

## Cartilaginous Metaplasia of the Thoracic Aorta of Control Turkeys and Exacerbation by $\beta$ -Aminopropionitrile<sup>1</sup> (41942)

CHARLES F. SIMPSON AND W. J. TAYLOR

*Colleges of Veterinary Medicine and Medicine, Box J-137, JHMHC,  
University of Florida, Gainesville, Florida 32610*

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**Abstract.** Seventeen of sixty distal extremities of the thoracic aortas of 12-week-old control male turkeys and 37 of 40 distal extremities of the aortas of turkeys fed 0.07%  $\beta$ -aminopropionitrile (BAPN) from 4 to 12 weeks of age contained areas of cartilaginous metaplasia when examined by light microscopy. The cartilaginous areas were generally elongated and located in the subendothelium of control turkeys, but a roundish area of cartilage was occasionally evident in the deep media. The magnitude of chondroplasia was enhanced by feeding BAPN; the extensive lesion usually extended from the subendothelium to deep in the media. Regardless of treatment, chondrocytes were pleomorphic, contained vacuoles, and had cytoplasmic processes. The cells were separated by pools of proteoglycans and connective tissue. The ultrastructure of chondrocytes in the aortas of both treatment groups was typical of this cell type. They had undulations or projections of the cell membranes. The cisternae of endoplasmic reticulum were dilated and contained electron-translucent material which was similar to extracellular proteoglycans. Golgi apparatus, free ribosomes, mitochondria, glycogen granules, filaments, and a centriole also were present in the cytoplasm. The extracellular matrix, which included collagenous and elastic fibers and also delicate fibrils and interconnecting matrix granules, separated adjacent chondrocytes by spaces of varying size. © 1984 Society for Experimental Biology and Medicine.

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The abdominal aorta of the male Broad-Breasted turkey, a muscular vessel, has been studied microscopically in great detail because of the spontaneous occurrence of dissecting aneurysms in this segment of the aorta (1, 2). The incidence of dissections and ruptures can be increased experimentally by agents which cause degeneration of the arterial wall (3, 4) or decreased by drugs which lower the high arterial blood pressure characteristic of the male Broad-Breasted turkey (5). The thoracic aorta of this strain of turkey, on the other hand, an elastic vessel, has not been studied extensively because degenerative changes are seen infrequently in this segment (6).

In the course of hemodynamic and pathologic studies of the thoracic aorta, it was observed on gross inspection that a firm area often was present in the wall of the distal extremity of this segment of the aorta and by microscopy it was determined that this area was the site of cartilage formation. Due

to the absence of electron microscopic studies of the thoracic aorta of the turkey, it was decided to study the cartilaginous area in detail. In addition, the lathyritic agent,  $\beta$ -Aminopropionitrile (BAPN) was found to increase the incidence and severity of cartilage formation in the thoracic aorta.

**Materials and Methods.** One group of 1-day-old Broad-Breasted White (BBW) male turkeys from a commercial hatchery was fed an unsupplemented (control) diet from 1 day until 12 weeks of age. A second group was fed the same diet to which 0.07% BAPN was added from 4 to 12 weeks of age, at which time all turkeys were sacrificed.

The distal extremities of the thoracic aortas of 60 control turkeys and 40 turkeys fed BAPN were examined by light microscopy. A thin ring of aorta, about 2 mm cephalad to the coeliac artery (7), was fixed in McDowell-Trump solution (8), embedded in paraffin, cut at 5  $\mu$ m, and stained with hematoxylin and eosin, orcein-Van Gieson, and toluidine blue stains. Correlative samples of the aortic rings found to contain cartilage when examined by light microscopy were prepared for electron microscopy by postfixing in 1% OsO<sub>4</sub> and embedding in Araldite.

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Ultrathin sections on grids were stained with uranyl acetate and lead citrate prior to examination with the electron microscope.

**Results.** Seventeen of sixty rings of the thoracic aortas from control turkeys contained areas of cartilaginous metaplasia by light microscopic examination. Generally, they were seen in the subendothelium and were elongated (Fig. 1), but cartilage was observed occasionally in the deep media, in which case it was more or less roundish.

