membrane intramembranous particles. Whether this is related to the stage of denervation or reinnervation is unknown (*8*).

Significant progress has been made in correlating function with intramembranous particle populations in skeletal muscle sarcoplasmic reticulum (*9*). It is anticipated that function will eventually be linked to the intramembranous particle populations seen in the muscle plasma membrane so that alterations in distribution and number of particles will be correlated with changes in specific plasma membrane functions in Duchenne dystrophy.

D. L. SCHOTLAND
E. BONILLA
M. VAN METER

Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia 19104

References and Notes

1. J. C. Dreyfus, G. Schapira, F. Schapira, *J. Clin. Invest.* **33**, 794 (1954); S. Mawatari, A. Takagi, L. P. Rowland, *Arch. Neurol.* **30**, 96 (1974).
2. H. Schmalbruch, *Acta Neuropathol.* **33**, 129 (1975).
3. B. Mokri and A. G. Engel, *Neurology* **25**, 1111 (1975).
4. D. Branton, *Proc. Natl. Acad. Sci. U.S.A.* **55**, 1048 (1966); V. T. Marchesi, T. W. Tillack, R. L. Jackson, J. P. Segrest, R. F. Scott, *ibid.* **69**, 1445 (1972); D. H. MacLennan, P. I. Seenian, G. H. Iles, C. G. Yip, *J. Biol. Chem.* **246**, 2702 (1971).
5. The specimens were removed at rest length, attached to a stick or U-shaped muscle clamp, and fixed immediately in 3 percent glutaraldehyde in 0.1*M* phosphate buffer, *p*H 7.4. Fascicles were dissected out and gradually infiltrated in glycerol in water up to a concentration of 30 percent. Freezing was carried out in Freon 22 and fracture and replication were performed in a Denton DF E5 freeze-etch unit and a Balzer BAF 301 freeze-etch apparatus. Tissue was digested in a commercial bleaching solution (Clorox). The detached replicas were washed twice in distilled water and finally picked up on uncoated 300-mesh grids. The material was examined and micrographed in an AEI EM-6B electron microscope operated at 60 kv. Particle counts per square micrometer were carried out on random areas of the protoplasmic (P) face and extracellular (E) face of the plasma membranes on micrographs enlarged to ×60,000. Plasma membranes from a minimum of eight fibers (1 μm^2 each) were counted in each biopsy. Particles were only counted when they were clearly above the surface of the replica. Particle size was not emphasized in this study. The randomness of the sample was ensured by photographing any area of the fracture that was recognized as plasma membrane. Individual square micrometers counted were chosen by blind selection.
6. J. E. Rash and M. H. Ellisman, *J. Cell Biol.* **63**, 567 (1974).
7. D. W. Deamer, A. Leonard, A. Tardieu, D. Branton, *Biochim. Biophys. Acta* **219**, 47 (1970).
8. J. E. Rash, M. H. Ellisman, L. A. Steahlin, *J. Cell Biol.* **59**, 280a (1973).
9. D. W. Deamer and R. J. Baskin, *ibid.* **42**, 296 (1969); R. Boland, A. Martinosi, T. W. Tillack, *J. Biol. Chem.* **249**, 612 (1974); T. W. Tillack, R. Boland, A. Martinosi, *ibid.*, p. 624.
10. We thank Dr. Clara Franzini-Armstrong and Dr. Robert Lisak for their helpful advice. We also thank the Neurology and Orthopedic staff of the University of Pennsylvania, Dr. Warren Grover of St. Christopher's Hospital, and Dr. Michael Mitchell of Hahnemann Medical College for their aid. Supported by NIH grants NS-08075 and 5-M01 RR00040 and the Henry M. Watts, Jr., Neuromuscular Disease Research Center.

