenal-cortical axis, he concluded that the response to stress was 'non-specific'. This led to the widely held view that the stress response largely involved non-specific production of corticosteroids, and it has subsequently led to the measurement of glucocorticoids as the primary indicator of stress and animal wellbeing. Mason (1968) showed that different types of stress, such as fasting and heat shock, produce dramatically different corticosteroid levels in experimental monkeys. It is now widely accepted that there are unique but different endocrine responses to different physical, chemical and psychological stressors, which vary within and between different species of animals (Dantzer and Mormede, 1985).

Fraser (1975) has defined stress as "...an abnormal or extreme adjustment in the physiology of an animal to cope with the adverse affects of its environment and management". In this report the adverse affect will be designated the **stressor**. The term stress has been further qualified by other workers (Selye, 1973 and Ewbank, 1985) to include the term **distress**. This term is used to identify the

extreme response to adverse stimuli which causes a damaging pathophysiological reaction in the host producing associative changes in behaviour, physiology and disease susceptibility. It has also been recognised that other stressors potentiate the physiological responses of the host without producing any adverse effect, and such responses are said to involve **eustress** (Selye, 1973).

Stressors include a wide variety of environmental stimuli which evoke significant homeostatic alterations in the host. They may result from natural changes in the environment, or artificial changes, imposed by management constraints, or caused by a range of experimental stimuli which have been used to study stress physiology. The effect produced by exposure to stress may be influenced by the severity of the stressor, the time during which it is applied (**acute** vs **chronic**), or whether the animal can escape the stressor, if it is applied repeatedly (**escapable** vs **inescapable**).

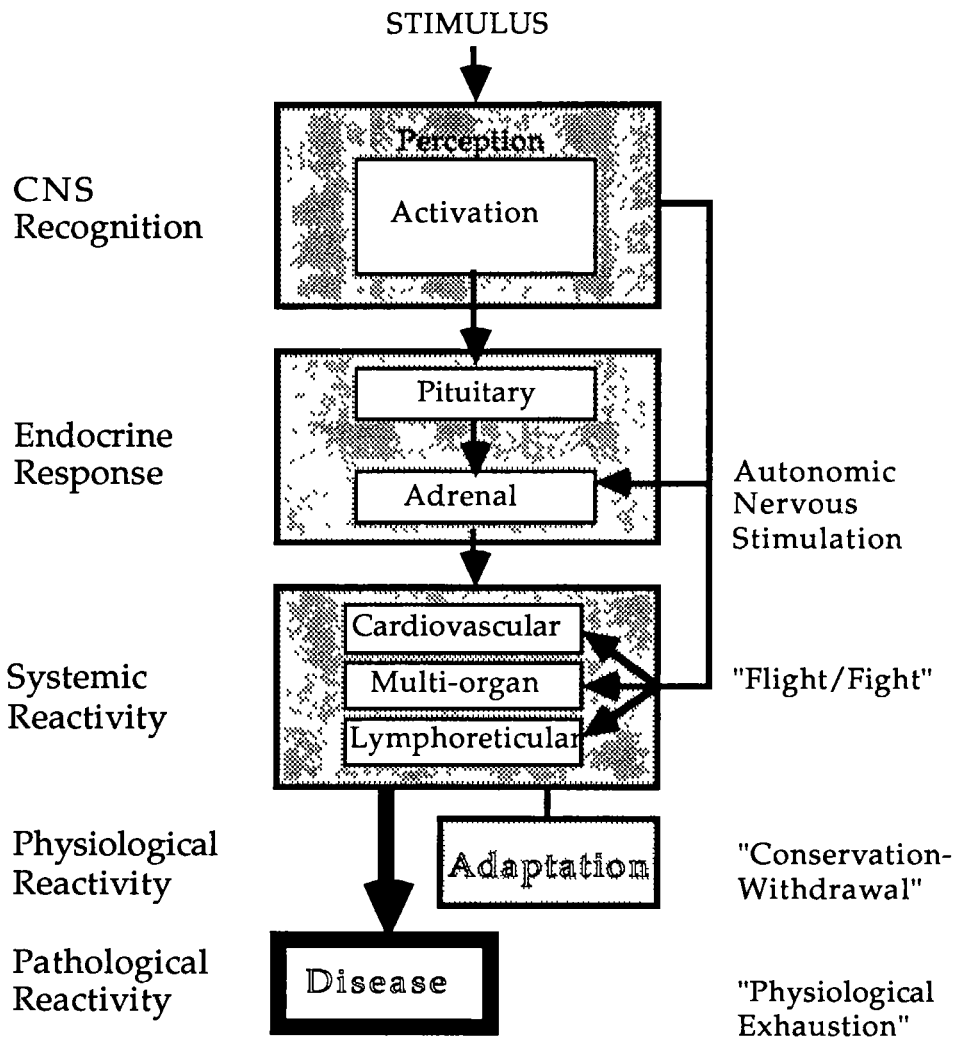
Natural exposure to extremes of heat or cold in the field or in animal housing or holding facilities, are amongst the commonest stressors. Physical restraint associated with separation, treatment or transport of animals is another important stressor. Environmental changes which produce strange or novel sounds, sights, odours or tastes can also evoke stress. Drugs or chemicals used in management or treatment of animals can act as stressors, while toxic products released by infectious agents, environmental pollutants or inadequate ventilation, can have a similar effect. Shearing, dehorning, docking and castration, carried out during routine management of domesticated animals also cause stress. Overcrowding, hierarchical challenge, weaning, exposure to unfamiliar surroundings or isolation can have a major behavioural impact on animals and evoke stress. Changes or restrictions in diet may also act as important stressors in farmed animals.