The magnitude and incidence of aortic chondroplasia were enhanced by feeding BAPN. Consequently, the lesion was extremely elongated, extended from the subendothelium to deep in the media (Fig. 2), and was detected in 38 of 40 thoracic aortas examined histologically. Aside from quantitative differences, cartilage was similar microscopically in both control and BAPN-fed turkeys. Chondrocytes at the periphery of the lesions were spherical. Cells in the central area were polygonal, had one or more cytoplasmic processes, and contained vacuoles and nuclei with prominent nucleoli (Fig. 3).

Centrally disposed chondrocytes were larger than those at the periphery, and, in addition, were surrounded by considerably more matrix. Cartilage cells were separated from each other by collagenous fibers, a few fragmented elastic fibers, and pools of proteoglycans which stained metachromatically with toluidine blue. At the confines of the cartilaginous areas were pale staining smooth muscle cells, most of which were swollen and appeared to be degenerated.

Thoracic aortas without cartilaginous metaplasia by light microscopy had a concentric arrangement of six to eight rows of closely aligned dense elastic lamellae immediately below the endothelium, and rows of circumferential smooth muscle cells in the midmedia which were separated from each other by dense elastic fibers, collagenous fibers, and small, diffuse accumulations of proteoglycans.

The ultrastructural morphology of chondrocytes in areas of cartilaginous metaplasia was typical of this cell type (9, 10). These pleomorphic cells were spherical to mildly

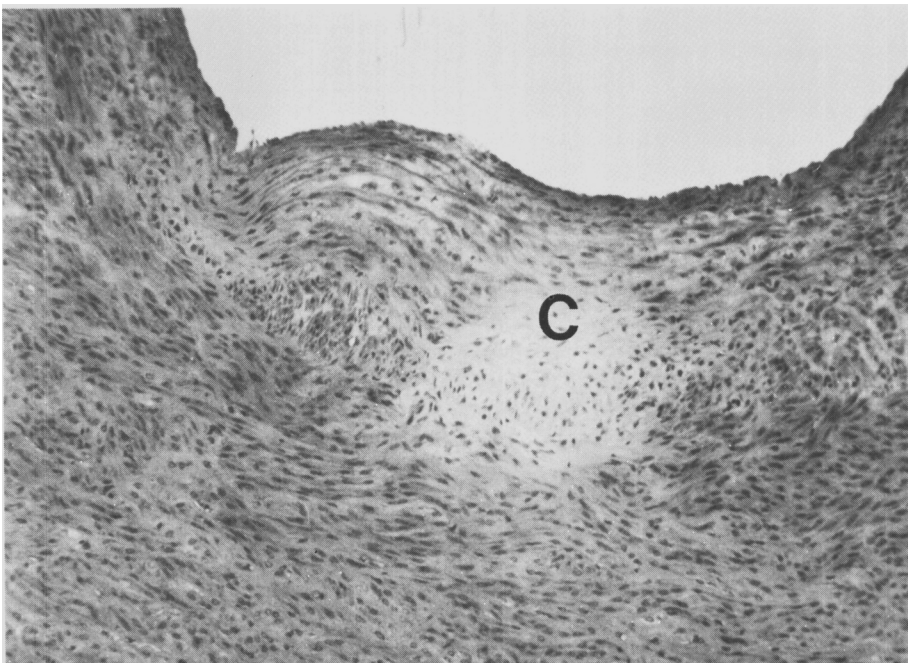


FIG. 1. There is cartilage (C) in the subendothelium of the distal thoracic aorta of a control turkey. H & E.  $\times 120$ .