13 January 1977

Endothelial Damage and Thrombocyte Adhesion in Pigeon Atherosclerosis

Abstract. *Scanning electron microscopy studies of spontaneously occurring atherosclerosis in pigeons reveal dramatic alterations in endothelial integrity. An irregular endothelium at the intimal cushion region of 5-week-old birds gives rise to extensive areas of pitted endothelium and subendothelial exposure. Thrombocytes, thrombocyte aggregates, and leukocytes are associated with the developing lesion.*

Ultrastructural changes in spontaneously occurring atherosclerosis have been described by several authors (*1*). Particular attention in these studies has been given to pathologic alterations in the arterial walls that climax in mature lesions composed of smooth muscle cells and connective tissue elements (*2*). Although the general sequence of events in spontaneous atherosclerosis has been elucidated, little specific information is available on the initiation of this disease. Recent observations using animal models, including chickens, dogs, swine, rabbits, and nonhuman primates (*3*) have been of value in providing more specific information on atherogenesis, and results obtained with these animal models suggest that both smooth muscle cell proliferation and lipid accumulation are manifestations secondary to continued endothelial injury (*4*). This hypothesis, however, is based upon experimentally induced atherosclerosis, since both the extent of arterial disease and the localization of spontaneous lesions vary in most species.

Arterial lesions in the White Carneau pigeon, unlike other animals, develop spontaneously and are highly predictable with respect to arterial localization (*5*). Furthermore, the similarity of pigeon intimal cushions to those found in humans (*5*, *6*), and the fact that the time course for lesion progression is well defined in this species (*7*), make the White Carneau a desirable model for identification of early events in spontaneous atherosclerosis. We report a scanning electron microscope study of aortas from the atherosclerosis-susceptible White Carneau pigeon. Our studies, focusing upon endothelial integrity and the role of circulating elements at the site of spontaneous lesion development, reveal significant endothelial damage, with thrombocytes and leukocytes adherent to both the damaged endothelial cells and exposed subendothelial fibrils.

Twenty White Carneau pigeons ranging in age from 5 weeks to 3 years were anesthetized by intravenous administration of pentobarbital (32 mg) and anticoagulated with 250 units of heparin prior to perfusion under pressure (110 mm-Hg) with 2.5 percent glutaraldehyde buffered to *p*H 7.2 with 0.1*M* phosphate. Subsequent to perfusion the excised aortas were fixed further by immersion overnight in fresh fixative before being dehydrated through graded alcohols and dried from CO_2 by the critical point method. Preparation of aortas by this technique permitted direct observation of aortic endothelium from below the celiac bifurcation, where spontaneous lesions develop, to areas 15 mm cephalad to the celiac origin (Fig. 1a). Included in such preparations are the vestigial ligament, openings of the intercostals, the intimal cushion region, and areas sufficiently far from the lesion to serve as control tissues.

Endothelium from the nonlesion control regions, as shown in Fig. 1b, was intact. The well-delineated endothelial cells had distinct marginal folds and multiple surface projections. This was true for birds of all ages and for birds with varying degrees of intimal cushion proliferation. Irregular patches positioned over the aortic surface near the intimal cushion region were occasionally found in birds as young as 5 weeks. When observed at high magnification, the endothelium in these irregular areas was disrupted and had extensive ruffling of luminal plasma membranes (Fig. 1c). In addition to the membrane ruffles, areas of endothelial desquamation were found in the 5-week-old birds. This damage, localized at orifices of the intercostals and in the area of the vestigial ligament, was most pronounced at the celiac bifurcation, where an extensively pitted and cratered endothelium resulted in large areas of subendothelial exposure (Fig. 1d). Blood cells adherent to the damaged surfaces frequently formed a semiconfluent cytoplasmic sheet. These observations were consistently found in pigeons of all ages, but were most conspicuous in older birds with identifiable lesions. Thrombocytes in the atheromatous area were attached to subendothelial fibrils and to altered endothelial cells by the extension of multiple cytoplasmic fingers (Fig. 1, e and f). Thrombocyte aggregates were also associated with necrotic endothelial cells (Fig. 1g). In addi-

tion to thrombocytes, significant numbers of leukocytes were observed in the lesion areas of mature birds. As shown in Fig. 1, h–j, the leukocytes, presumed to be mainly monocytes with a few granulocytes (see Fig. 1h, cell on the right), attached to the damaged endothelium and extended cytoplasmic fingers or ruffles while spreading across the endothelial surface.