Capture of wild animals, or the exposure of domesticated animals to restraint, transport or management extremes may cause stress, and evoke an adaptive response from the host. In the context of Selyes general adaptation syndrome (GAS), the response is recognizable initially as anxiety, which may progress to fright or terror. Failure of the animal to express the 'flight-fight' response results in the expression of anger or rage, and if the stimulus persists, frustration or helplessness. Because stress results largely from the individual's perception of the threat posed by the stimulus, rather than its nature *per se*, a cognitive or psychological component is central to all stress. The biological response within the host which results from exposure to stress is outlined in Figure 1, and produces an impact on many of the organs within the affected host.

Experimental studies carried out during the past 20 years have seen the marriage of psychology, neuroendocrinology and immunology into a new scientific subspeciality called psychoneuroimmunology. Research in this multifaceted system has identified and integrated the physiological factors produced by the neurological and immunological systems. Soluble factors produced by the neurological and immunological tissues include neurotransmitters, neuropeptides, ACTH, catecholamines, corticosteroids,

opioids and cytokines; lymphokines and monokines (Besedovsky *et al.*, 1985).

Figure 1: Systemic response to stress

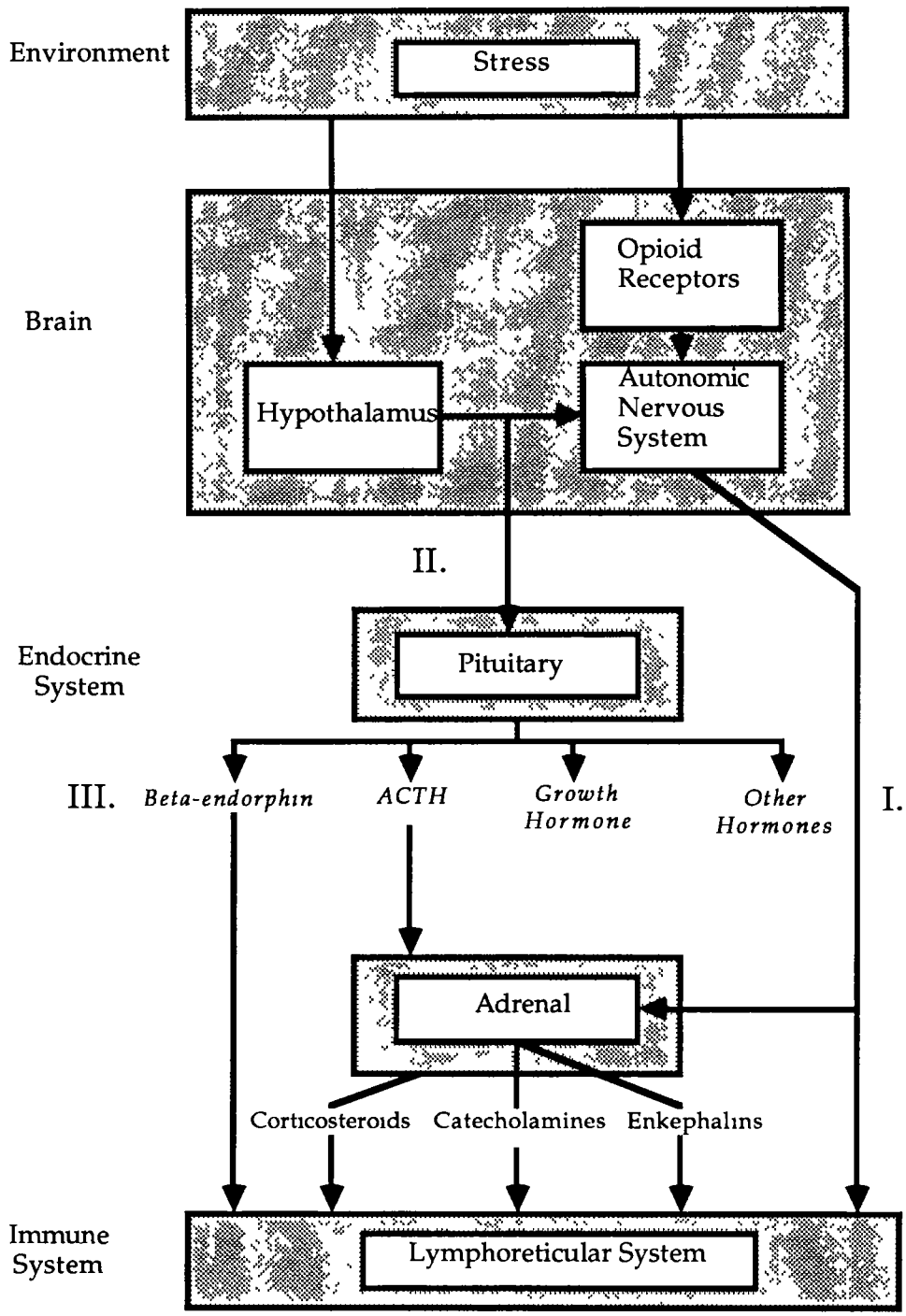


NEUROPHYSIOLOGY OF STRESS

Exposure to stress evokes the series of neuroendocrine responses outlined in Figure 2. A graded response occurs involving three discrete systems of neuroendocrine response. These include:

l. Autonomic nervous stimulation.
ll. Hypothalamic pituitary adrenal (HPA) axis responses.
lll. Neuropeptide and neurotransmitter production.

Figure 2: Stress and immune function



1. Nervous Stimulation

The 'Flight-Fight' Syndrome

Cognitive stimuli received by the cerebral cortex of the brain (Figure 2) produce neurological impulses which cause hypothalamic stimulation of the autonomic nervous system with an associated production of sympathetic

neurotransmitters. Activation of the autonomic nervous system produces an immediate response which results in the production of catecholamines by two pathways;

a) Directly, by release of nor-epinephrine from sympathetic nerve endings.
b) Indirectly, by release of epinephrine and small amounts of nor-epinephrine from the innervated adrenal medulla.

The somatic response to catecholamines cause dramatic increases in cardiovascular function and metabolism to effect the increased physical potential of the host which is manifest by the 'flight-fight' response. More specific responses are manifest within the haematological compartment where epinephrine may cause vasoconstriction and splenic contraction with an associated release of splenic erythrocytes and leukocytes into the blood vasculature (Cross *et al.*, 1988).

2. Hypothalmic-pituitary-adrenal stimulation

The 'Conservation-Withdrawal' Syndrome

Engel (1967) identified two distinct behavioral responses to stress in which the acute 'flight-fight' reaction was followed by a 'conservation-withdrawal' reaction. It has subsequently been demonstrated that the 'flight-fight' reaction is a result of the physiological changes due to the production of catecholamines by the sympathetic-adrenal medullary system (Axelrod and Reisine, 1984). Failure of this system to resolve stress leads to activation of the hypothalamic-pituitary-adrenocortical (HPA) response which is manifest behaviourally by the 'conservation-withdrawal' reaction. Persistence of HPA activation causes biological changes which may produce a pre-pathological state where the animal becomes susceptible to disease (Figure 1). If this latter state persists pathological changes will occur in the host (Kagan and Levi, 1974).

Harris (1948) first demonstrated that stimulation of the anterior hypothalamus produced corticotropin releasing factor (CRF), which activated the anterior pituitary gland to secrete adrenocorticotropins (ACTH). The target organ affected by ACTH is the adrenal cortex which is stimulated to produce significant levels of circulating glucocorticoids (cortisol/corticosterone). Glucocorticoids (GC) have an effect on most of the homeostatic systems in the body, through their impact on gluconeogenesis, and on the inflammatory and immunological systems. The name glucocorticoid implicates the primary effect produced by activation of the HPA system which is the production of a hyperglycaemia through enhanced hepatic gluconeogenesis. This metabolic response, and the associated increase in available energy, facilitates the 'conservation-withdrawal' response.

Depending on the species, different responses are produced by the action of cortisol on inflammation and blood leukocyte dynamics. Animals with relatively

high lymphocyte numbers, such as mice, rabbits, and chickens respond with a lymphopaenia and neutrophilia but produce a nett decrease in leukocytes. Those with relatively low lymphocyte numbers (dogs, cats, horses, cattle, pigs and man) respond with a leukocytosis because of the neutrophilia (Blecha *et al.*, 1982 and Burton and Beljan, 1970 and Gwazdauskas *et al.*, 1980). There is generally an eosinopaenia in most animals which produce high levels of cortisol. GC exerts a generalized anti-inflammatory action by causing a reduction in capillary permeability and endothelial swelling, reducing vascular flow. Activation of the HPA system and the associated increase in the levels of the plasma cortisol has been used widely to monitor stress levels in domestic animals (Franzmann *et al.*, 1975, Johnston and Buckland, 1976 and Leach, 1982). In cattle, elevated plasma cortisol levels have been found following handling (Crookshank *et al.*, 1979), weaning (Gwazdauskas *et al.*, 1978), castration and dehorning (Johnston and Buckland, 1976), acute pain (Stephens, 1980), forced exercise (Arave *et al.*, 1978), and transportation (Crookshank *et al.*, 1979 and Shaw and Nichols, 1964). However, it must be recognised that corticosteroids are only one hormone among many which are produced in the stress syndrome in animals (Roth, 1985). Thus single blood samples analysed for corticosteroids are unlikely to give a meaningful measure of the relevance of such factors in the overall response to stress.