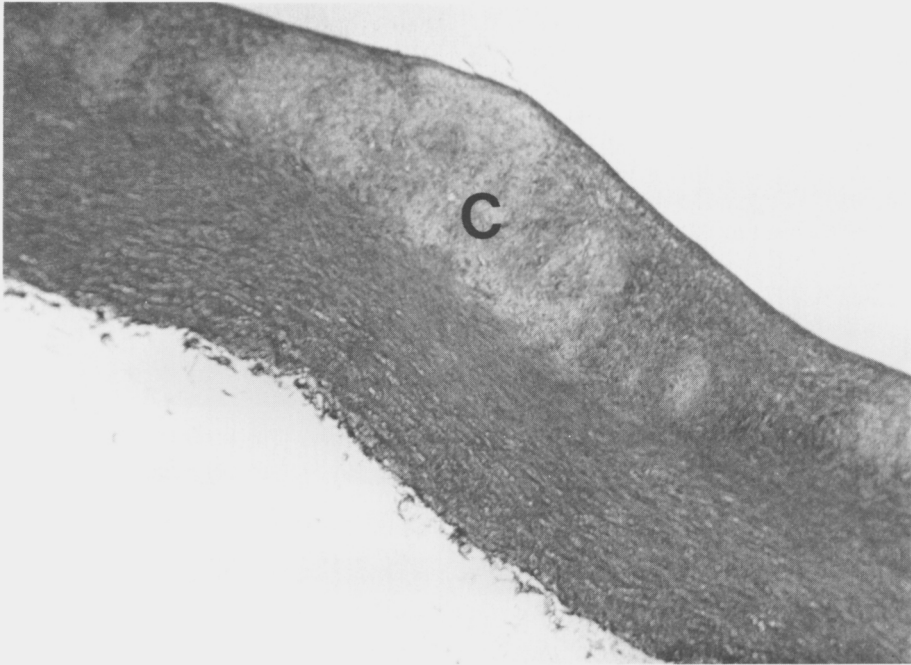


FIG. 2. Chondroplasia (C) of the aorta is exacerbated by feeding BAPN. H & E.  $\times 16$ .

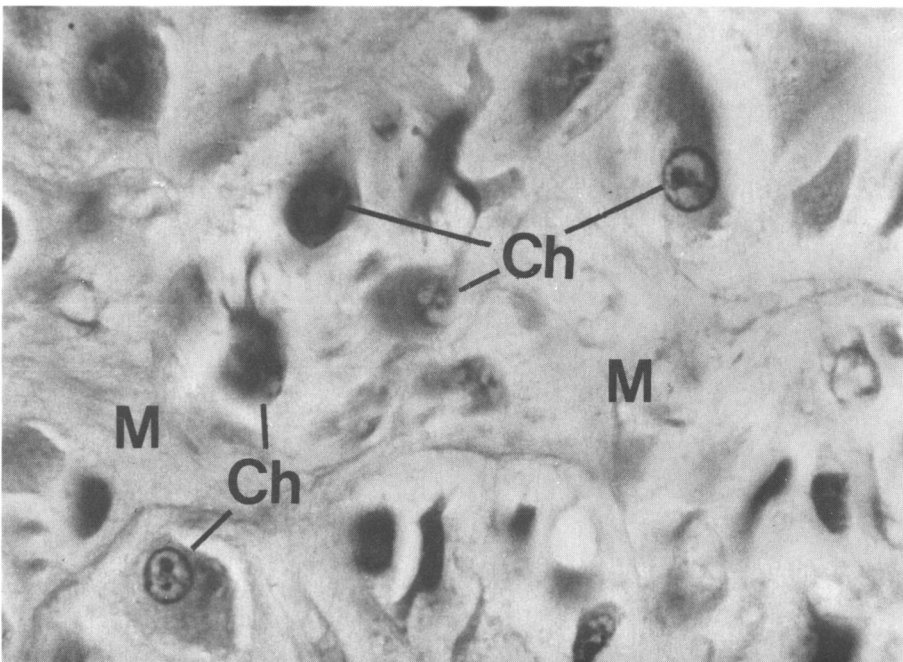


FIG. 3. Chondrocytes (Ch) are polygonal and are separated from each other by large deposits of matrix (M). H & E.  $\times 200$ .

elongated and had undulations or projections of their cell membranes (Fig. 4). Their nucleolus was prominent and the cisternae of endoplasmic reticulum were dilated and contained electron-translucent material. Golgi complex, free ribosomes, mitochondria, glycogen granules, juxtannuclear filaments, and a centriole were present in the cytoplasm (Figs. 5, 6). Vacuoles on the plasmalemma contained electron-translucent material similar to proteoglycans in the extracellular matrix (Fig. 6). Chondrocytes were widely separated from each other by extensive deposits of extracellular material. Such material consisted of dense bundles of collagenous fibers and an occasional fragmented elastic fiber. The matrix also included a network of branched, nonstriated fibrils and interconnected matrix granules which were separated from each other and from intertwined collagenous fibers by pools of proteoglycans. These accumulations of proteoglycans were electron lucent and were similar to the contents of vacuoles on or near the plasmalemma of chondrocytes; this material apparently was released into the extracellular spaces (Fig. 6).