Our observations clearly document structural alterations in arterial endothelium during the early stages of spontaneous atherosclerosis in White Carneau pigeons. These alterations, including plasma membrane ruffling and extensive endothelial desquamation, were evident in the youngest birds studied, but were more pronounced with an increase in age of the birds and were most severe at the celiac bifurcation, where intimal cushions develop. Evidence recently presented by Subbiah *et al.* (*7*) has established the critical period of spontaneous sterol accumulation in pigeon aortas. This critical period, occurring at 9 to 12 months of age, chronologically coincides with other events such as changes in intracellular metabolism relating to sterol esterification (*8*), increases in blood pressure (*9*), and elevations in the activity resembling that of platelet factor 4 (*10*). In light of these previous studies our present observation is particularly significant and suggests that endothelial damage is one of the earliest identifiable events in atherogenesis in the pigeon. The underlying cause of this damage and the explanation for its specific localization remain to be determined. It is conceivable, however, that genetic factors early in the development of the bird are instrumental in determining the susceptibility of specific endothelial sites to physical damage. Such regional variations in endothelium, as recently documented for human aortas (*11*), would correspond to other genetic features of aortas from the atherosclerosis-susceptible pigeons (*12*).

The role of platelets in atherogenesis has recently been reviewed (*4*), and a substantial amount of evidence is accumulating to link these circulating elements with experimentally induced mammalian atherosclerosis. According to one hypothesis (*4*), mitogenic factors