From the earliest studies it was recognised that GC production in many species of animals was associated with adrenal hypertrophy, thymic involution, lymphocytopaenia, eosinopaenia and neutrophilia (Cupps and Fauci, 1982 and Jensen, 1969 and Riley 1981 and Selye, 1946). The administration of GC to animals of many different species has been shown to markedly increase their susceptibility to infectious disease and cause activation of latent infection. The specific examples evident from their use in cattle show that GC injection decreases resistance to infectious bovine rhinotracheitis virus (IBRV) (Davies and Duncan, 1974), coccidiosis (Niilo, 1970 and Stockdale, 1976), herpesvirus (Sheffey and Davies, 1971), fatal bovine viral diarrhoea (BVD) (Shope *et al.*, 1976) and parasites (Callow and Parker, 1969). GC also appears to influence immunocompetence in cattle which show reduced levels of humoral immunity (Gwadzdauskas *et al.*, 1978 and May *et al.*, 1979), and *in vitro* lymphocyte blastogenesis (Roth *et al.*, 1982). By contrast the administration of GC simultaneously with BVD vaccine causes increased levels of resistance to subsequent challenge (Roth and Kaeberle, 1983). The activation of adrenocortical production of GC by injection of cattle with ACTH does not always produce a lymphopaenia (Roth *et al.*, 1982) and may have variable effects on immunocompetence (Roth and Kaeberle, 1983). Riley (1981) has found that the timing of administration of GC relative to immune challenge, also appears to be of considerable importance. In tumour rejection studies, he showed that administration of GC to rats from 3-7 days prior to challenge with tumour increases the rate of tumour rejection while administration of GC after (4-6 days) implantation of tumour decreases rejection rates. The level of GC hormone may

also be important in that a moderate increase in GC levels may cause immune enhancement rather than immunosuppression (Croiset *et al.*, 1987).

Although there is no doubt that steroids can have a major influence on immune function when present at significantly elevated levels, their effects may be much more varied when present at lower doses, or in species which are relatively resistant to the effects of steroids. It may be that, in the veterinary context, the role of steroids has been overestimated in stress, because most experimental studies have been carried out on laboratory rodents (Parillo and Fauci, 1979), which are classified as corticosteroid sensitive animals. By contrast primates (Parillo and Fauci, 1979) and domestic animals such as cattle (Roth and Kaeberle, 1982) and pigs (Westly and Kelley, 1984) belong to the corticosteroid resistant species.

3. Neuropeptide Production

Learning, Behaviour and Pain Disolution

Because stress is manifest mainly by the individual's perception of the external stimulus, it is important to consider the production and function of the brain peptides and transmitters which may influence the adaptive response following stimulation of the CNS. In recent years a large number of peptides have been characterised and reclassified (Kreiger, 1983) as neurotransmitters and neuropeptides. Whereas a small number were discovered in association with CNS tissue and called the 'hypothalamic releasing hormones', others were first discovered in neurohypophyseal tissue or as hormonal factors associated with the pituitary or adrenal glands, or intestinal tissue.

A prototype molecule which has been widely studied is proopiomelanocortin (POMC). This precursor molecule can, following cleavage, produce a series of important neuropeptides, which play an important role in the sensory function of the CNS. Products generated from POMC include melanocyte stimulating hormone (MSH), ACTH and β endorphin (Kreiger, 1983). Whereas the precise role of these peptides is not fully understood, a number of independent studies have implicated them as factors which are produced in increased amounts in animals subjected to stress (Takayama *et al.*, 1986). The opioid peptides (Lewis *et al.*, 1985), β endorphin (Haynes and Timms, 1987), vasopression and oxytocin (Wideman and Murphy, 1985 and Williams *et al.*, 1985) and aminobutyric acid (Yoneda *et al.*, 1983) have all been found at increased levels in stressed animals.

Evidence for the link between the CNS, endocrine and immunological systems, is seen from the extensive repertoire of receptors on immune cells for hormones and neuropeptides. These include receptors for corticosteroids (Cake and Litwak, 1975), β andrenergic agents (Bourne *et al.*, 1974 and Hadden *et al.*, 1970 and Singh *et al.*, 1979), acetylcholine (Richman and Arnason, 1979 and Strom *et al.*, 1974), endorphins (Hazum *et al.*, 1979) and enkephalins (Wybran

et al., 1979), have been demonstrated on the surface of immunologically active mononuclear cells.

Taken together the presence of hormone receptors and the modulation of immune reactivity by altered levels of hormones, analogous to that found in stress, provide a link between stress and immunocompetence through production of the neuropeptides.

STRESS AND DISEASE SUSCEPTIBILITY

The year 1878 provided not only the foundation statement in physiology by Claude Bernard, which has become the basis for our understanding of homeostasis and stress physiology, but in that year Pasteur (cited by Nicol, 1974) also made the empirical observation that hypothermia increases the susceptibility of fowl to anthrax infection, during the experimental demonstration of his 'germ theory' for infectious disease. This work first identified the link between the host, the microrganism and the environment in the response to infectious disease.

