

## STRESS-INDUCED IMMUNOLOGIC IMPAIRMENT IN ROCKY MOUNTAIN BIGHORN SHEEP

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Rocky Mountain bighorn sheep serve as hosts for a wide variety of parasitic, bacterial and viral pathogens (Forrester, 1971). A major pre-occupation in past years has been the incrimination of each of these agents as a prime etiologic agent in large-scale die-offs. Pneumonia caused by lungworms has been identified as a major pathogen in a number of outbreaks of respiratory disease. Some workers have emphasized bacterial organisms, particularly Pasturella, whereas others have postulated the involvement of viruses (Howe et al., 1966) or Mycoplasma (Wolf et al., 1970).

On the basis of present knowledge, there does not appear to be one pathogen that can be considered a common denominator. However, environmental stress (nutritional, meteorologic, psychologic or otherwise) is emphasized in a large proportion of disease outbreaks. Perhaps the association is more imagined than real - it may have been adopted in desperation since it is a convenient term accounting for the "all others" category. However, there is probably enough circumstantial evidence to warrant some time and effort in determining the mechanisms by which stress may influence the frequency and severity of such infections.

A good starting point is the development of methods to monitor general immunologic status. With such a tool, the role of a variety of environmental stressors in the respiratory diseases of bighorn sheep can be evaluated.

A number of methods, such as experimental infection or immunization, are available for assessing immunologic status. Such procedures permit a direct evaluation of the ability of animals to resist disease or produce antibody. However, they are obviously impractical for examining free-ranging populations of bighorn sheep. A technique is required which involves only brief handling of animals at a single capture.

Functional characteristics of peripheral lymphocytes have been used successfully in human medicine as correlates of immunologic reactivity (Revillard, 1971). The lymphocyte is a basic cellular element involved in both humoral and cellular immune responses. Phytohemagglutinin (PHA), an extract of the kidney bean (Phaseolus vulgaris), acts as a blastogenic agent in cultures of these cells, inducing sequential metabolic changes culminating in morphologic transformation to blastoid cells. This study was conducted to determine whether changes in in vitro lymphocyte responsiveness to PHA occurred in bighorn sheep subjected to stress associated with captivity.

### Experimental

Six adult Rocky Mountain bighorn ewes were captured in Jasper National Park in November, and were transported to Vancouver for nutritional and immunologic studies. They were offered medium quality hay for the duration of the study.

