released from platelets subsequent to endothelial injury are involved with smooth muscle cell migration and proliferation into the intima. The presence of thrombocytes, thrombocyte aggregates, and mononuclear cells at the sites of endothelial damage in the pigeon is consistent with this hypothesis, and suggests that spontaneous pigeon atherosclerosis parallels the development of experimental lesions in other species. Clearly a more thorough understanding of the early events in atherosclerosis is needed, but it is becoming progressively more obvious that both endothelial damage and blood cell response are integral to atherogenesis.

JON C. LEWIS
Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103
BRUCE A. KOTTKE
Mayo Clinic/Foundation, Rochester, Minnesota 55901

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Elk in the Shrub-Steppe Region of Washington: An Authentic Record

Abstract. *For the first time in recorded history, the American elk is established in the treeless interior region of Washington. The protective isolation provided by the large buffer zone around the Hanford facilities of the U.S. Energy Research and Development Administration is the important factor in maintenance of suitable habitat for elk.*

In the judgment of wildlife biologists, the American elk (*Cervus canadensis* L.) has not been found in the treeless interior region of Washington within recorded history (*1*). Since the turn of this century, elk have been restricted to the Cascade Mountains and other mountainous forested areas. During winter months, elk regularly move into the bordering nonforest areas to avoid deep snow and to seek forage; in the spring they retreat into the forests to bear young. Over the past 100 years, the Columbia Basin has been greatly modified by agricultural activities. Only three sizable land tracts still support native shrubs and grasses. These are the Yakima Indian Reservation, the U.S. Army's Yakima Firing Range, and the Hanford Reservation of the U.S. Energy Research and Development Administration (ERDA). Of these, only the Hanford Reservation is not subjected to livestock grazing. The portion of the Hanford Reservation that includes the Rattlesnake Hills contains some of the most extensive and least disturbed stands of the *Artemisia tridentata–Agropyron spicatum* association remaining in the Pacific Northwest (*2*). Approximately 260 km^2 of the Rattlesnake Hills is designated as a reserve for scientific and educational purposes and is protectively managed as the Arid Lands Ecology (ALE) Reserve; it also serves as a buffer zone for ERDA's Hanford research and production facilities.

Although the area has been under aerial surveillance since 1943, it was not until 1972 that the tracks of a single elk were reported (*3*). In the early autumn of 1974, aerial surveillance revealed a group of about 14 elk using the ALE Reserve (*4*). Since then, elk have been present throughout the year. It was suspected that calves were dropped in the spring of 1975, but young calves were not actually seen. In the autumn of 1975, mangled bushes were in evidence as the rutting bulls rubbed velvet from hardening antlers. On 15 June 1976, two cows, each with a newborn calf, were observed during a special helicopter flight.

We believe that the original group of elk wandered onto the ALE Reserve from the Cascade Mountains more than 60 miles to the west. The protective isolation, abundant forage, and drinking water available at several small natural springs now make the treeless ALE Reserve acceptable as year-round elk habitat. In this instance, the cessation of live-