Since then many workers have identified links between the stress associated with physical and psychological stimuli, and the increased susceptibility of animals to infectious, organic and neoplastic disease (Reviewed by Kelley, 1980 and Riley, 1981). Because of the complex interplay between factors, such as risk exposure to infectious agents and host response to infection, there are remarkably few documented reports using domesticated species which provide objective scientific information which implicate management stress as the causal factor in increased disease susceptibility. To compound the complexity of these systems, sophisticated management practices involving vaccination and therapy often obscure the natural susceptibility of domesticated animals to clinically obvious infectious diseases.

Because pigs are one of the most intensively farmed domesticated species of animals, considerable work has been carried out on the effect of management on susceptibility to disease in pig herds. Perinatal stress has been shown to cause an increase of incidence of gastroenteritis and diarrhoea in piglets (Curtis, 1974). Exposure of piglets to cold in the first days of life is of special importance in decreasing disease resistance (Kelley *et al.*, 1982). Shimizu *et al.* (1978) have demonstrated a direct link between the temperature at which pigs are housed and their susceptibility to transmissable viral gastroenteritis (TGE). The temperature at which the pigs are housed at after exposure to TGE is also important, as is adaptation of the animals to a given temperature prior to exposure.

'Shipping-fever' is a well documented disease (Hoerlein, 1980) associated with transportation of cattle. The onset and severity of individual animals response to transport may be influenced by a number of variables, including the quality of transport, the ambient temperature, the stock density, the novelty of transport and the time involved in transportation. Hoerlein and Marsh (1957) first suggested that the infectious complex causing 'shipping-fever' was due to

reduced resistance of animals, precipitated by transport stress, which caused establishment of secondary viral and bacterial infections. The age at which animals are exposed to stress appears to be extremely important because young calves are much more susceptible to stress associated episodes of infection, found during transport (Staples and Haugse, 1974), or following metreological changes (Martin *et al.*, 1975) or wetting (Jennings and Glover, 1952) than older animals. Calves transported during winter have a higher mortality than when transported under less severe weather conditions (Staples and Haugse, 1974).

Lactation in cattle involves an increased metabolic demand which may predispose cows to secondary stress. Increased susceptibility to infection is seen early in lactation in high milk producing cows (Newbould, 1974). Temperature extremes (McDowell and McDaniel, 1965) and nutritional stress (Morrow, 1976) cause further increase in the incidence of infection in lactating cows.

DEER AS A LARGE ANIMAL MODEL FOR STRESS STUDIES

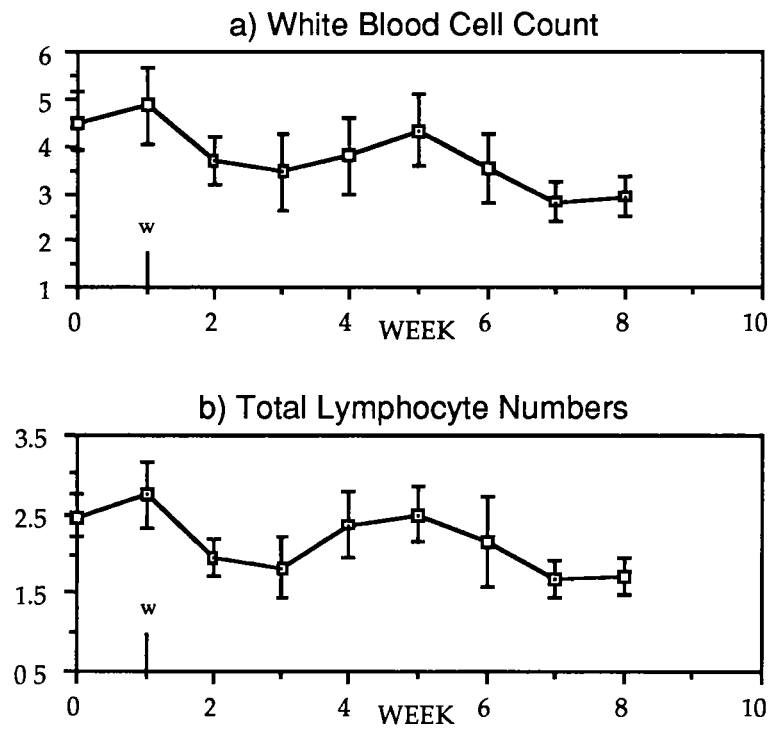
In the past 30 years New Zealand has been involved in one of the largest experiments in animal behaviour seen in recent centuries, involving the domestication of wild deer for farming under intensive husbandry conditions. This represents a uniquely accessible system to study the stress associated with domestication of a wild animal. Not only has it proved itself to be a viable alternative form of agricultural enterprise, but it has highlighted many unique problems imposed by husbandry, which invariably challenges the 'free and independent existence' of a wild animal. The success of this enterprise, which has resulted in the emergence within N.Z. of a national farmed herd of 500, 000 deer (1987), has been dependent primarily on the development of management and handling systems which allow farmed deer to be managed under a system of minimal stress. The primary response of these animals to the stress associated with human intervention and management is manifest majestically by their physical expression of 'flight' reflex as a component of their 'flight-fight' response to stress. The stresses imposed on this recently domesticated species are now well recognised, and vary in severity from the severe form of acute stress; in the wild capture of feral animals, to the chronic and insidious farming stress; during physical restraint, transport, handling, adverse climate, inadequate nutrition, weaning, social hierarchy and breeding (Griffin, 1987).