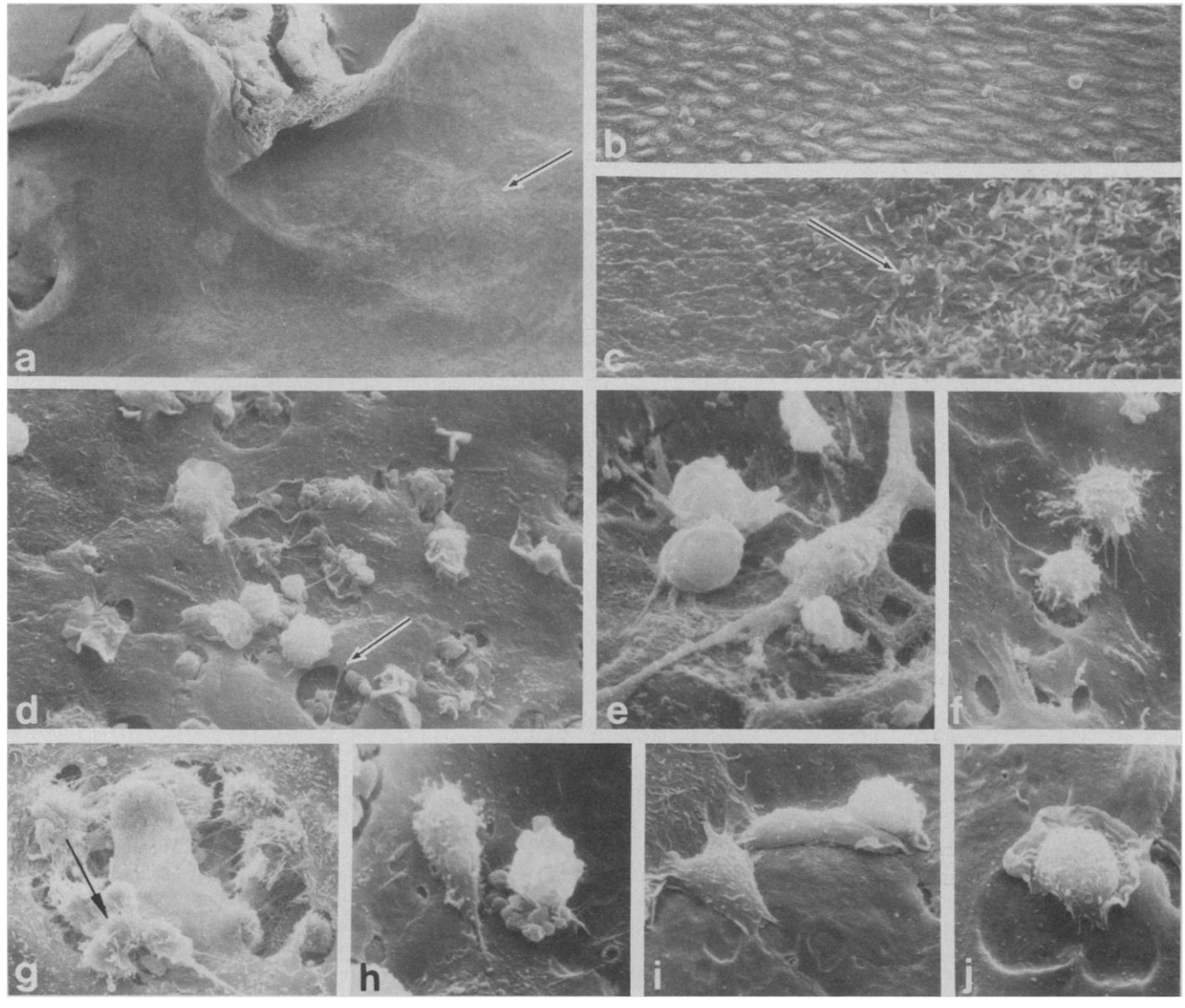

Fig. 1. Scanning electron micrographs of aortas from White Carneau pigeons. (a) Overview of perfused aorta showing celiac bifurcation, where intimal cushion (arrow) develops; (b) intact endothelium in region 15 mm cephalad to celiac bifurcation; (c) ruffled endothelium (arrow) found at celiac origin of 5-week-old pigeon; (d) pitted endothelium with subendothelial exposure (arrow) overlying the intimal cushion from 18-month-old bird; (e and f) thrombocytes adherent to damaged endothelium; (g) thrombocyte aggregate (arrow) associated with necrotic endothelium; (h–j) leukocytes adherent to damaged endothelial cell in cushion region. Magnifications: (a) ×10; (b) ×650; (c) ×900; (d) ×1600; (e) ×4100; (f) ×3000; (g) ×1800; (h–j) ×3000.