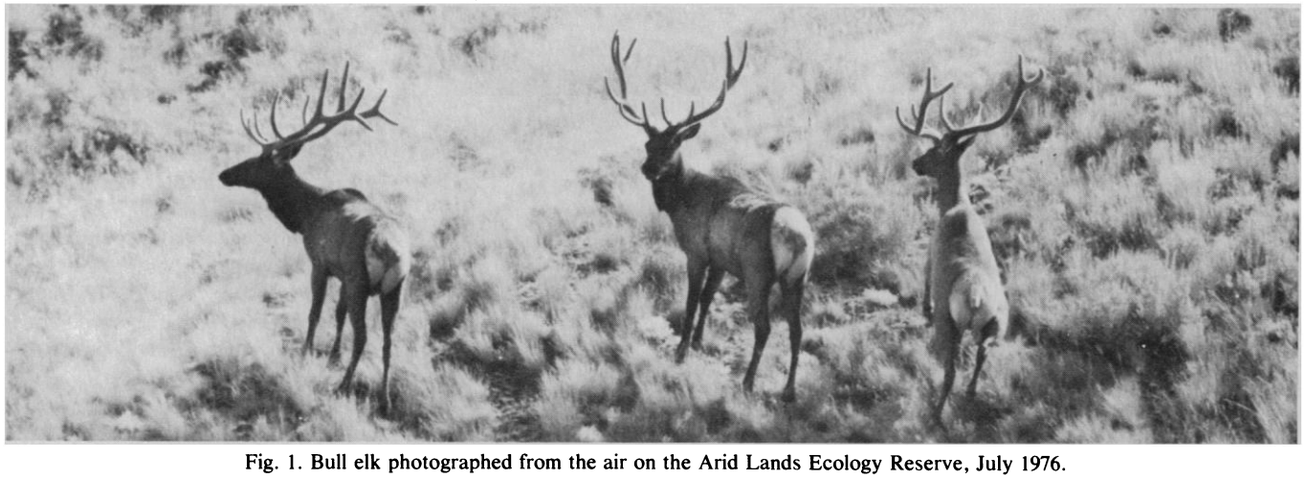

Fig. 1. Bull elk photographed from the air on the Arid Lands Ecology Reserve, July 1976.

stock grazing and the creation of a large buffer zone around ERDA's Hanford facilities has encouraged a large native ungulate to establish itself in a historically unoccupied range.

W. H. RICKARD
J. D. HEDLUND
R. E. FITZNER

Ecosystems Department, Battelle, Pacific Northwest Laboratories, Richland, Washington 99352

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4. Aerial surveillance takes place at weekly intervals to maintain fence integrity and to discourage off-road vehicle trespass.
5. Research conducted by Battelle, Pacific Northwest Laboratories for the Energy Research and Development Administration under contract E(45-1)-1830.

28 October 1976

Cytochrome c: Immunofluorescent Localization of the Testis-Specific Form

Abstract. *Mouse testes contain a unique form of cytochrome c. As demonstrated by the indirect immunofluorescence technique, the testis-specific cytochrome c is detectable in the primary spermatocyte and in cell types comprising the later stages of spermatogenesis. Interstitial cells, Sertoli cells, and spermatogonia contain the somatic form of cytochrome c, as does heart muscle.*

The germinal epithelium of mature testes is a highly specialized tissue designed for the production of spermatozoa. This tissue has many unique features, not the least of which is the presence of a number of proteins that are synthesized only in cells committed to spermatogenesis (*1*). The best example of such a protein is the testis-specific form of lactate dehydrogenase (LDH-C_4 or LDH-X; E.C. 1.1.1.27). This isozyme is composed of four C subunits, distinct from the A and B subunits found in other tissues (*2*). It first appears in the primary spermatocyte during midpachytene (*3*) and ultimately becomes the predominant if not the sole LDH of sperm. It now appears that an analogous phenomenon can be observed with cytochrome c, the electron-transport protein of the mitochondrial respiratory chain.

Hennig (*4*) recently reported the isolation of two different cytochromes c from mouse testes. One is identical to the cytochrome c found in mouse heart, while the other protein differs from the first by approximately 13 residues in the amino acid sequence (*4*). We shall refer to these molecules as cytochrome c_s, for the protein presumably found in all tissues, and cytochrome c_t for that form isolated from testes. In this report, we demonstrate that mouse cytochrome c_t is strictly confined to spermatogenic elements of the seminiferous epithelium.