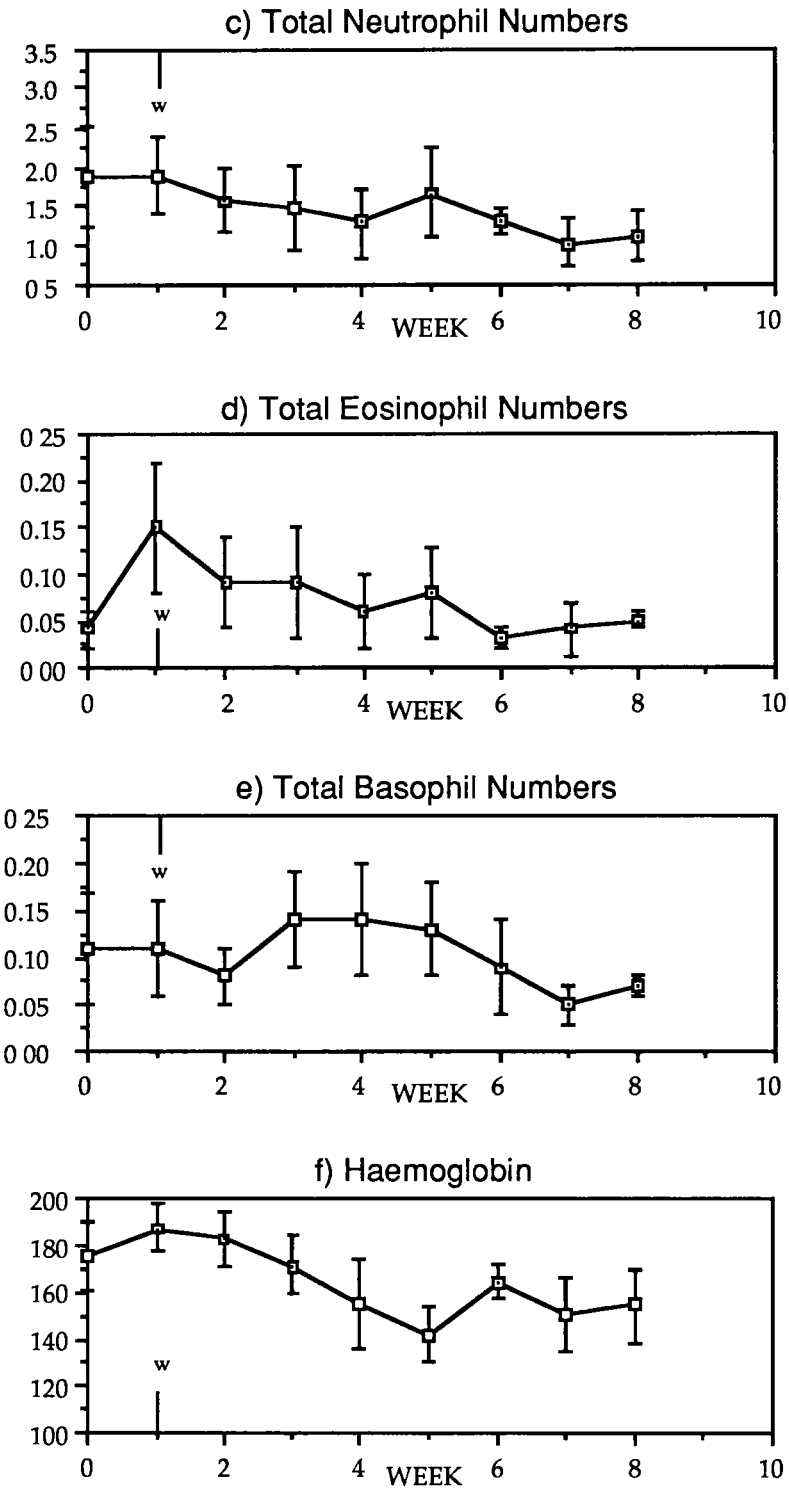
An array of infectious diseases, found in increased incidence in farmed deer are exacerbated by management stress. They range in severity from acutely fulminating bacterial disease involved in foot abscesses, which produce a rapidly fatal toxaemia in animals post capture (Griffin, 1987), to acutely lethal infection caused by malignant catarrhal fever (MCF) in animals following transport or after exposure to adverse climatic changes (McAllum, 1980). Episodic outbreaks of yersiniosis in weaned deer (Mackintosh and Henderson, 1984) and exacerbation of tuberculosis in breeding females (Griffin, 1988) have also been found in farmed deer.