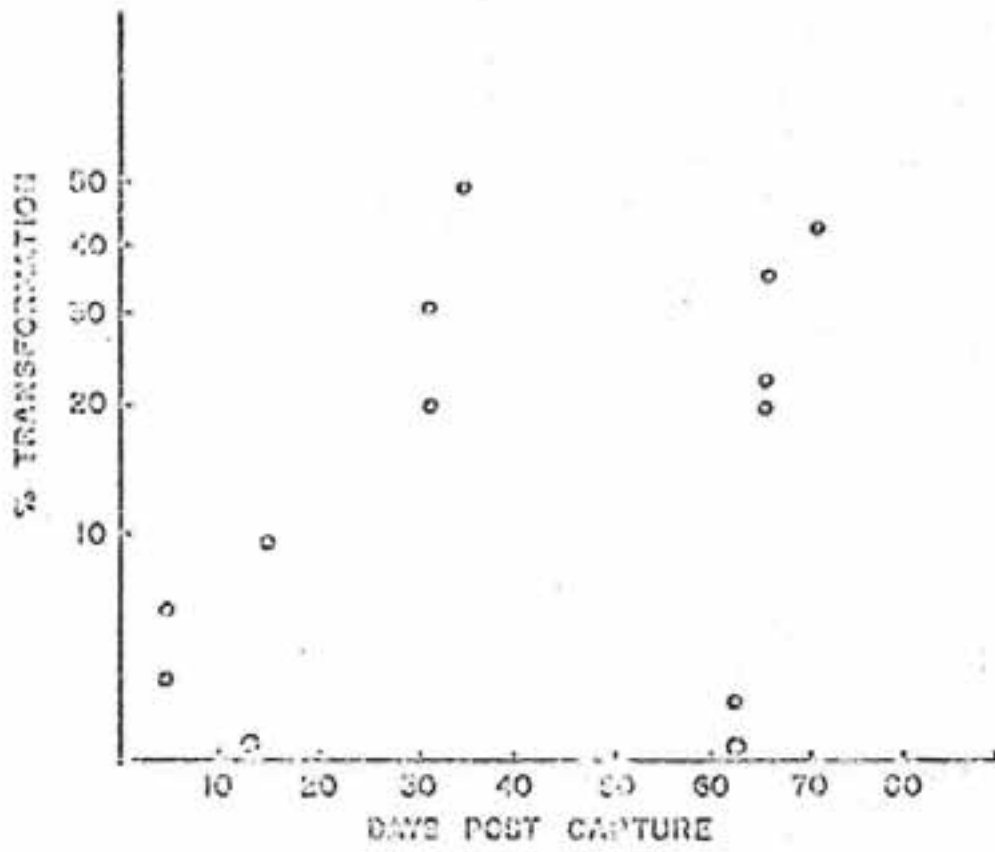


Figure 2

The functional activities of lymphocytes were evaluated by culturing blood lymphocytes with PHA, according to a procedure which will be reported in detail elsewhere. Following incubation for 6 days, the cells were stained with acridine orange and examined for morphologic changes by fluorescent microscopy.

### Results and Discussion

Lymphocytes responding to PHA were characterized by their large size, bright yellow-green nucleus and red cytoplasm (Fig. 1). In contrast, unstimulated cells were small, with a deep green nucleus and unstained cytoplasm.

In the present study, stress associated with capture, transport and nutritional changes was accompanied by an apparent depression of lymphocyte responsiveness to PHA. Lymphocyte stimulation, measured by per cent transformation, was low during the first two weeks following capture, but increased markedly over the next two months (Fig. 2). Two animals with clinical signs, including slight scouring, loss of condition, and high erythrocyte fragility, exhibited very low levels of lymphocyte transformation. However, one animal sampled 62 days following capture, exhibited a marginal degree of lymphocyte transformation although clinical signs, other than slight loss of condition, were not evident.

Lymphocyte responsiveness to phytomitogens has been subjected to extensive clinical evaluation in man as an in vitro correlate of immunologic status. Depressed transformation has been found in malnutrition and in a variety of disease conditions which involve immunologic impairment, including chronic lymphocytic leukemia, Hodgkin's disease, sarcoidosis, and advanced cancer (Naspitz and Richter, 1968, Revillard, 1971). Lymphocyte transformation has also been used, with some success, in the evaluation of immunosuppressive drugs and antilymphocyte globulins (Naspitz and Richter, 1968).

Although alterations of in vitro characteristics of peripheral lymphocytes of bighorn sheep were demonstrated during stress associated with introduction to captivity, the actual significance of this observation remains speculative. Since bighorn sheep are particularly susceptible to disease when brought into captivity, it is possible that depressed in vitro lymphocyte reactivity reflected impaired immunity. Such an interpretation is consistent with known lymphocyte behaviour in man and laboratory animals. However, further studies are required to establish the actual applicability of this procedure in the assessment of general immunologic status of bighorn sheep.

### Acknowledgements

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PREPARATION AND TESTING OF PASTEURELLA BACTERINS  
ON CAPTIVE BIGHORN SHEEP

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Abstract

Experimental testing was done on two multi-valent Pasteurella bacterins: one a formalinized cell bacterin and the other an extracted cell bacterin. Two groups of captive-penned bighorn sheep were used for the tests. One group consisted of newborn full-blood and hybrid (F<sub>2</sub>) bighorn sheep at Rachelwood Wildlife Research Preserve, Pennsylvania. This group received either antibiotics, domestic

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