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

References and Notes

1. R. J. Jones, *Evolution of the Atherosclerotic Plaque* (Univ. of Chicago Press, Chicago, 1963).
2. J. R. A. Mitchell and C. J. Schwartz, *Arterial Disease* (Davis, Philadelphia, 1965).
3. S. Moore, *Lab. Invest.* **29**, 448 (1973); R. J. Friedman, S. Moore, D. P. Singal, *ibid.* **30**, 404 (1975); M. B. Stemerman and R. Ross, *J. Exp. Med.* **136**, 769 (1972); E. Nelson, S. D. Gertz, M. L. Rennels, M. S. Forbes, M. A. Kahn, T. M. Forber, F. P. Heald, F. L. Earl, *Circulation* **51–52**, II-103 (1975); D. K. Detweiler, H. L. Ratcliffe, L. Luzinbuhl, *Ann. N.Y. Acad. Sci.* **149**, 868 (1968).
4. R. Ross and J. Glomset, *N. Engl. J. Med.* **295**, 369 (1976); L. A. Harker, R. Ross, S. J. Slichter, C. R. Scott, *J. Clin. Invest.* **58**, 731 (1976).
5. W. D. Wagner, T. B. Clarkson, M. A. Feldner, R. W. Prichard, *Exp. Mol. Pathol.* **19**, 304 (1973); R. F. Santerre, T. N. Wright, S. C. Smith, D. Brannigan, *Am. J. Pathol.* **67**, 1 (1972).
6. P. H. Cook and S. C. Smith, *Exp. Mol. Pathol.* **8**, 171 (1968); N. T. Lauper, K. K. Unni, B. A. Kottke, J. L. Titus, *Lab. Invest.* **32**, 536 (1975); T. B. Clarkson, R. W. Prichard, M. Netsky, H. B. Lofland, *Arch. Pathol.* **68**, 1 (1959).
7. M. T. R. Subbiah, K. K. Unni, B. A. Kottke, I. A. Carlo, D. M. Dinh, *Exp. Mol. Pathol.* **24**, 287 (1976).
8. M. T. R. Subbiah and B. A. Dickie, *Atherosclerosis* **24**, 575 (1976).
9. Z. J. Rymaszewski, M. Langelier, I. A. Carlo, M. T. R. Subbiah, B. A. Kottke, *ibid.* **23**, 111 (1976).
10. V. Fuster, B. A. Kottke, R. E. Ruiz, J. C. Lewis, E. J. Bowie, *Thromb. Res.* **10**, 169 (1977).
11. A. L. Robertson, *Circulation* **53–54**, II-56 (1976).
12. T. N. Wright, P. H. Cook, S. C. Smith, *J. Cell Biol.* **67**, 455a (1975); T. Zemplenyi and A. J. Rosenstein, *Exp. Mol. Pathol.* **22**, 225 (1975).
13. We thank Curtis Grabau and Margo Roberts for their technical assistance. Supported in part by the Mayo Foundation and NIH grant HL-14196 for a specialized center in research in atherosclerosis.

6 December 1976; revised 1 February 1977

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-

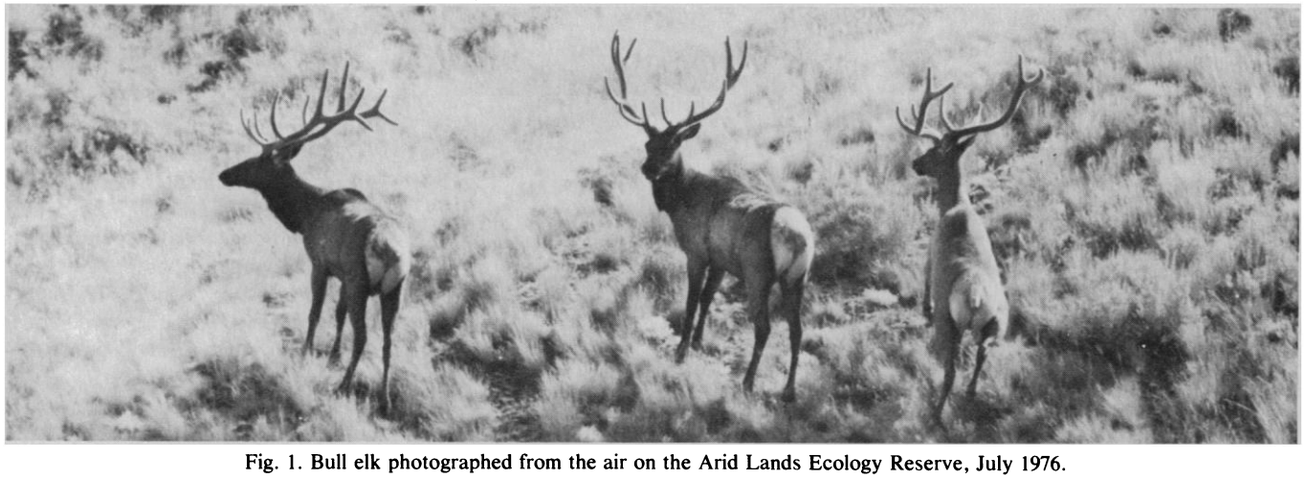

Fig. 1. Bull elk photographed from the air on the Arid Lands Ecology Reserve, July 1976.