WEANING, ENVIROMENTAL AND HANDLING STRESS IN FAWNS AND HINDS

A preliminary study was carried out to examine haematological and immunological changes in 28 young deer immunised with BCG one week before weaning. The results given in Figure 3 show that weaning (w) caused a leucocytopaenia involving a significant reduction in lymphocytes (Figure 3a). neutrophils (Figure 3b), basophils (Figure 3c), and eosinophils (Figure 3d). Leukocyte numbers recovered consistently in all animals in the succeeding four weeks. By five weeks post-immunization there was again a significant decrease in total white cell counts which included all cell types. The second cycle of leucocytopaenia was probably associated with adaptation to grain feeding and due to associated severe cold stress, induced by an extremely cold spell of weather, with temperature shifts ranging between +10^{o} C and -20^{o} C. A significant drop in blood erythrocyte numbers was also found in the animals between two and four weeks after weaning. Together these results suggest that weaning had an effect on the haematological parameters in fawns for the first week after seperation and that changes found in the animals in the subsequent weeks were more likely due to enviromental stressors associated with nutritional adaptation and cold weather.

Figure 3: Changes in haematological parameters in fawns following weaning (x10^{9}/l)

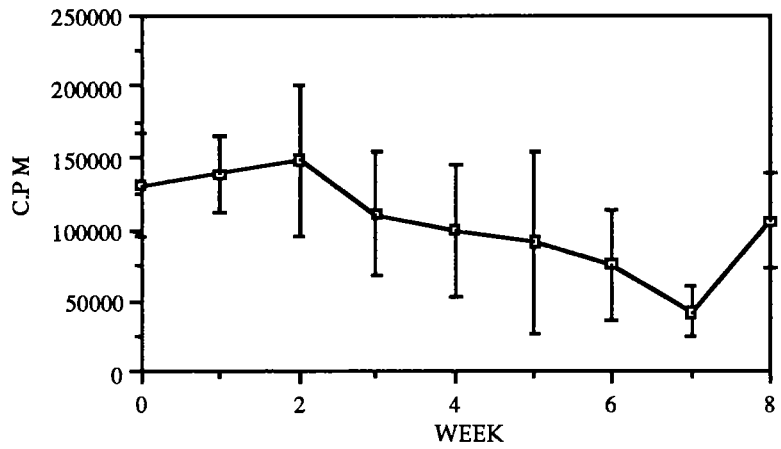




Measurement of lymphocyte response in the animals in the weeks following weaning (Figure 4) showed that there was a consistent fall off in T-cell reactivity, as measured by Concanavalin A stimulation, which was down 50% of the pre-weaning value at six weeks post-weaning. The fact that no significant drop in reactivity was evident in the immediate period following weaning suggested that the adverse climatic conditions and nutritional adaptation in the weeks after weaning had a more significant impact on lymphocyte recovery than

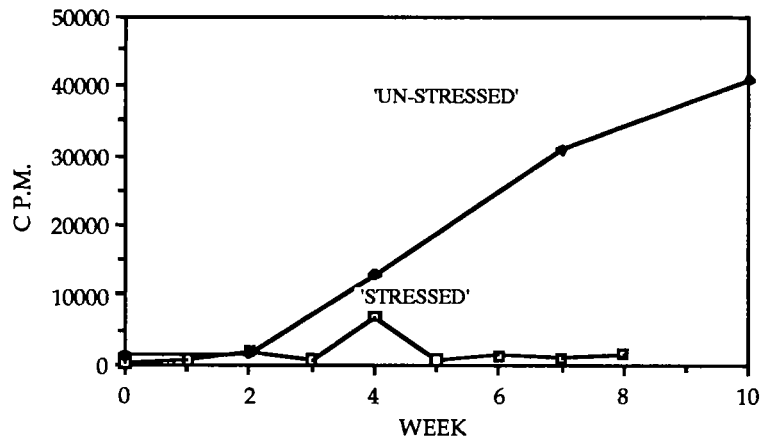
the immediate impact of separation at weaning.

Figure 4: The effects of nutrition and weather on lymphocyte transformation in response to Concanavalin-A



The composite effects of weaning , changed nutrition, and poor weather showed that a significantly impaired response was evident in weaned fawns immunised with killed BCG in oil adjuvant (Figure 5), when compared with six-month old animals immunized two months post-weaning. Adaptation of animals for two months following weaning appeared to produce significantly increased levels of lymphocyte reactivity to BCG immunization.

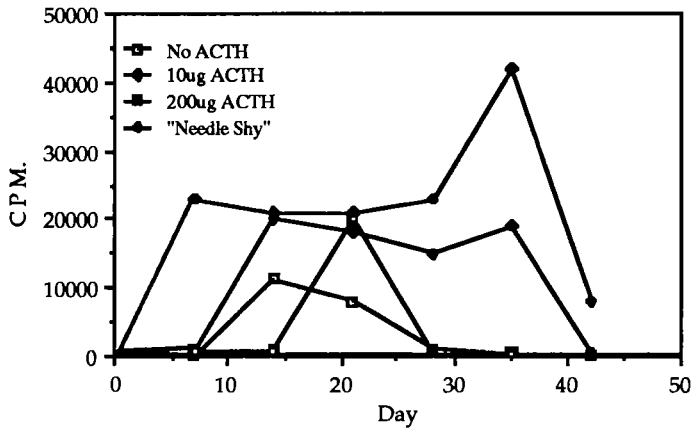
Figure 5: Environmentally 'stressed' vs 'unstressed' animal's lymphocyte responses to antigen (PPD-bovine)