**Discussion.** Cartilage is rarely present in the cardiovascular system; therefore, the high frequency with which it was seen in this study of the distal thoracic aorta of the turkey was completely unexpected. It is probably significant that cartilage only was seen in the area of the aorta where it is transformed from an elastic to a muscular blood vessel, presumably a point of stress (7). Cartilage has been described previously at the base of the cardiac valve cusps and edges of ruptured atherosclerotic plaques in the posterior aorta of birds, attributable, apparently, to stress and strain (11).

The cartilage cells in the turkey aorta had all of the organelles which are associated with active protein synthesis and secretion; for example, the dilated cisternae of the endoplasmic reticulum appeared to contain proteoglycans, comparable ultrastructurally to those of the extracellular matrix. The more peripheral lesions appeared to have less secretory apparatus and to be more pleomorphic with cytoplasmic extensions, characteristics which are associated with cellular proliferation in chondrocyte tissue cultures (12).

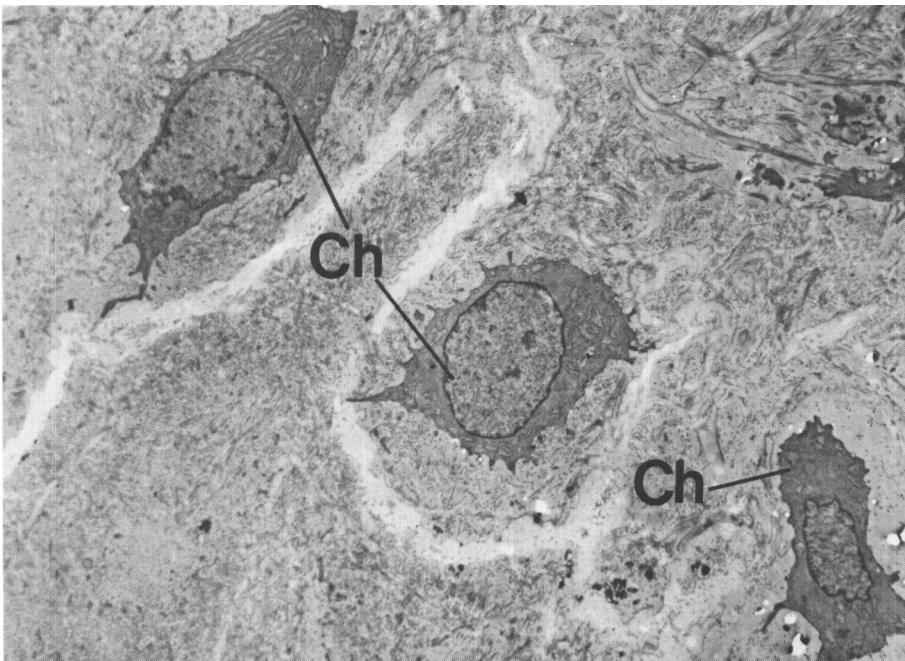


FIG. 4. Chondrocytes (Ch) are spherical to elongated and contain abundant endoplasmic reticulum.  $\times 3000$ .