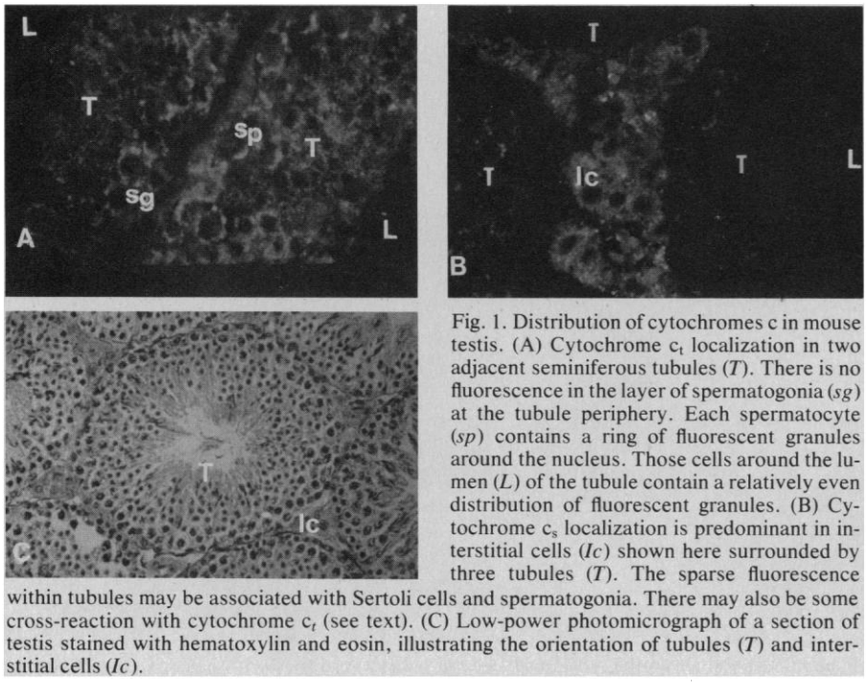

Fig. 1. Distribution of cytochromes c in mouse testis. (A) Cytochrome c_t localization in two adjacent seminiferous tubules (*T*). There is no fluorescence in the layer of spermatogonia (*sg*) at the tubule periphery. Each spermatocyte (*sp*) contains a ring of fluorescent granules around the nucleus. Those cells around the lumen (*L*) of the tubule contain a relatively even distribution of fluorescent granules. (B) Cytochrome c_s localization is predominant in interstitial cells (*Ic*) shown here surrounded by three tubules (*T*). The sparse fluorescence within tubules may be associated with Sertoli cells and spermatogonia. There may also be some cross-reaction with cytochrome c_t (see text). (C) Low-power photomicrograph of a section of testis stained with hematoxylin and eosin, illustrating the orientation of tubules (*T*) and interstitial cells (*Ic*).

Approximately 40 mg of pure cytochrome c_t was prepared from 1.9 kg of testes dissected from about 10,000 sexually mature, random-bred mice (*5*). Antiserums were raised in male New Zealand White rabbits by injecting them with glutaraldehyde–cross-linked polymers of either cytochrome c_s or cytochrome c_t (*6*). The specificity of the serums was tested by double diffusion in agar gels (*7*). The antiserum to cytochrome c_s reacted strongly with cytochrome c_s and weakly with cytochrome c_t. Conversely, the antiserum to cytochrome c_t reacted much more strongly with cytochrome c_t than cytochrome c_s. The cross-reacting antibodies in antiserum to cytochrome c_t were removed by absorption on a column consisting of cytochrome c_s bound to Sepharose 4B (*8*). The absorbed serum reacts only with cytochrome c_t and not cytochrome c_s. Because of the limited amount of cytochrome c_t available, it was not possible to obtain an antiserum to cytochrome c_s that did not cross react to a slight extent with cytochrome c_t.

Localizations of the cytochromes c were performed by the indirect fluorescent antibody technique (*9*) on sections cut from paraffin-embedded tissue which had been fixed in Bouin's fluid. After reaction with rabbit antiserum to cytochrome c, the slides were washed, treated with fluorescein isothiocyanate conjugated goat antiserum to rabbit immunoglobulin G, and examined by transmitted light dark-ground-fluorescence microscopy (*10*). Appropriate controls showed that the fluorescence in the sections treated with antiserum was due solely to specific interaction with the cytochromes c, and not to fluorescence of the tissue itself or to fluorescence induced by nonspecific binding of rabbit gamma globulins to the mouse tissue.

Immunofluorescent analysis of mouse testis from a sexually mature animal revealed that cytochrome c_t was confined to cells of the germinal epithelium. The antibody reaction appears as a discrete granular fluorescence in the cytoplasm, strongly suggestive of mitochondrial localization (Fig. 1A). From examination of adjacent sections stained with hematoxylin and eosin, the cells closest to the tubule periphery which are positive for cytochrome c_t are primary spermatocytes. Spermatogonia which occupy the basal