A separate experiment was carried out to measure the impact of handling and ACTH treatment to induce cortisol production in a group of ovariectomised hinds. Four groups, each containing four hinds, were treated as follows; group A

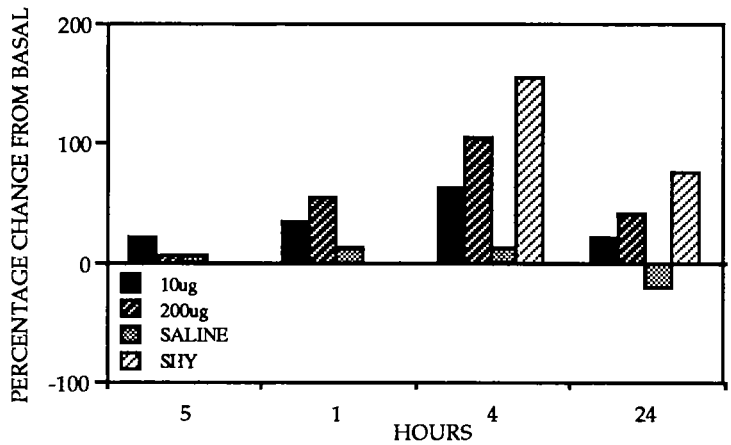
received saline, group B 10 μg ACTH intravenously, group C were given 200μg ACTH, and group D were a group of hypersensitive hinds which were 'needle-shy' and actively resisted restaint and bleeding. Four hours following treatment with ACTH or saline, all animals were immunised with a strong antigen; key-hole limpet haemocyanin (KLH), and their immune reactivity measured for the ensuing eight weeks. The results (Figure 6) showed the surprising findings that the 'needle-shy' animals gave the strongest lymphocyte reactivity to KLH following immunization. The next best response was seen in the animals treated with 10 μg ACTH, and the poorest response was seen in the untreated controls.

Figure 6: Lymphocyte activation by KLH



The classical acute increase in blood neutrophils was seen (Figure 7) in the 'needle-shy' animals and animals treated with high doses (200μg) of ACTH. No changes were seen in the neutrophil levels of control animals or those treated with low doses (10μg) of ACTH.

Figure 7: Neutrophil changes post-treatment



These preliminary experimental findings suggest that weaning and enviromental adaptation may produce a temporary reduction in immunocompetence as measured by lymphocyte reactivity. By contrast, activation of moderate levels of cortisol production, using low doses of ACTH or handling of 'needle-shy' animals, may potentiate immune reactivity rather than cause immunosuppression. It will be extremely important to further monitor how different management practices influence host physiology and immunity if we are to efficiently farm deer in a world which will continue to place new restrictions due to the need to balance the profit-cost-loss equation.

CONCLUSIONS

The physiological changes which occur under the influence of CNS activation during stress produce widespread changes within the host. The initial 'flight-fight' response is due to an increase in metabolic and cardiovascular activity, under the control of the sympathetic adrenomedullary system. This is followed by the 'conservation-withdrawal' response, under the influence of adrenocortical stimulation. The cycle is completed by the 'learning-adaptation' response conditioned by the production of a wide array of neuroendocrine factors.

Does this increased capacity to run, hide, tolerate pain and adapt, place undue demands on other biological systems within the host? The evidence forthcoming from the extensive studies which have examined the direct influence of stress on immune physiology and the current experiments, would suggest that immunity is impaired transiently during the acute phase of the stress response. However, because the generation of a protective immune response *de novo* requires 1 to 2 weeks to become fully activated, it may be of little relevance during the acute phase of stress, which demands an immediate effector response.

The integrity of the inflammatory system, and it's activation by preexisting immune cofactors, may be of far greater significance in the protection of the host during acute challenge. The increase in circulating blood neutrophils may contribute to enhanced non-specific protection during acute stress. There is also a significant body of data which suggests that resolution of acute stress has contingent side effects which can potentiate subsequent immune reactivity.

However, when stress is of extreme severity or persists chronically, it may place demands on the host which have more telling effects on immune function. Should chronic stress compromise immune function, it is of considerable importance as the persistence of stress may threaten the integrity of immunity and immunosurveillance. Data available from the study of chronic stress suggest that the inability of the host to cope with or counteract chronic aversive stimuli produces severe immune compromise through physiological imbalances, which result from chronic activation of the neuroendocrine pathways. Climatic stress in the weaner animals appears to have produced such an effect. By contrast the ability of the host to cope with chronic stress evokes the production of a different

array of neuropeptides and neuroendocrine factors, which are produced during learning and adaptation. Coping or adaptation may be expressed behaviourally by avoidance or escape from the aversive stimulus or the development of an assertive or aggressive response to challenge. The physiological factors produced during adaptation appear to cause potentiation of immunocompetence and may be of significant benefit for the host. The assertive adaptation response in the 'needle-shy' hinds appears to fit into this category.

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