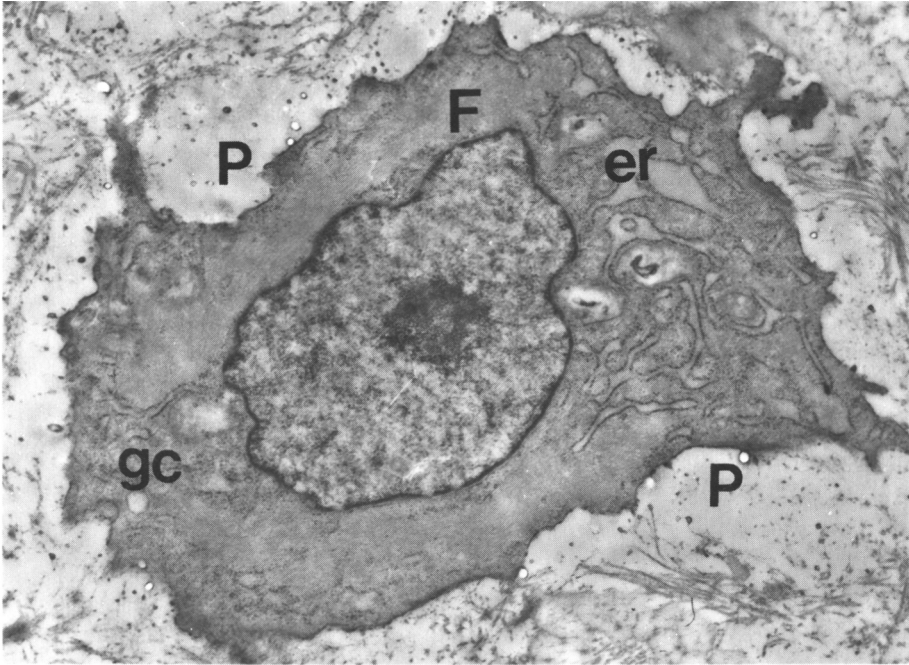


FIG. 5. Chondrocytes contain dilated cisternae of endoplasmic reticulum (er), Golgi complex (gc), and filaments (F). There is much extracellular proteoglycan (P).  $\times 5000$ .

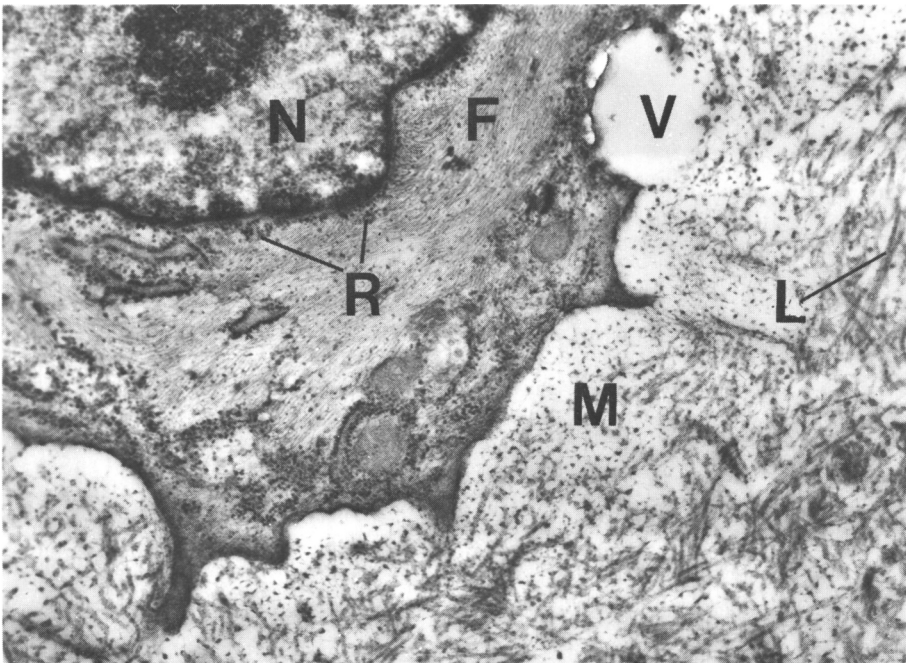


FIG. 6. There are filaments (F) and ribosomes (R) in the vicinity of the nucleus (N) and vacuoles (V) on the plasmalemma. Matrix granules (M) and collagen (L) are present extracellularly.  $\times 8000$ .

The ultrastructural appearance of the chondrocytes was quite distinct from that of smooth muscle cells, which are normally the only cell type present in vascular media (13). Filaments in the cytoplasm of chondrocytes are restricted to the perinuclear area and are more delicate than myofilaments in smooth muscle cells, which are also associated with cytoplasmic attachment points (14). Moreover, endoplasmic reticulum is not dilated in smooth muscle cells and they do not contain cytoplasmic vacuoles filled with proteoglycan-like material. It is known that the phenotypes of smooth muscle cells are quite variable, ranging from cells which have numerous contractile elements to those which do not contract but proliferate rapidly (15). The conversion of contractile smooth muscle cells to noncontractile multiplying cells has been termed modulation in contrast to dedifferentiation to a pluripotential stem cell which can then produce multiple cell lines. The presumed development of chondrocytes from smooth muscle cells would appear to require dedifferentiation and subsequent differentiation into another cell type. The explanation for exacerbation of cartilaginous metaplasia by BAPN is subject to conjecture. Possibly this influence is indirect and is mediated through the production of vascular weakness and hemodynamic stress which results from the known effect of BAPN on connective tissue (4).

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